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DYNAMICS OF MILK PRODUCTIVITY AND BODY WEIGHT OF COWS BY AGE AND PERIODS OF LACTATION

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The research was conducted on the farms of the Stepne State Enterprise and the Agroprogres Private Enterprise (stall housing) and on the dairy complex of the Kutuzivka State Enterprise (free housing).

It was established that the shape of the lactation curve of first-borns significantly differed from the classical one during untethered confinement. The average daily milk yield reached a maximum at 3-4 months and almost did not decrease until the 7th month of lactation. In older cows, fertility increased and reached a maximum at 2-3 months of lactation, and then gradually decreased. The relative increase in milk yield in comparison with the first month of lactation was 31% in first-born cows, 17% in second-calving cows, 7% in third-calving cows, and 18% in older animals, and in stall housing – 33%, 17%, 10 % and 2 %, respectively. The power of influence (η^2) of the month of lactation on the fat content in milk during untethered confinement was 8.5% in first-born cows, 14.5% in the second lactation, 13.1% in the third lactation, 12.2% in older cows, and for tethered confinement 14.8%, 36.9%, 33.6%, and 11.6%, respectively ($p<0.001$). Similar to the fat content in milk, the protein content of cows also changed during lactation. At the same time, the influence of the month of lactation on this indicator was much smaller than on the fat content, and in some cases it was improbable for stall housing.

The influence strength of the month of lactation on the daily yield of cows that were culled from the herd was 23.8% for free housing, 1.9% for body weight, and 8.0% for stall housing and 11.6 % ($p<0.001$). Correlation coefficients are 0.181, +0.411, and –0.223, +0.335, respectively ($p<0.01$).

Age-related changes in the body weight of animals depended on the technology of their husbandry. In free housing, it was higher both in the sample as a whole (6%) and in animals of different lactations (3-14%). The increase in body weight of cows with age in free housing was: 31 kg in the second lactation, 71 kg in the third lactation, and 100 kg in adult cows, while in stall housing these differences were only 6, 11, 35 kg, respectively.

Key words: husbandry technology, dairy cattle, age, month of lactation, daily yield, fat content, protein content, body weight.

ДИНАМІКА МОЛОЧНОЇ ПРОДУКТИВНОСТІ ТА ЖИВОЇ МАСИ КОРІВ ЗА ВІКОМ ТА ПЕРІОДАМИ ЛАКТАЦІЇ

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Встановлено, що за безприв'язного утримання форма лактаційної кривої первісток значно відрізнялась від класичної. Середньодобові надої досягали максимуму на 3-4 місяці і майже не знижувались до 7 місяця лактації. У корів старшого віку надій зростав і досягав максимуму на 2-3 місяць лактації, а в подальшому поступово знижувався. Відносне збільшення надоїв у порівнянні з першим місяцем лактації у первісток складало 31 %, у корів другого отелення – 17 %, третього – 7 %, а у старших тварин – 18 %, а за прив'язного утримання – 33 %, 17 %, 10 %, та 2 %, відповідно. Сила впливу (η^2) місяця лактації на вміст жиру в молоці за безприв'язного утримання була у первісток – 8,5 %, на другій лактації 14,5 %, на третій лактації 13,1 %, у старших корів 12,2 %, а за прив'язного утримання 14,8 %, 36,9 %, 33,6 %, та 11,6 %, відповідно ($p<0,001$). Аналогічно вміст жиру в молоці на протязі лактацій змінювався і вміст білка у корів. При цьому сила впливу місяця лактації на цей показник була значно менша, ніж на вміст жиру, а за прив'язного утримання у ряді випадків невірогідною.

Сила впливу місяця лактації на добовий надій корів, яких було вибракувано зі стада, за безприв'язного утримання складала 23,8 %, на живу масу – 1,9 %, а за прив'язного утримання – 8,0 % та 11,6 % ($p<0,001$). Коефіцієнти кореляції відповідно –0,181, +0,411, та –0,223, +0,335 ($p<0,01$).

Вікові зміни живої маси тварин залежали від технології їх утримання. За безприв'язного утримання вона була вища як у цілому по виборці (6 %), так і у тварин різних лактацій (3-14 %). Зростання живої маси корів із віком за безприв'язного утримання складало: у другу лактацію 31 кг, у третю лактацію - 71 кг та у повновікових корів – 100 кг, а за прив'язного утримання ці відмінності були лише 6, 11, 35 кг, відповідно.

Ключові слова: технологія утримання, молочна худоба, вік, місяць лактації, добовий надій, вміст жиру, вміст білка, жива маса.

The milk productivity of cows is significantly related to a number of factors, in particular the genotype of the animals, the conditions of their cultivation and maintenance, as well as the characteristics of each individual animal. It is known about the presence of age variability of cows in terms of milk yield. The research of domestic scientists revealed that the milk productivity of cows of the Ukrainian black and white dairy breed increased on average up to 4 calvings, after which it decreased. By this age, more than 56% of animals died, which made it impossible to achieve maximum livestock productivity (Klymkovetskyi and Nosevych, 2020). Other scientists (Vedmedenko and Alimova, 2021) established that the achievement of the highest level of milk yield is typical for cows in the sixth lactation, which is 18.8% higher compared to first-born cows and 3.7-10.5% higher than other age periods. According to the results of Turkish researchers (Eşki and Kurt, 2021), milk production increased in the first three lactations,

remained stable in the 3rd and 4th lactations, and decreased in the 5th and 6th lactations. In the studies of Korean scientists, a reliable relationship between milk yield and lactation number was found, with the maximum yield of cows in the third lactation (Vijayakumar et al., 2021). However, domestic scientists indicate that Holstein cows achieved maximum milk yield in the first lactation, when gross milk production exceeded that of animals in the third lactation by 2,407.1 kg or by 15.40% ($P < 0.05$) (Kapshuk, 2020). In studies (Mazur et al., 2018), the age of achieving the best lactation performance in cows of the Holstein breed was 1.86 lactations, and in animals of the Ukrainian black-and Red-and-White dairy breeds it was 1.83 and 1.79 lactations, respectively. Nevertheless, according to the results of other studies (Hartanto et al., 2020), it was concluded that dairy cows of the Holstein-Friesian breed had the highest productivity in the 4th lactation period. It has been established that the earlier a cow reaches high milk yields and keeps them stable longer, the higher its productivity per lactation (Sharapa and Boiko, 2018; Calsamiglia S. et al., 2020). However, other domestic scientists outline that the fact that with intensive exploitation technology, the level of milk productivity in cows increases from the first to the fourth calving, after which it slightly decreases, indicates the physiological exhaustion of the organism (Hutsuliak, 2019).

Some researchers (Gorelik et al., 2021) note a low negative relationship between body weight and milk yield of cows, which increases with age up to 3 lactations. The greatest decrease in milk yield was observed in the fifth lactation. A moderate positive genetic correlation between lactation constancy and 305-day milk yield indicates that selection for increased milk yield may slightly improve lactation constancy (Shokri-Sangari et al., 2019). According to (Hoy et al., 2012), body weight of all cows decreased from an average of 581 kg after calving to an average of 576 kg in the first week. The smallest body weight (532 kg) was reached after 42 days of lactation. The greatest loss of body weight was observed in first-born cows (9.7%), and in cows of the 2nd and 3rd lactation – the least (6.9%). Cows with above-average body weight loss were found to have a longer service period and required more inseminations for insemination than cows with below-average body weight loss. Similar data were obtained by other researchers (Řehák et al., 2012), who indicated that the loss of live masiz 1 to 8 weeks after calving was probably negatively correlated with the length of the service period between calving and fertile insemination. Changes in body weight during the first 120 days of lactation in high-yielding cows have been studied in detail in studies (Straten et al., 2008; Hazel et al., 2017). However, the authors note the large individual variability in these characteristics in the groups and the need for further research to clarify the relationship between changes in body weight, milk yield and reproductive function.

Some studies have shown that heifers and cows that produced more milk at the beginning of lactation often had a greater decline in body condition during this same period (Manríquez et al., 2021; Tribout et al., 2023). This rapid mobilization of energy reserves can lead to debilitating metabolic disturbances in the short term and have long-term effects on fertility and immunity (Stevenson, et al., 2020). French researchers (Faverdin, et al., 2017) point out that we should not neglect the fact that the amount of feed consumed, which is minimal during calving and increases sharply during lactation, partly hides the weight loss of cows at the beginning of lactation and can lead to an underestimation of the body's mobilization. Cows with a high body fat index also tend to consume more feed, which is a favorable characteristic when trying to limit disturbance in early lactation (Lefebvre et al., 2023). In the studies of Turkish scientists (Kul et al., 2021) it was established that body weight and milk yield increased until the fourth lactation, then they decreased. The lowest body weight was observed in the first two months

of lactation, and increased linearly with the following period of lactation. Based on the results of this study, it is suggested that monitoring body weight changes at the beginning and in the middle of lactation can be used as a management tool to improve milk yields in dairy herds.

Although dairy cattle breeding has focused on improving performance in leading dairy countries, there are also opportunities to improve the shape of lactation curves and milk composition related to human health (Cole et al., 2023). The quality of cow's milk remains a key issue that determines the success of dairy farming as a whole. Research (De Vries and Marcondes, 2020) found that herds with the longest productive lifespan had the highest somatic cell count in milk, the highest age at first calving and the highest conception rate.

At the same time, the above-mentioned factors in the conditions of various technologies of maintenance in Ukraine have not been sufficiently studied.

The purpose of the work is to determine the dynamics of body weight, milk productivity and milk quality of cows by age and lactation periods in the conditions of different husbandry technologies.

Research materials and methods. The research used the data of control milking and weighing of cows of three farms over the past 10 years. On the farms of the Stepne State Enterprise and the Agroprogres Private Enterprise, stall housing was used, and at the dairy complex of the Kutuzivka State Enterprise, free housing was used. Cattle feeding was separated according to the husbandry technology by different types of fodder and full-rational fodder mixes, and cows were milked in stalls and milking halls.

When conducting the study, only the data of cows that had weighing records in the month of control milking were used. All data was divided into gradations. The first group includes animals that have not been eliminated during the current month, the second group includes culling animals in the current month. The average values of daily milk yield, fat and protein content in milk of cows of different ages and stages of lactation were determined. We conducted a comparative analysis of the data that were obtained using different retention technologies.

Data processing was carried out according to basic statistical methods using the SPSS-20 computer program. Average values and their errors, correlation coefficients were calculated. The forces of influence were determined based on the results of variance analysis, and the probability was determined using the Fisher test. The comparison of average values and the probability of correlations was carried out according to the Student's test.

Research results. At the first stage of research, age-related differences in milk productivity and body weight of cows under different technologies of their husbandry were studied (tables 1-2).

Cows that did not drop out in the control month of lactation had an average daily weight yield of 1.6 kg lower in stall housing compared to tethered animals. But according to the average content of fat and protein in milk, as well as according to body weight, cows that were kept free had an advantage by 0.15%, by 0.01% and 33 kg ($p<0.001$).

By comparing the age-related changes in milk productivity, it was established that with free housing, the daily yield increased with age from the first to the second lactation by 2 kg, and then decreased by 1.4 kg in the third lactation, and in cows of the fourth lactation and older by 4.5 kg. During stall housing, the maximum average daily yield was obtained from cows of the 3rd lactation, which was higher by 1.1 kg in comparison with the second lactation and 2.6 kg – with the third ($p<0.001$). Age differences in the content of fat and protein in milk have not been established.

Table 1

Milk productivity of cows and their body weight in the month of control milking under untethered conditions

Lactation number	Daily yield, kg		Fat percentage		Protein percentage		Body weight, kg	
	n	M±m	n	M±m	n	M±m	n	M±m
No culling animals in the current month								
1	11687	20.3±0.06	10002	3.69±0.003	10002	3.11±0.002	11687	561±0.7
2	5303	22.3±0.12	4613	3.71±0.005	4610	3.14±0.004	5303	592±0.9
3	3265	20.9±0.16	2858	3.69±0.007	2859	3.13±0.005	3265	632±1.2
4-12	5102	17.8±0.13	4522	3.69±0.006	4527	3.12±0.004	5102	661±1.0
Total	25357	20.3±0.04	21995	3.69±0.002	21998	3.12±0.002	25357	597±0.5
Culling animals in the current month								
1	180	14.4±0.72	98	3.68±0.043	98	3.08±0.025	180	484±6.4
2	217	14.4±0.74	129	3.69±0.040	130	3.07±0.025	217	518±6.4
3	157	14.0±0.85	82	3.84±0.056	84	3.04±0.026	157	544±9.0
4-12	239	10.5±0.62	107	3.73±0.045	108	3.05±0.025	239	604±7.6
Total	793	13.2±0.34	416	3.73±0.021	420	3.06±0.012	793	541±3.0

Table 2

Milk productivity of cows and their body weight in the month of control milking under stall housing

Lactation number	Daily yield, kg		Fat percentage		Protein percentage		Body weight, kg	
	n	M±m	n	M±m	n	M±m	n	M±m
No culling animals in the current month								
1	3539	20.9±0.11	3472	3.60±0.007	3489	3.09±0.003	3539	545±1.2
2	1549	22.0±0.23	1533	3.55±0.009	1536	3.12±0.004	1549	551±1.4
3	1569	23.5±0.20	1525	3.50±0.010	1532	3.11±0.005	1569	556±1.4
4-12	5794	22.1±0.10	5739	3.50±0.005	5751	3.11±0.003	5794	580±0.9
Total	12451	21.9±0.06	12269	3.54±0.004	12308	3.11±0.001	12451	564±0.6
Culling animals in the current month								
1	104	13.5±0.76	97	3.65±0.047	98	3.09±0.010	104	495±8.6
2	129	13.5±0.73	120	3.65±0.026	121	3.09±0.009	129	553±8.6
3	112	12.6±0.75	107	3.59±0.028	107	3.06±0.012	112	578±9.7
4-12	193	11.9±0.48	188	3.60±0.021	188	3.10±0.010	193	589±7.3
Total	538	12.7±0.33	512	3.62±0.021	514	3.09±0.004	538	560±3.8

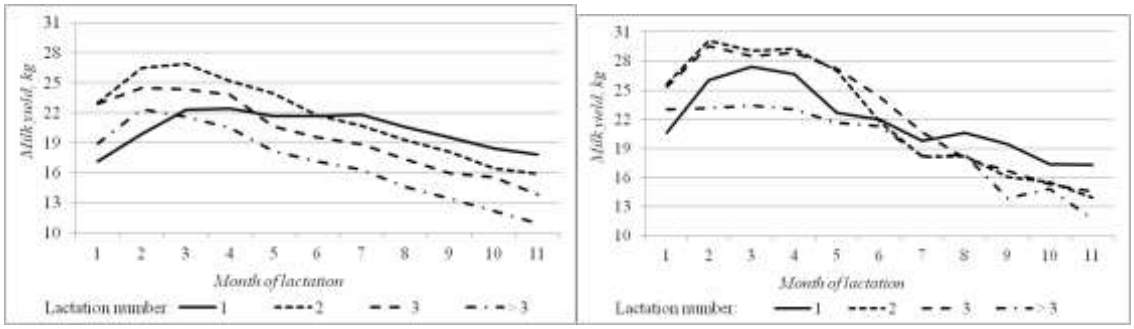
As for the body weight of cows of different ages, it depended on the technology of husbandry. During free housing, body weight was higher both in the sample as a whole (6%) and in animals of different lactations (3-14%). It is important to pay attention to the fact that the increase in the weight of cows with age was greater in free housing. Thus, the weight of animals increased with age for free housing by 31 kg in the

second lactation, by 71 kg in the third lactation, and by 100 kg in adult cows, while in stall housing these differences were only 6, 11, 35 kg, respectively ($p<0.001$).

Next, the differences in performance indicators of cows that were culled from the herd were considered. When kept untethered, these animals had an average daily weight of 7.1 kg less ($p<0.001$), fat content 0.04% more ($p<0.05$), protein content 0.06% less ($p<0.001$) and body weight was 56 kg lower, compared to cows that remained in the herd ($p<0.001$). Similar trends were established for stall housing. The differences, respectively, were –9.2 kg ($p<0.001$), +0.06% ($p<0.01$), –0.02% ($p<0.01$) and –4 kg ($p>0.05$). It should be mentioned that the average daily milk yield of cows that were left out was probably higher when kept without tethering compared to stall housing. This indicates that a lower percentage of animals were eliminated due to low milk production in free housing than in stall housing. Average daily milk yield and body weight of older cows that died in the current month were lower compared to first-borns.

In contrast to free housing, in which the body weight of cows that left the herd, regardless of age, was lower in first-borns by 77 kg, cows of 2 lactations by 77 kg, cows of 3 lactations by 88 kg, 4 lactations and older by 57 kg ($p<0.001$) compared to animals that remained in the herd. In stall housing, only the weight of first-borns was lower by 50 kg ($p<0.001$), and the culled animals of older age had a body weight of 2-22 kg higher ($p>0.05$).

The second stage of research was aimed at studying the characteristics of changes in the productivity of cows and body weight during different lactations under different husbandry technologies. The dynamics of changes in the average daily milk yield of animals depending on the number of calving is shown in Figure 1.



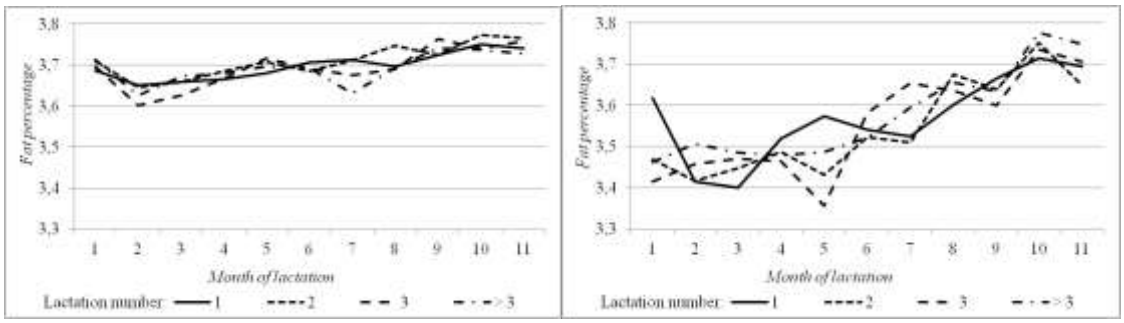
Free housing *Stall housing*

Fig. 1. **– Dynamics of daily milk yield of cows under different technologies of husbandry**

The lactation curve of first-borns significantly differed from the classical one during free housing. The average daily milk yield reached a maximum at 3-4 months of lactation and almost did not decrease until the 7th month. In older cows, the shape of the lactation curve was similar to the classical one. At the beginning of lactation, yield increased and reached a maximum at 2-3 months of lactation, and then gradually decreased. So, in cows of the second lactation, in the first month after calving, it was 23.0±0.38 kg, the maximum yield – 26.9±0.42 kg, and on the 11th month of lactation, it decreased to 15.9±0.48 kg. Accordingly, in the third lactation, milk yield was: 22.9±0.48, 24.5±0.67 and 13.8±0.63 kg, and in older animals: 18.9±0.41, 22.4±0.57 and 11.0±0.46 kg. The relative increase in milk yield compared to the first month of lactation was 31% in first-born cows, 17% in second-calving cows, 7% in third-calving cows, and 18% in older animals. The influence (η^2) of the month of lactation on daily fertility was: at the first calving – 8.5%, at the second lactation – 14.5%, at the third lactation – 13.1%, and in older cows – 12.2% ($p <0.001$).

The power of influence (η^2) of the month of lactation under stall housing on daily yield was higher and was equal to: at the first calving – 14.8%, at the second lactation – 36.9%, at the third lactation – 33.6%, and at more older cows – 11.6% ($p<0.001$). This indicates a smaller influence of other factors and more comfortable conditions of detention during tethered detention. The relative increase in milk yield compared to the first month of lactation was 33% in first-born cows, 17% in second-calving cows, 10% in third-calving cows, and 2% in older animals.

Figure 2 shows the changes in the fat content in the milk of cows according to the data of daily milk yield under different husbandry technologies.



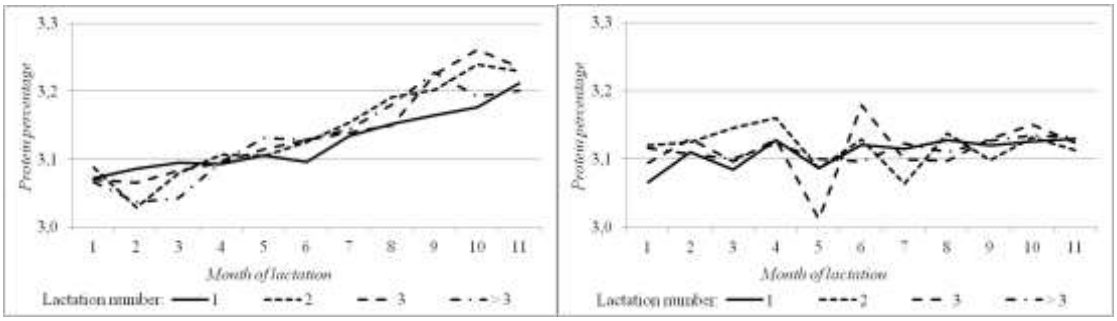
Free housing *Stall housing*

Fig. 2. **– Changes in the fat content in the milk of cows according to the data of daily milk yield under different technologies of husbandry**

During free housing, changes in milk fat content were small but plausible. The influence (η^2) of the month of lactation on this indicator was: at the first calving – 8.5%, at the second lactation 14.5%, at the third lactation 13.1%, and in older cows 12.2% ($p<0.001$). At the same time, the following clear trend was observed: in the first month of lactation, the fat content was at the level of 3.7%, in the second month of lactation, it decreased by 0.04-0.10%, then gradually increased and reached a maximum at 10-11 months of lactation (3.74-3.77%). This is due to changes in daily milk yield and the well-known relationship between milk yield and milk fat content.

Changes in the fat content in the milk of cows during husbandry were more significant. The influence (η^2) of the month of lactation on this indicator was: at the first calving – 14.8%, at the second lactation 36.9%, at the third lactation 33.6%, and in older cows 11.6% ($p<0.001$). Although the aforementioned trend was less clearly expressed in different lactations: in the first month of lactation, the fat content was at the level of 3.42-3.62%, in the second month it was equal to 3.42-3.51%, then it gradually increased and reached a maximum at 10-11 months of lactation (3.55-3.71%).

Similar to the content of fat in milk during lactation, the protein content of milk in cows kept without tethering also changed (Fig. 3). In the first month of lactation, it was equal to 3.06-3.09%, in the second month it slightly decreased and by the end of lactation it increased to 3.11-3.24%. At the same time, the influence (η^2) of the month of lactation on this indicator was much smaller than on the fat content and was: at the first calving – 2.4%, at the second lactation 5.1%, at the third lactation 4.8%, and at older cows 4.0% ($p<0.001$).



Free housing *Stall housing*

Fig. 3. **– Changes in the protein content in the milk of cows according to the data of daily milk yield under different technologies of husbandry**

During stall housing, the protein content practically did not depend on the month of lactation of the cow. The probable influence of the stage of lactation on the protein content of 3.1% and 2.5% ($p<0.001$) was established only in cows of the 1st and 3rd calving. The value of this indicator increased in cows of the above-mentioned lactations from 3.07-3.09% in the first month of lactation to 3.12-3.13% at the end of lactation.

The nature of changes in the body weight of cows during lactation depended on the number of calving (Fig. 4). Unfortunately, body weight data were incomplete for a number of months of lactation during stall housing, so they were not analyzed.

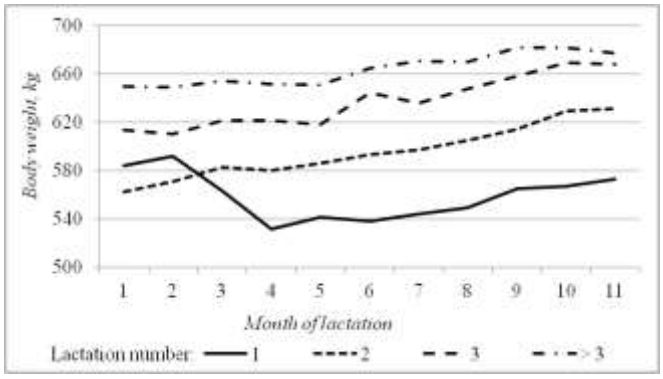
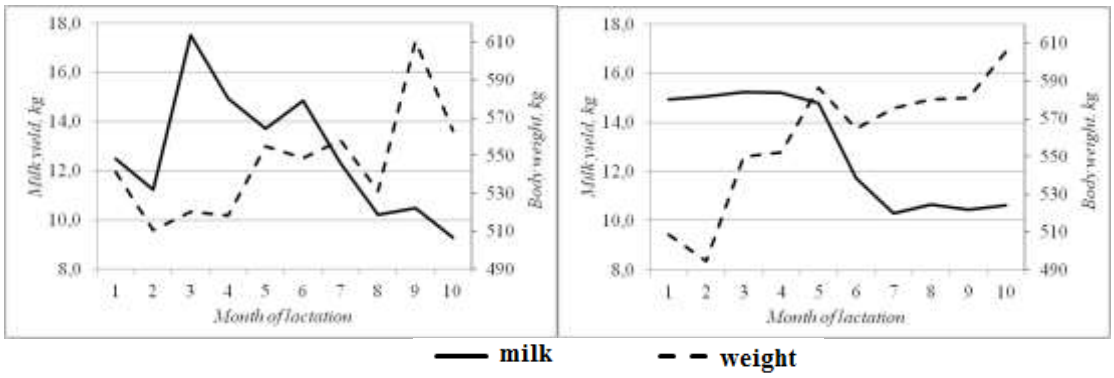


Fig. 4. **– Dynamics of body weight of cows during lactations under free housing technology**

During the period of lactation, the body weight of the first-borns changed as follows when they were kept untethered. In the first two months of lactation, it almost did not change (584±1.7 and 592±1.9 kg). In the third-fourth month after calving, their weight decreased by 29-61 kg, and starting from the fifth month, it gradually increased. At the same time, at the 11th month of lactation, it did not reach the value of the second month and was 19 kg lower. This indicates a negative energy balance at the beginning of lactation in first-borns. Nevertheless, this phenomenon was not observed in older lactating cows. The body weight of cows of the second calving gradually increased by 68 kg during 11 months of lactation, of the third – by 55 kg, and of the fourth – by 27 kg.

The influence (η^2) of the month of lactation on body weight was: at the first calving – 9.7%, in the second lactation – 10.1%, in the third lactation – 7.0%, and in older cows – 3.0% ($p<0.001$).

Figure 5 shows the dynamics of daily milk yield and body weight of cows at weaning in different months of lactation.



Free housing *Stall housing*

Fig. 5. **–Daily milk yield and body weight of cows at culling in different months of lactation under different technologies of husbandry**

As the graphs show, during the first two months of lactation, the average daily weight yield of cows that were left was at the level of 12.5±0.64 -11.2±1.3 kg, then in the 3rd month it probably increased to 17 .5 ± 1.25 kg and was at the level of 13.7 ± 1.41 - 14.9 ± 0.95 kg at 4-6 months of lactation. In the future, it gradually decreased to 9.3±1.57 kg. Changes in the body weight of cows were as follows: in the first month of lactation, cows with a body weight of 541±7.4 kg were eliminated; in 2-4 months of lactation, the animals that dropped out had a weight of 21-30 kg lower; in the following months of lactation, their body weight increased. The power of influence (η2) of the month of lactation on daily yield was 23.8%, and on body weight – 1.9% ($p<0.001$), correlation coefficients were -0.181 and +0.411 ($p<0.01$), respectively.

During stall housing, the average daily weight yield of cows that died during the first five months of lactation was 14.8±1.34 - 15.2±1.06 kg, and subsequently decreased to 10.3±1.03 - 10.6±0.77 kg. The body weight of cows that died in the first month of lactation was 509±13.4 kg, in the second month of lactation animals with a slightly lower weight (495±12 kg) were died, and later their body weight increased to 605±11.9 kg. The power of influence (η^2) of the month of lactation on daily yield was 8.0%, and on body weight – 11.6% ($p<0.001$), correlation coefficients were -0.223 and +0.335 ($p<0.01$), respectively.

Discussion. According to our data, it was established that the daily yield of cows under free housing increased with age from the first to the second lactation, and then decreased, while the maximum average daily yield was obtained from cows in the third lactation during stall housing. At the same time, according to Ukrainian scientists (Klymkovetskyi A. A. and D. K. Nosevych, 2020; Hutsuliak, 2019), animals with 4 calvings had the highest yield, and according to (Vedmedenko and Alimova, 2021), the achievement of the highest level of yield is characteristic of cows in the sixth lactation. However, our results are consistent with Eşki and Kurt, 2021; Vijayakumar et al., 2021; Gorelik et al., 2021. Nevertheless, some researchers (Kapshuk, 2020) indicate the highest yield for the1st lactation, and others for the second (Mazur et al., 2018). Daily milk yield of cows depends significantly on paratypic and genetic factors. Under different conditions of their maintenance and the level of annual genetic improvement of dairy cattle, age-related changes in milk productivity differ.

As for the dynamics of the body weight of cows during lactation, we have established that in the third-fourth month after calving, the weight of the first-borns decreased by 29-61 kg during free housing. Other researchers point to the loss of body weight of cows in a third of lactation (Hoy et al., 2012). They also indicate that the greatest loss of body weight was observed in first-born cows, and the least in cows of

the 2nd and 3rd lactation. Similar data were obtained by other researchers (Řehák et al., 2012; Stevenson, et al., 2020; Manríquez et al., 2021; Tribout et al., 2023). It should be noted that the phenomenon of negative energy balance is primarily related to the inconsistency of the feeding level with the genetic potential of the animal's milk productivity.

Conclusions:

1. The technology of husbandry, feeding and milking dairy cattle affects the nature of changes in milk productivity and body weight of cows with age and during lactation.

2. The daily yield of cows increased with age from the first to the second lactation under free housing, and during tethered husbandry the maximum average daily yield was obtained from cows of 3 lactations.

3. The increase in body weight of cows under free housing was 31 kg in cows of the second lactation, 71 kg in cows of the third lactation, and 100 kg in older cows, while in tethered husbandry these differences were only 6, 11, 35 kg, respectively.

4. Cows, which were culled from the herd, under untethered conditions had an average daily yield of 7.1 kg less, a fat content of 0.04% more, a protein content of 0.06% lower, and a body weight lower by 56 kg. than the cows that remained in the herd, the differences were – 9.2 kg, +0.06%, –0.02% and –4 kg, respectively.

5. In free housing, the body weight of cows that left the herd was lower by 77 kg in first-born cows, by 77 kg in the 2nd lactation cows, by 88 kg in the 3rd lactations, and by 57 kg in the 4^{th} lactation and older cows compared to animals that remained in the herd. Only the weight of the first-borns was lower by 50 kg during tethered husbandry.

6. The decrease in the body weight of the first-borns was established for the 3-4th month of lactation under free housing, which indicates a negative energy balance at the beginning of lactation. In older cows, fertility increased and reached a maximum at 2-3 months of lactation, and then gradually decreased.

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INFLUENCE OF MANURE STORAGE AND PROCESSING TECHNOLOGIES ON THE CHEMICAL PARAMETERS OF WATER

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Studies to study the impact of various options for storing and processing manure on the content of chemical compounds in the catchment area of farms were carried out in two farms in the Kyiv region with loose housing of cows. At AIS LLC, animals are kept in an easy-to-collect room, and manure is stored in a room with a deep, long-changing straw litter separately from waste from the milking and dairy block. In Terezine ALC, cows are kept in an easily assembled room with boxes. At the same time, manure is stored and processed in a bioreactor-fermenter. Water sampling for analysis on farms, springs within a radius of 1 km from farms, and in the nearest natural reservoirs (Ros and Protoka rivers, Bila Tserkva district, Kyiv region) was carried out during the second decade of September 2021. With the option of storage and processing of manure in a bioreactor-fermenter, the concentration of chlorides was 16.36 mg/dm3 higher compared to the option of storage and processing of manure in a room with deep, long-lasting straw litter. The sulfate and phosphate content was also higher – by 11.97 and 0.01 mg/dm3 Under. The content of chlorides, phosphates, and sulfites in water samples taken within a radius of 1 km from farms was 17.22 higher than the option of storage and processing of manure in a bioreactor-fermenter; 0.02 and 11.27 mg/dm 3. The content of chlorides, phosphates, and sulfites in drinking water samples taken from drinking bowls for animals was also slightly higher for the option of storage and processing of manure in a bioreactor-fermenter by 17.22; 0.02 and 12.91 mg/dm 3. In samples of drinking water taken from drinking bowls for animals under the option of storage and processing of manure in a room with deep, long-changing straw litter, The ammonium content was slightly higher than the indicators obtained from the farm where manure processing takes place in a bioreactor-fermenter (by 0.05 mg/dm3). So, with the option of storing and processing manure in a room with a deep, long-lasting straw litter, wastewater after washing milking equipment does not get into organic waste but settles and is disposed of. With this technology, the concentration of chemical compounds in the drinking water of the farm and the springs used by the population within a radius of 1 km from the research object and in the nearest natural reservoirs with running water is lower than in the case when the water after washing the milking equipment enters the general storage of manure (bioreactor-fermenter).

Key words: dairy cows, water, deep litter, bioreactor-fermenter, chemical compounds.

ВПЛИВ ТЕХНОЛОГІЙ ЗБЕРІГАННЯ ТА ПЕРЕРОБКИ ГНОЮ НА ХІМІЧНІ ПОКАЗНИКИ ВОДИ

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Дослідження з вивчення впливу різних варіантів зберігання та переробки гною на показники вмісту хімічних сполук водозбірного басейну ферм проводили у двох господарствах Київської області з безприв'язним утриманням корів. У ТОВ «АІС» тварин утримують у легкозбірному приміщенні, а зберігання гною відбувається у приміщенні з глибокою довгонезмінюваною солом'яною підстилкою окремо від відходів з доїльно-молочного блоку. У ТДВ «Терезине» корів утримують у легкозбірному приміщенні з боксами, при цьому зберігання та переробка гною відбувається у біореакторі-ферментері. Відбір зразків води для аналізу у господарствах, джерелах у радіусі 1 км від ферм та у найближчих природних водоймах (річки Рось і Протока Білоцерківський район Київська область) здійснювали упродовж другої декади вересня 2021 року. За варіанту зберігання та переробки гною у біореакторі-ферментері концентрація хлоридів була на 16,36 мг/дм3 вищою порівняно з варіантом зберігання та переробки гною у приміщенні з глибокою довгонезмінюваною солом'яною підстилкою. Також вищими були й значення вмісту сульфатів та фосфатів – на 11,97 та 0,01 мг/дм3 відповідно. Вміст хлоридів, фосфатів і сульфітів у зразках води, відібраної в радіусі 1 км від ферм, був вищим за варіанту зберігання та переробки гною у біореакторі-ферментері на 17,22; 0,02 та 11,27 мг/дм3. Вміст хлоридів, фосфатів і сульфітів у зразках питної води, відібраних із напувалок для тварин, був дещо вищим також за варіанту зберігання і переробки гною у біореакторі-ферментері на 17,22; 0,02 та 12,91 мг/дм3. У зразках питної води, відібраних із напувалок для тварин за варіанту зберігання і переробки гною у приміщенні з глибокою довгонезмінюваною солом'яною підстилкою, вміст амонію дещо переважав показники, отримані із ферми, де переробка гною відбувається у біореакторі-ферментері (на 0,05 мг/дм3). Отже, за варіанту зберігання і переробки гною у приміщенні з глибокою довгонезмінюваною солом'яною підстилкою стічна вода після процедури промивання доїльного обладнання не потрапляє до органічних відходів, а відстоюється та утилізується. За такої технології концентрація хімічних сполук у питній воді ферми та джерелах, якими користується населення у радіусі 1 км від об'єкту дослідження та у найближчих природніх водоймах з проточною водою, нижча, ніж за варіанту, коли вода після промивання доїльного обладнання надходить до загального зберігання гною (біореактор-ферментер).

Ключові слова: молочні корови, вода, глибока підстилка, біореактор-ферментер, хімічні сполуки.

Entry. The problem of providing the population of Ukraine with high-quality and safe dairy products has become a priority at this time (Borshch et al., 2020; Ruban, et al., 2022). This is especially true in areas that have been and continue to be subjected to significant anthropogenic pressure. As a result of the accident at the Chornobyl nuclear power plant, a large part of Ukraine was subjected to radioactive contamination (Rozputnii et al., 2018). At the same time, almost 9% of agricultural land with different types of soils and their moisture level were polluted. These factors have affected the

health of the country's population and the agricultural sector, particularly animal husbandry (Krysinska & Klymenko, 2021).

In the current conditions of intensive development of industrial production, as a result of the activities of metallurgical, chemical, fuel and energy, processing, and other enterprises, the concentration of toxic substances dangerous to human health in soil, water, air, and food products is several times higher than the permissible norms (Hejna et al., 2018; Chirinos-Peinado & Castro-Bedriñana, 2020). One of the sources of environmental pollution is agriculture, particularly dairy cattle breeding (Zhao et al., 2019; Libisch et al., 2022; Lin et al., 2023). Traditionally, environmental issues associated with the dairy industry have been considered to relate to impacts on water quality in reservoirs and springs close to animal housing sites (Cortés, et al., 2020; Fusco et al., 2020). However, due to global warming trends, global scientists have recently argued that dairy cattle are one of the primary biological sources of greenhouse gas production (Kou et al., 2021).

During their life, cattle produce waste accumulated in feces, urine, steam, etc., which, under certain conditions, can cause significant harm to nature (Portiannyk & Mamenko, 2022). Modern technologies for the production of livestock products can significantly reduce the risks of environmental pollution. However, it is necessary to adapt these technologies to specific production conditions in the most rational way, depending on natural and climatic conditions (Ny et al., 2022). A dairy farm with 500 cows of average productivity emits more than 3 tons of carbon dioxide, almost 5 tons of water vapor, a significant amount of ammonia, and other gases into the atmosphere daily, producing about 20 tons of effluent and about 30 tons of excrement. Therefore, the acute problem of reducing environmental pollution by livestock waste makes it necessary to look for ways to more rationally utilize and efficiently use manure and effluents from dairy farms and complexes (Borshch et al., 2021).

Harmonization of the development of technology and nature is possible only due to scientifically based compromises between human economic activity and the problem of sustainability of environmental conditions (Borshch et al., 2020). To date, the only acceptable option for the progressive movement of humanity is the movement "according to the principles of sustainable development", which does not destroy the natural biotic mechanism of self-regulation of the natural environment. Therefore, when creating and implementing technological and technical systems in production, one should strive to minimize the artificial impact on the environment (Saber et al., 2022).

Technological processes of livestock production associated with the processing of natural and energy resources are accompanied by the emergence of a significant amount of waste. Hence, a cattle farm is a manufactured object that can significantly impact the environment, being a source of air, soil, and water pollution (Cortés, et al., 2020). The chemical composition of drinking and river water, along with agricultural sources of pollution, Heavy industry enterprises, infrastructure facilities, and the location of cities or settlements along rivers, also have a significant impact (Esterhuizen et al., 2012).

The research aimed to study the effect of different options for storing and processing Manure on the indicators of the chemical compounds content in the farms' catchment area.

Materials and methods. The study was carried out in two farms in the Kyiv region with loose housing of cows but with different options for storing and processing Manure. The object of the study was the chemical parameters of water on farms with different technologies for the storage and processing of Manure. At AIS LLC, animals are kept in an easy-to-collect room with deep, long-lasting straw bedding. Manure is

removed from the premises twice a year. In Terezine ALC, animals are kept in an easily assembled room in boxes. At the same time, storage and processing of Manure, together with waste from washing and disinfection of milking and dairy equipment, takes place in a bioreactor-fermenter (Fig. 1). Manure is removed from the room using a delta scraper with a frequency of 12 times a day. The application of Manure to the fields in the studied farms takes place in August-October and March-April. Under the option of storing and processing Manure in a room with deep, long-term unchanging straw litter (AIS LLC), in contrast to Terezin ALC, waste from washing milking and dairy equipment enters a separate container and is disposed of without getting into the soil.

Rice. 1. **Manure storage and processing options: a) deep straw bedding; b) bioreactor-fermenter**

The location of the nearest natural reservoirs and agricultural lands from farms with various options for storing and processing manure, where the research was carried out, is shown in Table. 1 and in Fig. 2.

Table 1

The location of the nearest natural reservoirs and agricultural lands in relation to farms with different options for storing and processing manure

Indicator	Manure storage and processing option	
	deep, long-lasting litter	Bioreactor-fermenter
Name of the reservoir	Ros River	Protoka River
Reservoir basin area, m2	12 575	580
Distance from the reservoir to the farm, km	2,3	5,5
Distance from the reservoir to the agricultural land in which organic fertilizers were applied, km	0,080	0,150

Water sampling for analysis on farms, springs within a radius of 1 km from the farm, and in the nearest natural reservoirs (Ros and Protoka rivers, Bila Tserkva district, Kyiv region) was carried out at an average daily ambient air temperature of +15.2 °C (second decade of September 2021). Eight samples were taken at each of the three locations (the nearest natural reservoir from the farm, spring water within a radius of 1 km from the farm, and water from the farm). Determination of water quality indicators was carried out in the State Institution "Bila Tserkva City Sanitary and Epidemiological Station" of the Ministry of Health of Ukraine. The volume of water samples for analysis was 5 liters. Bottles were plastic, cleanly washed, and rinsed with distilled water.

a) b)

Rice. 2. **Location of farms about water bodies and agricultural lands: a) with storage and processing of manure in a room with deep, long-lasting straw bedding; b) with the storage and processing of manure in a bioreactor-fermenter**

Water samples from rivers were taken above the potential source of pollution (agricultural land), against it, and downstream. Water sampling from open reservoirs of rivers was carried out following DSTU ISO 5667-1-2003. Water was taken at a depth of 0.5–1 m from the reservoir's surface at a distance of 1–2 m from the shore. Water samples from open reservoirs (wells) were taken at a depth of at least 1 m from the surface – in the morning when water had not yet been taken from it. The content of chlorides and sulfates the values of total hardness, nitrates, and nitrites in water were determined according to generally accepted methods (Malina et al., 2014). The permanganate oxidation and alkalinity of the studied water samples were detected according to the Kubel method and titrimetric study, respectively. The content of phenols in water was estimated by the photocolorimetric method.

The research materials were processed by the method of variational statistics based on the calculation of the arithmetic mean (M), the standard error (m), and the significance of the difference between the compared indicators (P). To show the probability in the tables, the symbols $P \geq 0.95$; $R \geq 0.99$; and $P \geq 0.999$, are respectively marked with asterisks (*;**;***).

Research results. Natural water contains significant impurities, ranging from dissolved metals and their salts to mechanical inclusions like rust, sand, and clay. The chemical composition of impurities is significant and affects water's total amount of chlorides, sulfates, and phosphates (Rozputnii et al., 2018).

The relationship between the variant of storage and processing of manure and the content of chemical compounds in river water samples has been established. In particular, the content of chlorides, which in high concentrations cause respiratory and skin diseases in animals (Malina et al., 2014), in the water of the studied rivers was the lowest than in the case of storage and processing of manure in a room with deep long-lasting straw litter by 36.25 mg/dm3 (Table 2).

As for the content of sulfates (salts of sulfuric acid), which, when exceeding the permissible norms in the body of both humans and animals, can cause allergic reactions and gastrointestinal disorders (Krysinska & Klymenko, 2021), their slightly higher concentration in river water was observed with the option of processing and storing manure in a bioreactor-fermenter – 52.31 mg/dm3, which is 11.97 mg/dm3 more than in a room with deep, long-lasting straw litter. The content of phosphates, which, at high concentrations, cause eutrophication (enrichment of water bodies with nutrients, accompanied by a decrease in their productivity) in natural objects and cause a nerve

agent in the body (Zhovnir & Grebin, 2018), was the lowest for the option of storage and processing of manure in a room with deep long-changing straw litter and amounted to 0.03 mg/dm3, which is 0.01 mg/dm3 lower than the values obtained in the fermenter bioreactor. In general, the concentration of the three main chemical compounds in the water samples of the zone of the studied farms was within the maximum permissible norm.

Table 2

Water quality indicators in the nearest natural reservoir from the farm depending on the option of manure storage and processing, mg/dm3, (M±m)

Indicator	Maximum permissible norm (not more than) mg/dm3	Manure storage and processing option	
		deep, long-lasting litter	Bioreactor-fermenter
Chlorides	350	36,25±1,032	52,61±0,680***
Sulphates	250	40,34±1,188	52,31±1,282***
Phosphates	3,5	0,03±0,001	0,04±0,002***

*Note. *** P≥0.999 – compared to the option of storing and processing manure on deep long-changing litter.*

Similar research results were observed in drinking water samples taken within a radius of 1 km from the location of the farms. The content of chlorides and phosphates was higher in the bioreactor-fermenter by 17.22 and 0.02 mg/dm3. A higher concentration of sulfites in the studied water samples was also found with this variant of manure storage and processing – 43.46 mg/dm3, which is 11.27 mg/dm3 more than with the option of processing and storage of manure in a room with deep long-changing straw litter (Table 3).

Table 3

Indicators of drinking water quality within a radius of 1 km from farms depending on the options for storage and processing of manure, mg/dm3, (M±m)

Indicator	Maximum permissible norm (not more than) mg/dm3	Manure storage and processing option	
		Deep long-immutable Litter	Bioreactor-fermenter
Chlorides	350	26,05±0,235	43,27±0,142***
Sulphates	250	32,19±0,531	43,46±0,810***
Phosphates	3,5	0,01±0,001	0,03±0,001***

*Note. *** P≥0.999 – compared to the option of storing and processing manure in a room with deep, long-lasting straw litter.*

The goal was to study the content of those indicators that directly depend on the activities of agricultural enterprises to determine the quality indicators of drinking tap water from drinking bowls on farms. Concerning the content of chemical compounds in samples taken from animal drinkers, there was a general trend towards the highest values for the content of chlorides and phosphates for the option of storing and processing manure in a bioreactor-fermenter, and slightly lower - in a room with deep, long-lasting straw bedding. However, in terms of the ammonium content, which at high concentrations affects the increase in blood pressure and respiratory rate and leads to

disorders in the liver and kidneys (UNESCO, 2019), the highest value was registered for the option of storage and processing of manure in a room with deep long-changing straw litter – 0.08 mg/dm3, which exceeded similar values for storage and processing of manure in a bioreactor-fermenter – by 0.05 mg/dm3 (Table 4).

Table 4

Indicators of the quality of drinking water on the territory of the farm depending on the option of storage and processing of manure, (M±m)

Indicator	Maximum permissible norm (no more)	Manure storage and processing option	
		deep, long-lasting litter	Bioreactor-fermenter
Chlorides, mg/dm^3	350	28,33±0,172	45,55±0,203***
Sulfates, mg/dm^3	250	32,54±0,773	45,45±0,927***
Phosphates, mg/dm^3	3,5	0,02±0,001	0,04±0,001***
Ammonium, mg/dm^3	0,5	0,08±0,0005	0,03±0,0001***
Nitrites, mg/dm^3	0,002-0,003	0,006±0,002	0,016±0,004*
Nitrates, mg/dm^3	45	0,88±0,09	7,19±2,04***
Permanganate oxidation, mg/dm^3	2,0	0,67±0,03	0,72±0,09
Phenols, mg/dm^3	0,001	0,0001±0,00001	0,0002±0,00001
Alkalinity, mmol/dm^3	6,5–8,5	7,09±0,08	7,95±0,05
Total hardness, mEq/l	1,5-7,0	8,10±0,22	9,40±0,34**

*Notes: * – P≥0.95; ** – R≥0.99; ¯ P≥0.001 – compared to the option of storing and processing manure in a room with deep, long-lasting straw bedding.*

In terms of the content of nitrites in water, which are capable of destroying red cells in the blood of mammals (Damania et al., 2019), the highest concentration was observed with the option of storage and processing of manure in a bioreactor-fermenter of 0.016 mg/dm3, which was 0.010 mg/dm3 higher than the content in water samples obtained from a farm with deep litter. High nitrate content in water increases the risk of chronic stomach upset, causes endocrinological disruption to the human body and can lead to abortion and stillbirth in farm animals (Malina et al., 2014). According to this indicator, the highest value was noted for the variant of storage and processing of manure in a bioreactor-fermenter of 7.19 mg/dm3, which is 8.17 times more than the indicators obtained in water from a farm with storage and processing of manure in a room with deep long-changing straw bedding. Permanganate oxidation determines the content of organic and mineral substances in water (Barnwal et al., 2017). It makes it possible to establish a specific indicator for water pollution in general. The smaller the permanganate oxidation, the more likely water will be used not only for operational needs. A high index of permanganate oxidation indicates the presence of a significant proportion of bacteria among organic matter. As a rule, an indicator higher than 2.0 mgO 2/dm3 negatively affects the body's reproductive function, liver, and kidneys (UNESCO, 2019). A slightly higher value of the permanganate oxidation of water among the studied farms was recorded during the storage and processing of manure in a bioreactor-fermenter of 0.72 mg/dm3, which by 0.05 mg/dm3 exceeded the value of indicators from samples obtained from a room with deep long-changing straw litter. Phenols are organic aromatic compounds used in the production of various aldehyde resins, polyamides, epoxy resins, antioxidants, etc.; when ingested by humans or animals, they can cause devastating effects on the kidneys and brain (Boyle et

al., 2010). Compared to the bioreactor-fermenter, a slight decrease in the phenol content was found in the variant of storage and processing of manure on deep litter (by 0.0001 mg/dm3). The alkalinity of water is a measure of the ability of a water solution to neutralize strongly, that is, those that completely dissociate into their ions when mixed with water acids. When they come into contact with mucous membranes or on the skin, highly mineralized alkaline waters cause them to dry out excessively (Keiser & Shapiro, 2019). In the studied farms, the total alkalinity of drinking water on the farm was within the permissible norm. It amounted to 7.09-7.95 mmol/dm3 with a slight advantage (less by 0.86 mmol/dm3) in the option of storing and processing manure in a room with deep long-changing straw litter, compared to a bioreactor-fermenter.

The total hardness of the water depends on the amount of soluble minerals, particularly calcium and magnesium. Hard water does not pose a danger to human and animal health. At the same time, hard drinking water in drinking bowls of farm animals can cause mold on the walls (Malina et al., 2014). The highest value of the total hardness of drinking water was stated for the option of storage and processing of manure in a bioreactor-fermenter – 9.40 mEq/l, which is 1.30 mEq/l higher than in a room with deep long-changing straw litter. At the same time, the total hardness of water in both farms where the study was carried out exceeded the maximum permissible norm by 1.1–2.4 mEq/l.

Discussion. In their life, cattle produce waste, which, under certain conditions, can cause significant harm to nature. Modern technologies for the production of livestock products can significantly reduce the risks of environmental pollution. However, it is necessary to adapt them most rationally, depending on natural and climatic conditions.

Hamill K. D. & McBride G. B. (2003), in studies conducted on the South Island of New Zealand in a subtropical climate, examined the phosphate content of six small rivers located near farmland or dairy farms. They established a relationship between the phosphate content in river water and the location of dairy farms. In water samples from rivers adjacent to farmland, phosphate concentration was lower than in water samples from rivers near dairy farms. Similar data are provided by another group of New Zealand researchers, who found an increased sulfate content in river water samples during organic fertilizer application to the fields (Wilcock et al., 1999).

The data obtained coincide with the data of Indonesian scientists who studied the content of chlorides, sulfates, and phosphates in the water of the Solo River (Java Island, equatorial climate), as well as in the drinking water of settlements located in its basin, where dairy farms were located (Widiastuti et al., 2015). They established an increased content of chlorides, sulfates, and phosphates in rivers and drinking water. At the same time, waste from washing milking equipment and washing the pre-milking area on the farms under study fell into the soil and water bodies. Scientists from the Republic of South Africa (subtropical climate zone) reported increased chlorides, sulfates, and phosphates in drinking spring water in settlements near the farm (Esterhuizen et al., 2012).

A decrease in the concentration of phosphates in water from Lake Champlain (Vermont, USA, continental climate) after constructing a sedimentation system on a nearby farm is reported in the work of American scientists (Kominami & Lovell, 2012).

Conclusions. The dependence of the indicators of the content of chemical compounds chlorides, sulfates, and phosphates in water from the nearest natural water bodies on storing and processing manure on the farm has been established. With the option of storing and processing manure in the room for keeping animals on deep, long-changing bedding and regulated disposal twice a year, wastewater after washing

milking equipment does not get into organic waste and is settled and disposed of separately. With this technology, the concentration of chemical compounds in the drinking water of the farm, the sources of which are used by the population within a radius of 1 km from the farm and in the nearest natural reservoirs with running water, is lower than in the case when the water after washing the milking equipment enters the general manure storage (bioreactor-fermenter).

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USE OF ANIMAL PRODUCT PROCESSING WASTE IN OYSTER MUSHROOM PRODUCTION TECHNOLOGY

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The article presents a study of the possibility of using animal husbandry processing waste as a nutrient additive to compositions of synthetic fermented substrate for the cultivation of oyster mushrooms. Wastes that are of low value for use in other branches of agriculture and industry and are mainly disposed of as solid waste by burial were investigated.

In modern economic conditions, it is important to reduce the cost of the substrate, one of the possible ways to achieve this goal is the use of cheaper raw materials. Meals and bran are traditionally used to balance the substrate in terms of nitrogen content. These products are waste products from the processing of agricultural crops and are also classically used to balance the rations of farm animals and poultry. This creates competition between industries and increases the price of raw materials, which ultimately causes an increase in product prices. Therefore, the search and research of alternative balancing components for the preparation of oyster mushroom substrate, which are not competitive for animal husbandry, is relevant. The analysis of the obtained results made it possible to form the following conclusions: their use as additives for the preparation of the substrate will allow them to be used in further processing, with the obtaining of additional products of mushroom production, the study of the growth of colonies of oyster mushrooms using the selected additives made it possible to determine the best options: yes, the addition of skin shavings and the addition of pores It does not inhibit the colonization of these additives, but, on the contrary, increases the growth rate of colonies compared to colonies developed without additional nutrition; the obtained data of the laboratory experiment can be considered primary and those that require further research, including when using other methods of preparation of additives (sterilization, solid-state fermentation), as well as verification in scientific and economic experiments on the determination of technological properties in substrate compositions and the calculation of biological effectiveness and productivity of oyster mushrooms and other saprophytes.

Key words: waste, leather, feathers, animal husbandry, technology, mushrooms, oyster mushroom.

ВИКОРИСТАННЯ ВІДХОДІВ ПЕРЕРОБКИ ПРОДУКЦІЇ ТВАРИННИЦТВА В ТЕХНОЛОГІЇ ВИРОБНИЦТВА ГЛИВИ

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В статті наведено дослідження можливості використання відходів переробки продукції тваринництва в якості поживної добавки до композицій синтетичного ферментованого субстрату для вирощування гливи звичайної. Досліджувались відходи, що представляють низьку цінність для використання в інших

галузях сільського господарства та промисловості і в основному утилізуються як тверді відходи шляхом поховання.

В сучасних економічних умовах важливим є зменшення собівартості субстрату, одним з можливих шляхів досягнення цієї мети є використання більш дешевої сировини. Для балансування субстрату за вмістом азоту традиційно використовують шроти та висівки. Ці продукти є відходами переробки сільськогосподарських культур і також класично використовуються для балансування раціонів сільськогосподарських тварин і птиці. Це створює конкуренцію між галузями виробництва і підвищує ціну на сировину, що в кінцевому результаті спричиняє ріст цін на продукцію. Тому пошук і дослідження альтернативних балансуючих компонентів для приготування субстрату гливи, таких, що не є конкурентними для тваринництва є актуальним. Аналіз отриманих результатів дозволив сформувати наступні висновки: використання в якості добавок для приготування субстрату дозволить використати їх в подальшій переробці, з отриманням додаткової продукції грибовиробництва, дослідження росту колоній гливи звичайної з використанням обраних добавок дозволив визначити найкращі варіанти: так, додавання стружки шкіри та додавання пір'я не гальмує колонізацію цих добавок, а, навпаки, збільшує швидкість росту колоній в порівнянні з колоніями, що розвивались без додаткового живлення; отримані дані лабораторного досліду можна вважати за первинні та такі, що потребують подальшого дослідження, в тому числі при використанні інших способів підготовки добавок (стерилізації, твердотільної ферментації), а також перевірки в науково-господарських дослідах з визначення технологічних властивостей в композиціях субстрату та розрахунку біологічної результативності і урожайності гливи та інших сапрофітів.

Ключові слова: відходи, шкіра, пір'я, тваринництво, технологія, гриби, глива.

The processing of agricultural products involves the complex use of raw materials without the generation of production waste and the introduction of technologies for the use of generated waste to obtain additional products (Kirylenko I.V., 2020; Kovtunyuk Z.I., 2021).

Today, oyster mushroom production is one of the most intensively developing industries. The consumption of artificially grown mushrooms is constantly increasing. This is connected, on the one hand, with the increase in the production of mushrooms and their transformation into an independent branch of agriculture, on the other hand, with the collection of mushrooms, which annually decreases in the places of their natural growth (Simakhina, 2023; Bandura I.I., 2020 , 2021)

According to I.I. Bandura (2020, 2019), the intensive development of industrial cultivation of edible mushrooms is due to a number of factors:

• High productivity of mushrooms, which is the highest yielding agricultural crop (the average yield of oyster mushrooms is 20-25% of the weight of the substrate).

• Mushrooms are a source of protein, vitamins, trace elements and medicinal substances.

• To grow edible mushrooms, agricultural, forestry and processing industry wastes are used, which are transformed by mushrooms into food and medicinal substances; that is, the problem of production of food and medicinal substances and the ecological problem of waste disposal are simultaneously solved.

• Mushroom cultivation technology is environmentally friendly, it can be fully mechanized, and in some cases automated.

• Mushroom cultivation is a zero-waste production, as the substrates after harvesting the mushrooms can be used as a protein-fortified additive for livestock feed or as fertilizers. Thus, today oyster mushroom production is a promising and profitable branch of agriculture (Bandura, I.I., Kulyk, A.S., et al., 2020; Shcherbak O.V., 2022; Shovdra V., 2023)

The oyster mushroom is very technological, has a high growth rate and significant competitiveness in relation to strange microflora. The mushroom grows on various cellulose- and lignin-rich plant wastes from agriculture, food and wood processing industries. In general, the oyster mushroom has no equal in terms of the number of substrates on which it is cultivated. The simplest substrate for intensive cultivation is wheat straw (Mykhaylova O. et al., 2011; Vdovenko S., 2020).

In our country, the following lignin- and cellulose-rich wastes of agricultural production are the main components of substrates for growing boletus fruit bodies: straw of cereal crops: wheat, rye, barley, oats, millet, rice; shredded leaves, corn stalks and cobs, waste from the cotton processing industry, sunflower and buckwheat husks, sawdust and shavings of deciduous trees (Yuskov D.S., 2020; Vlasenko K.M. et al., 2022). These wastes contain the main components of the energy supply of the oyster mushroom, as a wood-destroying saprophytic fungus: cellulose and lignin.

To achieve the optimal physical and chemical composition of the substrate, different compositions of the initial components are used. Thus, it is necessary to take into account the technology of thermal preparation of the substrate, since the physical and chemical properties of the components and the finished composition may change with different technologies.

In modern economic conditions, it is important to reduce the cost of the substrate, one of the possible ways to achieve this goal is the use of cheaper raw materials. Meals and bran are classically used to balance the substrate in terms of nitrogen content. These products are waste products from the processing of agricultural crops and are also classically used to balance the rations of farm animals and poultry. This creates competition between the branches of production and increases the price of raw materials, which ultimately causes an increase in product prices (Kovalyov M.M. et al., 2020). Therefore, the search and research of alternative balancing components for the preparation of oyster mushroom substrate, which are not competitive for animal husbandry, is relevant.

The analysis of numerous literary sources and Internet publications indicated the main requirements for the components of the substrate:

- the component should not impair the physical composition of the substrate: air permeability, absorption and retention of moisture, should not emit harmful substances that impair the growth and development of mycelium;
- the component must not contain harmful substances, which can deteriorate the quality and affect the safety of food products (commercial oyster mushroom);
- the component should be easily accessible for procurement (purchase) and use, not requiring technological complications when using it;
- the component should easily and evenly mix with other components of the substrate, without forming lumps and without interfering with the operation of the equipment for forming the substrate;
- the component must be cheap and distributed throughout the country, not requiring special and expensive storage conditions;
- the use of the component should improve the ecological situation and prevent environmental pollution (principle 5R). It is desirable that this is one of the production

wastes that can be used for further processing production (Recycle principle) (Chernyshov I., 2022).

Taking into account the specified requirements, among a large number of possible options, we chose the following possible alternative components:

- leather shavings - leather production waste,
- waste of the fur industry - substandard hides, furs and trimmings,
- remnants of feathers - waste from poultry slaughter.

The use of the selected components is used in the production technology of protein supplements to balance the rations of farm animals and poultry, but, taking into account the complexity of processing and the energy intensity of the technology, it is advisable to find other areas of use, in particular, as components of substrates in industrial mushroom cultivation.

The purpose of the study. The purpose of the work is the study of animal husbandry waste (leather shavings, fur industry waste, feather remnants) as substrate components for the cultivation of saprophytic mushrooms (oyster mushrooms).

To fulfil the set goal, the following tasks were expected to be performed:

- to investigate the nutritional and technological value of livestock waste as additives to the substrate;
- calculate the biochemical and technological indicators of waste as components of the substrate;
- to investigate the features of oyster mushroom mycelium growth on the proposed supplements.

The scientific novelty of the obtained results: new data were obtained on the possibility of using livestock waste for balancing substrates for growing oyster mushrooms.

The practical significance of the work lies in the possibility of introducing new balancing additives based on livestock waste into the production of substrates for saprophytic mushrooms.

Research materials and methods. The scientific work was carried out in the conditions of the laboratory complex of the department of technologies of processing and storage of agricultural products of the Kherson State Agrarian and Economic University.

The work was an integral part of the scientific and research work of the Department of Technologies for Processing and Storage of Agricultural Products of the Biological and Technological Faculty "Development and improvement of technologies for the production, processing, examination and quality control of livestock products using the best domestic and global gene pool in the farms of the Southern region of Ukraine" during 2021- 2022.

The selection and preparation of substrate samples was carried out according to the method of agrochemical examination of greenhouse soils and substrates

Samples were weighed on VLTK-500 quadrant laboratory scales with an accuracy of 0.01 g.

The preparation of the nutrient medium (potato agar) and the actual technique of oyster mushroom mycelium sowing were performed according to generally accepted methods (V.V. Yevlash et al., 2020).

To evaluate the growth of the mushroom culture, a method based on the study and analysis of the dynamics of the increase in the radius of the colonies from the time of cultivation was used. The radial growth rate (Vr) was calculated according to formula (1):

$$Vr = \frac{a-b}{t1-t0} \quad (1)$$

where: a is colony radius at the end of growth, mm;

b is radius of the colony at the beginning of the phase of linear growth, mm;

t_1–t_0 is duration of linear growth, days.

The modified growth coefficient (GCj) was calculated according to formula (2) (Bukhalo A.S., 1988):

$$PKj = d \cdot h \cdot g \cdot j/t \quad (2)$$

where, **d** is the diameter of the colony, mm;

h is colony height, mm;

g is colony density in points;

j is homogeneity of the colony in points;

t is the age of the colony, days.

The study of morphological and cultural features on different substrates was carried out using the criteria described by A.S. Bukhalo (1988).

The effect of the additive on the growth rate of mycelium was determined by sowing two growth points on one Petri dish, one of which remained without additional nutrition until the end of the experiment, and one of the selected additives was placed next to the other. The repetition of samples is threefold.

The average samples for conducting experiments were taken:

- leather shavings – from industrial waste dumps of PE "Novik V.P", Kherson, in the form of shavings after milling of leather;

- fur industry waste - from industrial waste of PE “Plishko T.O.”, Kherson, in the form of fur flaps of rabbit leather after cutting;

- feathers - from industrial waste dumps of "Avangard" LLC, Kherson, in the form of a wet mixture of cover feathers after processing chicken carcasses on beating machines.

The average sample of each sample was dried to a constant weight of the sample, after which it was crushed in a laboratory disintegrator to a fine fibrous mass. The obtained mass was pasteurized at 80 ºC for 15 min, imitating the temperature regime of substrate pasteurization according to the author's hydrothermal treatment technology[1].

Research results. After forming the experiment, Petri dishes with nutrient medium, mycelium and additives were placed in a thermostat with a constant temperature of 18 ºC. During the first two days, the mycelium adapted to the changed conditions of growth and nutrition. At the end of the second day (after 40 hours from seeding), microscopy of the experimental samples was carried out. The growth rate of oyster mushroom mycelium at this, initial, stage was the same, since the conditions of nutrition and growth were the same, which was required by the conditions of the experiment. During the further development of the mycelium, the hyphae approached the dosage of the additive, penetrated inside and colonized with assimilation of nutrients. The development of mycelium on the opposite side of the Petri dish occurred with a lack of nutrients on the depleted potato agar medium.

[1] Pat. 138577 Ukraina, MPC (2006) B09B 3/00 B01J 2/22 (2006.01) Sposib utylizatsii vidkhodiv silskohospodarskoho vyrobnytstva [The method of disposal of agricultural production waste] Zayavl. 10.12.2019 ; Opubl. 13.03.2019, Bul. № 23. 4 c. (in Ukrainan).

By changing the intensity of hyphae growth, it is possible to draw conclusions about the ability of the oyster mushroom enzyme system to break down the nutrients of additives; or, in the case of the same intensity of growth with the control point of growth, the impossibility of splitting and consumption of additional nutrients; or the slowing down of hyphal growth compared to the control point in the case of toxic effects of additive substances.

At the initial stages of the experiment, the mycelium of the mushrooms adapted and began active growth on the supplements of leather shavings and feathers. So, in the variant with fur production waste, mycelial growth stopped at the border with the additive and did not progress further. Obviously, the decomposition products of the raw leather had a toxic effect on the growth and development of the mycelium. This assumption was proven on the 5th day, when we observed the death of two out of three repetitions of this version of the experiment. The mycelial samples died at both growth points, and the agar had a distinct smell of hydrogen sulphide and ammonia. This proves that the use of fur processing waste in its raw form as additives to the oyster mushroom substrate is impossible with this method of processing the substrate (pasteurization).

The detailed analysis of the stages of colonization of the selected supplements with oyster mushroom mycelium at the initial stages of growth is given in previous publications (Chernyshov I.V., 2021, 2022).

In the course of research, we found that the densest mycelium of the mushroom with high aerial hyphae was observed in the variant with the addition of leather shavings. On this supplement, the oyster mushroom mycelium had a fluffy white colony with a clear and even edge, a dense opaque layer. The height of the aerial mycelium was at the level of 3–5 mm with well-developed hyphae. The smell is pleasant, mushroomy (Fig. 1).

On the feather supplement, the mycelium formed loose white colonies with weak radial zonation. The density of the colonies was somewhat lower, but the height of the aerial mycelium was higher (5-6 mm). The growth rate was the highest, at the end of the experiment the culture colonized most of the area of the nutrient medium. The smell is pleasant, mushroomy (Fig. 2).

The culture colony in the version of the experiment with fur waste had a clear demarcation line from the additive, predominant growth in the opposite direction, reduced growth intensity, dense hyphae. Two of the three repetitions of the experiment at the end of the experiment were affected by bacterial colonies, a sharp smell of ammonia and hydrogen sulphide was observed (Fig. 3).

The photos (Figs. 1, 2, 3) show bacterial colonies that developed parallel to the inoculated oyster mushroom, but this is explained by the fact that the experiment was not conducted in sterile conditions, the development of mycelium was close to production conditions.

To analyze the growth intensity of oyster mushroom mycelium in different versions of the experiment, according to the methodology, the size of the colonies was measured and compared with control points of growth. Measurements were performed on the 7th day from the beginning of the experiment.

Fig. 1. **Colony size of oyster mushroom with the addition of leather shavings (top) and growth control points on the 7th day of growth**

Fig. 2. **Colony size of the oyster mushroom with the addition of feathers (top) and growth control points on the 7th day of growth**

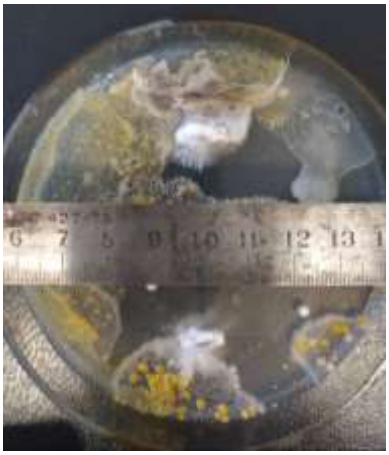

Fig. 3. **Colony size of oyster mushroom with addition of fur processing waste (top) and control point on the 7th day of growth**

The experiment was considered complete after colonization of half of the area of the nutrient medium of the Petri dish on the 7th day after inoculation. We measured the diameter of the colonies in the experimental variants and calculated the radial growth rate and growth coefficients. The appropriate indicators of the growth of colonies in

dishes with cultures were taken as the control variant. The calculations are shown in Table 1.

Table 1

The speed of radial growth of colonies and growth coefficients of the oyster mushroom using different animal husbandry and aquaculture wastes

Type of supplement	Rate of radial growth of colonies Vr, mm/day	Growth coefficient GCj,
Control (no additive)	0.8±0.1	28.4±1.5
Leather shavings	5.0±0.7	57.7±2.1*
Fur production waste	0.7±0.1	27.3±1.4
Feathers	6.4±1.2	156.0±12.2*

*Note: * statistically significant difference compared to control, P<0.05*

Therefore, the growth rate of oyster mushroom mycelium clearly reflects the ability of the mushroom enzyme system to break down complex organic compounds, using the breakdown products as additional nutrients. The highest growth rate was found for the variant of the experiment with bird feathers. The growth rate was 1.4 mm/day higher than in the variant with the addition of leather shavings and 5.7 mm/day higher than with the addition of fur industry waste.

The calculation of the growth rate of the mycelium colonies proved the results of the observation of the growth and development of the colonies and the characterization of the morphological signs of the growth of the colonies. The colonies grew almost as well as the no supplemented controls, but the photographs indicated that the growth was directed along the fur supplement and in the opposite direction, proving the toxic effect of this supplement.

Calculations of the growth coefficient, which additionally takes into account the growth of colonies in height, generally confirm the data obtained during observation and calculations of the growth rate. Thus, colonies with additional nutrition from leather shavings occupied an intermediate value, more than the control option and less than the option with the addition of feathers. Colonies with added fur waste had slightly lower growth rates, but the difference was not significant.

Colonies supplemented with feather supplements not only had greater radial colony growth, occupying a larger area of the Petri dishes, but also had larger colonies in height. Despite the lower density of mycelial hyphae growth, the significant area of capture of the nutrient medium in recalculation gave the value of the growth coefficient by 127.6 points more than in the control variant.

Discussion. The results of the conducted experiment as a whole prove the data obtained in the studies (Bandura I.I. et al., 2021, 2020).

The biological disposal of leather waste with the production of additional food products is undoubtedly the best option compared to the burial of these wastes, as it was outlined in the research of M.K. Kolyada et al. (2014). The use of enzymatic methods of processing has a number of advantages over chemical ones. Considering that the assimilation of nutrients from leather shavings in our experiments occurs under the influence of the enzyme system of the mycelium of mushrooms, it can be assumed that this is an enzymatic method of disposal.

The use of feathers in their natural form as a feed component of animal and poultry diets is limited by the low digestibility of keratin. According to V.S. Bomko et al. (2023), the structural protein of feathers (keratin) can be digested in the digestive

tract of animals only after hydrothermal hydrolysis. After that, the digestibility of raw protein increases significantly (75-80%). Meanwhile, the amino acid composition of feather protein is rather unbalanced, due to the low content of lysine, histidine and methionine and high content of cystine. This drawback significantly limits the use of feather meal in animal feeding. In addition, the technology for the production of feather flour is energy- and material-intensive. The use of native feathers without complex hydrolysis technology as a balancing additive in substrates for growing mushrooms can have a greater economic effect.

Therefore, higher mushrooms have an enzyme system that allows them to break down and consume the proteins of processed leather (leather shavings) and the biologically stable keratin of bird feathers. The use of such additives without additional industrial splitting, in their natural form, will theoretically increase the yield of mushrooms, but this requires additional scientific and economic experiments.

Conclusions. Thus, analyzing the development of oyster mushroom colonies grown with additives based on livestock waste, the following conclusions can be drawn:

- the study of the nutritional and technological value of livestock waste as additives to the substrate indicates a great potential in their use as balancing additives in the composition of substrate compositions for growing mushrooms, especially to supplement the lack of nitrogen in the main components of the composition;

- the analysis of the requirements for the substrate components made it possible to identify promising alternative additives, the use of which can reduce the cost of the substrate composition, since their use is not competitive with the animal feed base, and the selected additives are livestock waste;

- the use of selected additives will reduce the amount of waste that is not used or is used to a limited extent and is usually disposed of as solid industrial waste. The use as additives for the preparation of the substrate will allow to use them in further processing, with obtaining additional oyster mushroom production, which corresponds to the concept of waste management "5R";

- the most essentials growth and manifestation of cultural signs was observed in variants with the addition of leather shavings and feathers. Colonies that received additional nutrition from the specified additives in the first hours after contact with the additive colonized it and, as a result, had the largest sizes compared to the growth control points;

- the worst variant of development was observed with fur production waste. The biological availability of the protein of unwashed leather and the possibility of reproduction of microorganisms led to the rotting and decomposition of proteins with the release of hydrogen sulphide and ammonia, which inhibited the growth of the mycelium, and subsequently caused its death. Therefore, it can be concluded that with the selected method of preparation of additives (pasteurization), inoculation and incubation of mycelium, it is impractical to use these processing wastes as additives. But the use of these additives during sterile cultivation remains relevant and requires further research;

- the obtained data of the laboratory experiment can be considered primary and those that require further research, including when using other methods of preparation of additives (sterilization, solid-state fermentation), as well as verification in scientific and economic experiments on the determination of technological properties in substrate compositions and calculation of biological effectiveness and productivity of oyster mushrooms and other saprophytes.

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STRESS-PROTECTIVE PROPERTIES OF THE 1,2,4-TRIAZOLE-3-THIOL DERIVATIVES COMPOUND UNDER ACUTE STRESS CONDITIONS OF LAYING HENS

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A new synthesized substance codenamed ASP-34 was used as the research object. The stress-protective properties were studied during prophylactic oral consumption of the compound under conditions of 4-hour acute heat and immobilization stress of laying hens in comparison to the control groups and the reference substance with proven stress-protective, hepatoprotective and immunostimulating effects «Cyanophor» (cyanocobalamin+butaphosphane).

In serum and liver homogenate, the content of markers of the antioxidant-prooxidant system was determined: the quantitative content of diene conjugates (DC), thiobarbituric acid reactants (TBA-reactants), as well as the activity of catalase and superoxide dismutase (SOD).

The results of the study demonstrate the presence of probable stress-protective properties of the 1,2,4-triazole-3-thiol derivative compound ASP-34 on the model of acute 4-hour heat and immobilization stress of laying hens. The test sample compound of the 1,2,4-triazole-3-thiol derivative ASP-34 in this study contributed to the restoration of the levels of TBA-reactants and DC (both in the serum and in the liver parenchyma) to the levels of similar indicators in negative control group ($p>0.05$ vs. NC). Moreover, there was also a tendency to increase the serum activity of SOD by 2.4 times ($p=0.09$) from the values of this indicator in the positive control group. According to the results of the biochemical study, it is clear that both of the tested agents in the indicated doses showed an antioxidant effect against the background of acute thermal and immobilization stress of chickens, but no significant difference between the activity of the two agents was noted.

The practical value of the obtained results is the confirmation of the stress-protective properties of the compound of the 1,2,4-triazole-3-thiol derivative ASP-34 on the model of acute 4-hour heat and immobilization stress of laying hens and the following recommendation for conducting tests in production conditions.

Key words: 1,2,4-triazole-3-thiol derivatives, stress-protective action, acute stress, antioxidant system, laying hens.

СТРЕС-ПРОТЕКТОРНІ ВЛАСТИВОСТІ СПОЛУКИ ПОХІДНИХ 1,2,4-ТРИАЗОЛ-3-ТІОЛУ ЗА УМОВ ГОСТРОГО СТРЕСУ У КУРЕЙ НЕСУЧОК

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В якості об'єкта досліджування використовували нову синтезовану речовину під шифром «АСП-34». Досліджували стреспротекторні властивості за профілактичного перорального задавання сполуки в умовах 4 годинного гострого теплового та іммобілізаційного стресу у курей несучок у порівнянні до груп контролю та референтного засобу з доведеними стрес-протекторним, гепатопротекторним та імуностимулюючим ефектами «Цианофор» (ціанокобаламін+бутафосфан).

В сироватці і гомогенаті печінки визначали вміст маркерів антиоксидантно-прооксидантної системи: кількісний вміст дієнових кон'югатів (ДК), реактантів тіобарбітурової кислоти (ТБК-реактанти), а також активність каталази та супероксиддисмутази (СОД).

Результати дослідження демонструють наявність вірогідних стрес-протекторних властивостей у сполуки похідної 1,2,4-триазол-3-тіолу під шифром «АСП-34» на моделі гострого 4 годинного теплового та іммобілізаційного стресу у курей несучок. Тестовий-зразок сполуки похідної 1,2,4-триазол-3-тіолу під шифром «АСП-34» в даному досліджені сприяв відновленню рівнів ТБК-реактантів і ДК (як в сироватці, так і в паренхімі печінки) до рівнів аналогічних показників в групі негативного контролю ($p>0,05$ проти НК). При цьому також відмічалася тенденція до збільшення сироваткової активності СОД в 2,4 рази ($p=0,09$) від значень даного показника в групі позитивного контролю. За результатами біохімічного дослідження очевидно, що обидва досліджуваних засоби в зазначених дозах виявляли антиоксидантний ефект на тлі гострого стресу теплового та іммобілізаційного у курей, проте вагомої різниці між активністю двох засобів не відмічалося.

Практичною цінність отриманих результатів є підтвердження стрес-протекторних властивостей у сполуки похідної 1,2,4-триазол-3-тіолу під шифром «АСП-34» на моделі гострого 4 годинного теплового та іммобілізаційного стресу у курей несучок та наступна рекомендація до проведення випробувань у виробних умовах.

Ключові слова: похідні 1,2,4-триазол-3-тіолу, стрес-протекторна дія, гострий стрес, антиоксидантна система, кури несучки.

Introduction. The economic transformations that began in our country a few years ago affected all branches of the agricultural industry, not bypassing even the most stable and prosperous ones like poultry farming. In the process of reforming agriculture in Ukraine, there was a reduction in the number of animals and poultry, as well as their

productivity. As a result, only 60-70% of the population's needs for livestock and poultry products are met.

In connection with the importation of poultry from abroad, diseases that had not previously been encountered in our country were registered. The fate of Ukrainian poultry farming worries both practitioners and scientists. There is a constant search for a way out of the crisis. The ultimate goal is the efficient use of all reserves and the profitability of poultry farming.

In industrial poultry production, progress is closely related to the improvement of existing and the development of new methods of regulating the conditions of the external environment, taking into account the peculiarities of the biology of chickens, using the achievements of genetics and selection in the creation of highly productive breeds, crosses and lines with high and stable resistance to infectious diseases under strict compliance with the rules of veterinary protection of poultry health and adaptability to industrial technology.

Scientists are constantly working on improving standards, developing resource-saving technologies, the implementation of which will allow farms to increase the profitability of production.

The task of fully providing the population with poultry products is of great importance. The most important condition for increasing the production of these dietary food products is the intensification of production based on the use of techniques aimed at increasing productivity in poultry farming. Together with the increase of the fodder base and the improvement of the technology of keeping chickens, it is crucial to use various substances that accelerate growth and contribute to the preservation of its vitality and productivity when the poultry is affected by adverse factors.

The intensification of production in the poultry industry increases the load on the chicken's body, technological processes, veterinary treatments, poor-quality feed are stress factors for the chicken’s body in the conditions of poultry enterprises. The demand for stress-protective agents under conditions of profitability in the poultry industry is relevant.

Industrial poultry farming is the most sensitive branch of agriculture to heat stress. First of all, this is related to the peculiarities of the chicken's body. Sensitivity to high temperatures is facilitated by the dense covering of the skin with feathers, the absence of sweat glands, as well as the high density of stocking. More and more often, the summer months of the year are associated in Ukraine with abnormally high temperatures and droughts. In animal husbandry and poultry, losses from such weather conditions can reach enormous scales.

Under the influence of high temperature in the poultry house (35–40 °C), the body temperature of the chicken rises by 0.5–1 °C, which leads to an increase in water consumption by 2–3 times. Further increase in the temperature in the poultry house and, accordingly, the body temperature by 1.5–2 °C, leads to the death of the chicken. In addition, an excessively high temperature of the environment triggers a whole complex of negative reactions in the body in the form of oxidative stress: the balance in the chicken's body between the production of free radicals and the level of antioxidants that neutralize them is disturbed. Under normal conditions, the number of free radicals is about 5% of the total number of metabolites. In the event of stress, particularly thermal stress, their level increases sharply, and the functional capabilities of the body as a whole decrease rapidly.

Heat stress is accompanied by a decrease in poultry productivity. The level of feed consumption falls and, accordingly, increases and egg production decrease, feed

conversion, protein and shell quality deteriorate, mortality and culling of chickens increases. In other words, the economic efficiency of production deteriorates.

Accordingly, in the prevention of heat stress, it is important to comply with technological veterinary and sanitary requirements for keeping poultry, which relate to the arrangement of premises for keeping poultry (improvement of ventilation, water supply, etc.).

In addition, feed or medicinal supplements that provide energy balance in the chicken's body, protect against dehydration, and support the body in the fight against oxidative stress have a positive effect. Means of foreign production are high-cost, therefore, the creation and research of the effectiveness of stress-protective means of domestic production is an urgent issue.

Organic compounds containing nitrogen atoms represent a large group of chemical structures of both natural and synthetic origin that can exhibit a wide range of biological activity. In this regard, the 1,2,4-triazole system, a five-membered nitrogen-containing heterocycle, has attracted active scientific attention (Shcherbyna, R., 2019). Therefore, compounds that contain a 1,2,4-triazole cycle in their structure are among the most promising objects of pharmacological research.

1,2,4-triazole derivatives are widely used in pharmaceutical and medicinal chemistry as well as in organic synthesis (Gao, F., Wang, T., Xiao, J. & Huang, G, 2019). For example, such drugs as fluconazole, bevacizumab (Avestim), tripuzol-neo, itraconazole, thiotriazoline and many other substances containing a 1,2,4-triazole cycle have been used in clinical and veterinary practice.

In our opinion, the study of structures with two bridged 1,2,4-triazole cycles belonging to different chemical classes may lead to the discovery of quite promising compounds that demonstrate versatility.

Taking into account previous studies, it can be stated that a large number of 1,2,4-triazole derivatives show a significant ability to inhibit the growth of pathogenic bacteria (Onkol, T., Doğruer, D. S., Uzun, L., Adak, S., Ozkan, S., & Sahin, M.F., 2008). In 2020, the antimicrobial activity of morpholine 2-(5-(3-fluorophenyl)-4-amino-1,2,4-triazol-3-yl)thio)acetate was evaluated in a rat model of experimental pancreatitis (Bigdan, O.A., Parchenko, V.V. & Kyrychko B.P., 2016). Furthermore, a research group from India studied the antimicrobial properties of new asymmetric bis-1,2,4-triazoles, demonstrating moderate bacteriostatic activity of the synthesized compounds against strains of *B. Subtilis, S. Aureus, E. Coli* and *P. Aeruginosa*. Feng Gao et al. showed (Gao, F., Wang, T., Xiao, J., & Huang, G., 2019) that the hybridization of the 1,2,4-triazole with other antibacterial pharmacophore groups can yield even more effective substances in this regard. The study conducted in China describes the recent results of targeted quinoline/quinone hybridization of 1,2,4-triazole, which holds promise for the treatment of infections caused by resistant bacterial strains (Zhang, J., Wang, S., Ba, Y., & Xu, Z., 2019).

Oxidative stress plays a crucial role in the development of a large group of diseases and pathologies, increasing with age (Cunha Lima, J.A.D., DE Farias Silva, J., Santos, C. S., Caiana, R.R.A., DE Moraes, M.M., DA Câmara, C.A.G., & Freitas, J.C.R., 2021). Accordingly, the discovery and development of protective agents against oxidative stress are of great interest. One possible approach to the development of new antioxidants involves the use of virtual screening through molecular docking with the enzyme superoxide dismutase (SOD) (Gurunanjappa et al. 2016, Kumar et al. 2016, Hatai & Banerjee 2019). SOD acts as an endogenous antioxidant defense system by converting hydrogen peroxide-derived superoxide radicals into water and oxygen to neutralize these species and thus prevent their damaging effects on cellular components

(Moloney & Cotter 2017; AL A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S & Dhama K. 2014.).

Among the classes of organic compounds that increase SOD activity, 1,2,3-triazoles stand out (Atapute P, Sanakal RD, Mulla SI & Kaliwal B., 2019, Zhang L et al. 2016.). 1,2,3-triazoles are heterocyclic compounds that are readily prepared by the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. These compounds serve as analogues of various biological functional groups that act as donors and acceptors of hydrogen bonds (Dheer D, Singh V & Shankar R. 2017., Agalave SG, Maujan SR & Pore VS. 2011., Santos CS, Oliveira RJ, Oliveira RN & Freitas JCR. 2020).

The purpose of this work was to determine the stress-protective properties of compounds of 1,2,4-triazole-3-thiol derivatives on the model of acute thermal and immobilization stress of laying hens.

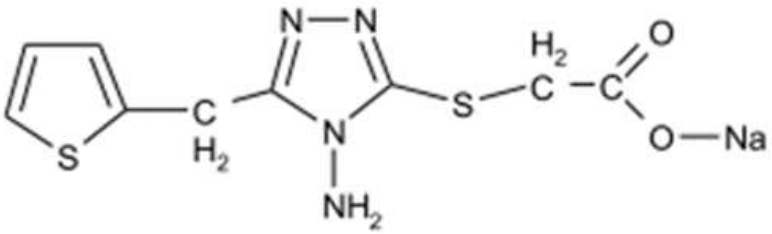
Materials and methods. The research was carried out on the basis of the "Veterinary Pharmacy" and "Innovative Technologies and Safety and Quality of Livestock Products" laboratories of the Department of Microbiology, Veterinary and Sanitary Examination, Animal Hygiene and Quality and Safety of Livestock Products of the Faculty of Veterinary Medicine of the Sumy National Agrarian University, Department of Toxicological and Inorganic Chemistry of Zaporizhzhya State University of the Medical and Pharmaceutical University, Educational and Scientific Institute of Applied Pharmacy of the National Academy of Sciences (Kharkiv).

The study was aimed at studying the stress-protective properties of a new synthesized substance under the code ASP-34 on poultry in conditions of acute immobilization and heat stress.

The substance was synthesized by the authors' team of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry of ZSMFU (Zaporizhzhia): Yevgeny Knysh, Oleksandr Ivanovych Panasenko, Andriy Andriyovych Safonov. (Patent of Ukraine UA 112619 C2 "Sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4h-1,2,4-triazol-3-yl)thio)acetate, which provides actoprotective activity") (Fig. 1).

The formula of the invention

Sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4h-1,2,4-triazol-3-yl)thio) acetate:



which exhibits actoprotective activity

Fig. 1. **Formula of the invention Sodium 2-((4-amino-5-(thiophen-2-ylmethyl)-4h-1,2,4-triazol-3-yl)thio) acetate**

The study of stress-protective properties was carried out on 28 laying hens of the Dekalb White cross, aged 430 days, which were kept under standard conditions (temperature 25-28 °C, relative humidity 60±10%, 8:16 hour day-night cycle, with free access to water and food) on the basis of the Sumy National Agrarian University vivarium. All stages of the research were carried out in accordance with Directive

2010/63/EU of the European Parliament and the Council of the EU dated September 22, 2010 "On the protection of animals used for scientific purposes" (Protocol of the Bioethics Commission No. 6 dated June 8, 2021) (Guide for the care and use of laboratory animals, 2011, Directive 2010/63/EU).

For the study, the chickens were randomized according to the indicator of minimization of differences in average body weight into 4 experimental groups (7 heads in each):

1. Negative control (NC) – chickens that were not subjected to immobilization stress (group I);

2. Reference group (RG) – chickens that received a reference sample under the immobilization stress (group II);

3. Positive control (PC) – chickens that were subjected to immobilization stress without the use of any means (group III);

4. Experimental group (EG) – chickens that received a test sample under the immobilization stress (group IV).

A new synthesized substance codenamed ASP-34 was used as the research object. Taking into account the results of previous studies, a dose of 100 mg/kg was chosen in this experiment, which has already demonstrated the presence of actoprotective properties. The drug with proven stress-protective, hepatoprotective and immunostimulating effects "Cyanophor" (cyanocobalamin+butaphosphan) was used as a comparison drug in the recommended dose according to the instructions (1 ml/head).

The studied substances were given orally (by the method of individual drinking) in the form of a suspension with purified water. Substances were given daily for 10 days in appropriate doses on an empty stomach from 9:00 a.m. to 10:00 a.m.

On the 10th day, 1 hour after the last consumption of the substances, simulation of thermal and immobilization 4-hour stress with withdrawal of food and water was carried out. For this, chickens from 3 groups (except the negative control group) were kept in tight boxes (without access to water and feed) for 4 hours from 7:00 to 11:00. The temperature in the boxes at the beginning of the test was 26^{o}C, after 20 minutes it rose to 31^{o}C, after 4 hours it was 32-33^{o}C.

39 hours after the end of the thermal and immobilization 4-hour stress, the poultry was slaughtered in accordance with the principles of humanity by the method of decapitation. The period before slaughter was determined by the period of manifestation of changes at the stress-reaction stage. Whole blood was collected during decapitation. Blood serum was obtained by centrifugation at 1500 g for 10 minutes on an Eppendorf 5702R refrigerated centrifuge (Eppendorf, Germany). All received serum samples were stored at -20^{o} C.

An autopsy of the chicken was carried out in order to record pathological and anatomical signs, and liver samples were taken for biochemical research.

In serum and liver homogenate, the content of markers of the antioxidant-prooxidant system was determined: the quantitative content of diene conjugates (DC), thiobarbituric acid reactants (TBA-reactants), as well as the activity of catalase and superoxide dismutase (SOD). Verification of the results of biochemical studies (absorption) was carried out by photometric methods on a KFK-3 photoelectrocolorimeter and a SF-46 spectrophotometer.

Quantitative DC content in samples of biological material was determined photometrically by a routine method after extraction with heptane at a wavelength of 233 nm (Katerji, M., Filippova, M., & Duerksen-Hughes, P., 2019; Facundo H. T. F., Brandt C. T., Owen J. S., Lima V.L.M., 2004).

Analysis of TBA-reactants was carried out according to a standard reaction with thiobarbituric acid, after protein precipitation with trichloroacetic acid, the optical density of the sample was measured against a control sample at 532 nm (Katerji, M., Filippova, M., & Duerksen-Hughes, P. , 2019; Borgesa A. C. P., Piassãoa J. F. G., 2018).

Catalase activity was determined by a generally accepted method based on the reaction of the formation of colored complexes of hydrogen peroxide and ammonium molybdate with an optimal absorption length of 410 nm (Korzhov V. I., Zhadan V. M., Polianska M. O., Opimakh S. H. & Ihnatieva, 2022; V. I. Nandi, A., Yan, L.J., Jana, C.K., & Das, N., 2019).

To determine the activity of SOD in samples of biological material, autooxidation of adrenaline at a wavelength of 347 nm was determined by dynamics every minute for 3 minutes; enzyme activity was expressed in conventional units, which corresponded to the percentage of inhibition (Kopanytsia, O.M., 2017).

The obtained results were expressed as arithmetic mean (M) and standard error of the mean (SEM). Comparisons between the studied groups were performed using parametric methods of analysis (ANOVA, post-hoc Tukey HSD test). The probability of differences was determined at the significance level of $P<0.05$. Statistical processing was carried out using the basic package of MS Exel 2007 and IBM SPSS Statistics 22 (Indrayan, A. & Malhotra, K. R., 2018).

Study of the influence of the 1,2,4-triazole-3-thiol derivative compound ASP-34 on the biochemical parameters of the antioxidant system of blood serum and liver tissues of laying hens under conditions of acute thermal and immobilization stress

Under the conditions of pathology in the chicken, the pro-/antioxidant system was predicted to be impaired, which was reflected in the imbalance of the serum and tissue content of lipid peroxidation products and the activity of antioxidant defence enzymes. The results of biochemical measurements are shown in Table 1.

Thus, in the blood plasma of animals of the positive control group, against the background of stress factors, there was a tendency to an increase in the content of TBA-reactants in the blood serum by 43.9% ($p=0.06$), a probable increase in the content of DC by 53.3%, and a probable decrease activity of SOD by 78.6% compared to similar indicators in the NC group. In turn, a statistically significant decrease in catalase activity by 19.4% and SOD by 55.4%, as well as an increase in the content of TBA-reactants by 134.9% and DC by 42.8% was observed in the homogenate of the liver of animals of the PC group ($p< 0.05$ against NC).

Against the background of the application of the reference agent to laying hens, there was a significant normalization of the content of TBA-reactants and DC in the blood plasma and the content of TBA-reactants in the homogenate of liver tissues, as well as a probable increase in the activity of SOD in the blood serum by 2.6 times and a decrease in the content of DC in the homogenate tissues by 18.1% ($p<0.05$ vs PC).

The test sample of the 1,2,4-triazole-3-thiol derivative compound ASP-34 in this study contributed to the restoration of the levels of TBA-reactants and DC (both in the serum and in the liver parenchyma) to the levels of similar indicators in negative control group ($p>0.05$ vs. NC). At the same time, there was also a tendency to increase the serum activity of SOD by 2.4 times ($p=0.09$) from the values of this indicator in the positive control group.

According to the results of the biochemical study, it is clear that both of the tested agents in the specified doses have an antioxidant effect on chickens under the acute thermal and immobilization stress, but no significant difference between the activity of the two agents was noted. Thus, it can be noted that the antioxidant effect of both agents

in the studied doses under conditions of acute stress of chickens is comparable (Table 1).

Table 1

The influence of ASP-34 on biochemical indicators of the antioxidant system of blood serum and liver tissues of chickens under conditions of acute stress (n=7, M±SEM)

Indicators	I (NC)	II (RG)	III (PC)	IV (EG)
In blood serum				
Catalase, μmol/(min*l)	24.324± 2.605	18.530± 2.767	17.053± 1.204	19.723± 1.989
TBA-reactants, μmol/l	0.264± 0.026	0.244± 0.037 [c]	0.380± 0.031 [b. d]	0.251± 0.028 [c]
DC, μmol/l	0.287± 0.024 [c]	0.312± 0.008 [c]	0.440± 0.017 [a. b. d]	0.344± 0.024 [c]
SOD, d. unit (% inhibition)	29.429± 3.470 [b. c. d]	16.571± 2.277 [a. c]	6.286± 1.426 [a. b]	14.857± 2.293 [a]
In the liver homogenate				
Catalase, μmol/(min*l)	2.573± 0.104 [b. c. d]	2.086± 0.079 [a]	2.074± 0.106 [a]	2.173± 0.101 [a]
TBA-reactants, μmol/l	77.653± 10.924 [c]	101.096± 15.967 [c]	182.410± 23.466 [a. b. d]	93.769± 15.193 [c]
DC, μmol/l	79.251± 4.472 [b. c]	92.726± 3.735 [a. c]	113.180± 2.172 [a. b. d]	83.896± 2.934 [c]
SOD, d. unit (% inhibition)	17.286± 1.886 [c]	12.571± 2.959	7.714± 1.584 [a]	14.571± 2.308

Notes:
[a] *– the differences are probable relative to the NC group (p<0.05);*
[b] *– the differences are probable relative to the RG group (p<0.05);*
[c] *– the differences are probable relative to the PC group (p<0.05);*
[d] *– differences are probable relative to the EG group (p<0.05).*

Discussion. Scientists around the world are drawing attention to a wide range of pharmacological properties of 1,2,4-triazole-3-thiol derivatives. For example, Zahid H. Chohan and Muhammad Hanif from Bahauddin Zakaria University investigated the antibacterial and antifungal activity of 1,2,4-triazole derivatives and showed that divalent metal complexes can be more powerful antibacterial and antifungal drugs than the precursors of Schiff's bases (Chohan , Z.H., & Hanif, M., 2013).

Kumar, H., Javed, S. A., Khan, S. A., & Amir, M. (2008) synthesized a series of 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxyacetic acid with the aim of obtaining new compounds with potential anti-inflammatory activity, analgesic activity and less ulcerogenic potential. All compounds were evaluated for their anti-inflammatory activity using the carrageenan-induced rat paw edema test. Compounds with strong anti-inflammatory activity were further tested for their analgesic, ulcerogenic and antioxidant effects. Of all the compounds tested, compounds 3, 7, 17, and 20 demonstrated a significant reduction in rat paw edema induced by carrageenan treatment. These compounds showed significant analgesic effects and, at equimolar oral doses compared to flurbiprofen, were also non-gastrotoxic in rats. Compound 17

was evaluated as the lead compound, which has higher anti-inflammatory activity (81.81%) than the comparator (79.54%), low ulcerogenic potential and protective effect on lipid peroxidation.

Cunha Lima, J. A. D., DE Farias Silva, J., Santos, C. S., Caiana, R. R. A., DE Moraes, M. M., DA Câmara, C. A. G., & Freitas, J. C. R. (2021). obtained the compounds semisynthetically via Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction between ethyl 2-azidoacetate and terminal acetylenes obtained from the natural products carvacrol, eugenol, isovanillin, thymol, and vanillin. The products were obtained in 50 to 80% yield and characterized using several spectrographic methods. Antioxidant activity was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The authors confirm that the products showed moderate antioxidant activity, with ethyl 2-(4-((4-formyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetate showing the highest antioxidant capacity (EC 50 = 75.5 μg/ml) among the 1,4-disubstituted 1,2,3-triazoles formed. As a result, the formation of these compounds opens up new opportunities for the development of new antioxidants.

The results of our study also demonstrate the presence of probable stress-protective properties of the 1,2,4-triazole-3-thiol derivative ASP-34 in the model of acute thermal and immobilization stress of laying hens.

Conclusions

Under the conditions of acute thermal and immobilization stress of 4-hour stress of laying hens (which also caused oxidative stress), under the influence of the compound 1,2,4-triazole-3-thiol derivative ASP-34 a probable decrease in peroxide markers was determined oxidation of lipids in the serum and liver of poultry, which indicates the presence of mediated antioxidant and membrane-protective properties of the studied agent.

Prospects for further research include studying the effectiveness of the 1,2,4-triazole-3-thiol derivative ASP-34 under industrial conditions.

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Conflict of interest

There is none

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EFFICIENCY OF THE USE OF TYPICAL AND INNOVATIVE RATIONS FOR FEEDING COWS IN THE SOUTH OF UKRAINE

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The paper presents an innovative view of the recommended feeding rations for effective milk production on an industrial basis in the conditions of southern Ukraine, taking into account the fact that this territory belongs to the zone of risky land use and the negative effect of global warming, which has been manifested in recent years. Scientific and economic experiments were carried out at the State Enterprise "SF Andriyivske" of the Bilhorod-Dnistrovsky district of the Odesa oblast according to generally accepted methods. The advantages of using winter rye as a crop whose growing season falls on wetter periods of the year have been established, which makes it possible to harvest high-quality silage with an energy content of 12.0 MJ, crude protein 12.7%, neutral detergent fiber 51.9% in the calculation per 1 kg of dry matter. A tendency to advantage in terms of milk yield was established from the cows of the experimental groups that received rye silage in combination with wet beer groats as the main fodder. Taking into account the ratio of fat and protein in the milk of the cows of the experimental groups in our studies, the optimal ratio of fat: protein was established in the cows of IV experimental group (1.2: 1), whose rations as the main forage contained only 45.0 kg of rye silage with the addition to this 6.0 kg of wet beer grain. The use of rye silage in combination with wet beer groats in the feeding rations of the cows of the experimental groups revealed a positive effect on indicators of rumen fullness, manure consistency, feed digestion, cow fattening, health status, reproductive capacity and animal survival. A tendency was established for the superiority of the milk yield of the cows of the experimental groups for 305 days of lactation by 95-258 l, respectively, compared to the animals of the control group, where the milk yield was 7,898 l, which, due to the use of cheaper ingredients in the rations, made it possible to reduce the cost of production of 1 liter of milk by 11.3-23.7%.

Key words: feed ration, rye silage, beer grain, yield, fat content, protein content, economic efficiency.

ЕФЕКТИВНІСТЬ ВИКОРИСТАННЯ ТИПОВОГО ТА ІННОВАЦІЙНИХ РАЦІОНІВ ГОДІВЛІ КОРІВ В УМОВАХ ПІВДНЯ УКРАЇНИ

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У представленій статті наведено інноваційний погляд щодо рекомендованих раціонів годівлі задля ефективного виробництва молока на промисловій основі в умовах півдня України з урахуванням належності даної території до зони ризикованого землекористування та негативної дії глобального потепління, що виявляється останнім роками. Науково-господарські досліди проведено в умовах ДП«ДГ«Андріївське» Білгород-Дністровського району Одеської області за загальноприйнятими методиками. Встановлені переваги використання озимого жита як культури, вегетаційний період якої припадає на більш вологі періоди року, що дає можливість заготовити достатньо якісний силос із вмістом енергії 12,0 МДж, сирого протеїну 12,7 %, нейтрально-детергентної клітковини 51,9 % в розрахунку на 1 кг сухої речовини. Установлено тенденцію до переваги за рівнем надою у корів піддослідних груп, що отримували в якості основного фуражного корму житній силос у поєднанні з вологою пивною дробиною. З урахуванням співвідношення жиру і білка в молоці корів піддослідних груп у наших дослідженнях оптимальне співвідношення жир : білок встановлено у корів IV дослідної групи (1,2 : 1), раціони яких в якості основного фуражного корму містили лише 45,0 кг житнього силосу з доповненням до цього 6,0 кг вологої пивної дробини. Використання житнього силосу у комбінації з вологою пивною дробиною у раціонах годівлі корів дослідних груп виявили позитивний вплив на показники наповненості рубця, консистенцію гною, перетравлення корму, вгодованості корів, статусу здоров'я, відтворювальної здатності і збереженості тварин. Установлено тенденцію до переваги за рівнем надою корів дослідних груп за 305 днів лактації відповідно на 95-258 л порівняно з тваринами контрольної групи, де надій склав 7898 л, що за рахунок ще й використання більш дешевих інгредієнтів раціонів дозволило зменшити собівартість виробництва 1 л молока на 11,3-23,7 %.

Ключові слова: раціон годівлі, житній силос, пивна дробина, надій, вміст жиру, вміст білку, економічна ефективність.

In the conditions of industrial-type domestic dairy cattle breeding, fodder such as corn silage and alfalfa hay play an important role in ensuring a stable and high level of productivity of the dairy herd, since currently most farms practice year-round monofarming (Drackley J. K. et al., 2014; Ruban S. Yu. et al., 2015; Kozyr V. S., 2019).

In fact, the analysis of corn silage shows that the main indicators of its quality such as dry matter content, exchangeable energy concentration, pH, level of digestibility of organic matter as a percentage of total dry matter, and starch content are lower than the existing norms, because silage is often harvested in suboptimal conditions due to the heat the phase of its maturity. We should outline that such a situation is not uncommon in the south in conditions of ongoing global warming. In connection with the existing difficulties of growing corn for silage and alfalfa for hay due to the lack of moisture in the south of Ukraine, which have recently been exacerbated by global warming, the

search for alternative fodder crops for dairy cattle breeding is an urgent task today (Elfeel A. A. A. et al., 2023).

The effective "healthy" ration for feeding dairy cows contains coarse and juicy forages of at least 60.0% of the dry matter in the ration (Pidpala T. V. et al., 2019; Riznychuk I. et al., 2023).

In addition to corn for silage, which is a feed rich in exchangeable energy, various cereal crops can be conserved, with winter crops being a priority, as their active vegetation occurs during wetter periods of the year. Quite often ensiled crops are rye or triticale. The question arises, which culture is better for silage and subsequent feeding to dairy cattle? Thus, studies in one of the US pilot projects did not reveal a significant difference between rye and triticale, not even significant changes in quality were noted between fresh and ensiled feed from these ingredients. The main thing is to harvest these crops for ensiling at the stage of flag leaf formation, this ensures low fiber content and high digestibility (*Crops and Forages. Hoard's Dairyman*).

Cereal crops can be alternative feed for small dairy farms in limited climatic conditions, given their short growing season (Burgess P. L., et al., 1973).

Research conducted in Mexico, where the climate is quite arid, found that both rye and triticale are good alternative forage crops for ensiling (Vega-García Jesús I. et al., 2023).

Cultivation of such forage crop as winter rye (Secale cereale L.) helps not only to meet the growing demand for feed ingredients, but also to improve crop rotation (Herbstritt S. et al., 2022).

The purpose of the work is to determine the efficiency of using typical and innovative feed rations for cows in the production of milk on an industrial basis in the conditions of the south of Ukraine, which belongs to the zone of risky land use and under the negative effects of global warming.

In order to achieve the goal, using the feeding technology based on feed rations with different proportions of rye silage and the use of wet beer groats as a corrective component of the ration in the conditions of the southern region of Ukraine, the following tasks were solved:

- to evaluate the milk productivity of cows (yield by stages of lactation, yield for 305 days of lactation, fat and protein content in milk, amount of milk fat and protein);
- to study the influence of the use of rye silage and wet beer groats in feed rations on indicators of rumen fullness, manure consistency, feed digestion, cow fattening, health status, reproductive capacity and survival of cows in the experimental groups;
- to determine the economic efficiency of the conducted research.

Research materials and methods. The object of research was indicators of milk productivity and reproductive capacity of cows using a feeding technology based on feeding rations with different proportions of rye silage and the use of wet beer groats as a corrective ingredient of the ration in the conditions of the southern region of Ukraine.

Scientific and economic experiments were carried out in the conditions of the State Enterprise "SF Andriyivske" of the Institute of Climate-Oriented Agriculture of the National Academy of Agrarian Sciences of Ukraine, Bilhorod-Dnistrovsky district, Odesa oblast, according to methods generally accepted in dairy cattle breeding, and laboratory research of silage samples in the conditions of a specialized laboratory for the research of fodder *Frank Wright LTD (Ashbourne, United Kingdom)* using the method of infrared spectroscopy.

To conduct the experiment, a control group and 4 experimental groups of cows of the Ukrainian red dairy breed of the Holstein type, 10 heads each, were formed using

the pair-analogue method. A total of 50 cows at the age of 2-3 lactations were involved in the experiment. The early lactation feeding rations of animals of the control group included a set of feeds traditional for dairy cows in industrial production conditions (corn silage – 25 kg, alfalfa haylage – 8.0 kg, alfalfa hay – 1.5 kg, compound feed – 11.3 kg). The compound feed included corn, wheat, barley, sunflower meal, soybean cake, salt, chalk, premix. The rations of experimental groups I-III, in addition to the specified traditional set of feeds, contained a reduced amount of corn silage (22.0; 16.0; 12.0 kg, respectively, for experimental groups I, II, III) and alfalfa hay (6.0; 3.0 kg, respectively, for I and II experimental groups). 13.0 and 25.0 kg of rye silage were included in the rations of cows of II and III research groups, respectively. The amount of compound feed for animals of I, II, III, IV experimental groups was 10.2 each; 10.1; 9.6; 10.9 kg respectively. The feeding rations of the cows of IV experimental group contained only rye silage in the amount of 45.0 kg, alfalfa hay – 1.0 kg and beer grain – 6.0 kg from basic fodder. Their feed also included corn, wheat, barley, sunflower meal, soybean cake, salt, chalk, and premix. The composition of the feed rations of the cows of I-III experimental groups included fresh wet beer grain in the amount of 10.0 kg.

The milk productivity of cows was evaluated for 305 days of lactation by the method of conducting control milking every decade. The content of fat and protein in milk was determined using an ultrasonic milk analyzer Ecomilk Standard-4600-00002. Average milk samples were taken from each cow during control milking (Soboliev O. I. et al., 2022; Ladyka V. I. et al., 2023).

The number of animals chewing the cud was determined by their relative number (%) in relation to the total number of animals in the experimental group.

The fullness of the rumen of cows in farm conditions was assessed on a 5-point scale, where the smallest amount corresponds to 1 point, and the maximum – 5 points. The optimal indicator is desirable for dairy cows with normal health status and appetite corresponds to 3 points. The individual feed intake of each cow was studied 1 time per 3 days in the period of early, middle and late lactation during herd inspection (Götze K. et al., 2019).

Methodology for assessing the consistency of manure and the level of digestibility of ration components in points from 1.0 to 5.0 (Cow Talk).

Fatness of dairy cows was assessed on a 5-point scale. At the same time, 1 point corresponds to a very thin animal, 5 to excessively fat and 3 to factory fattening (Cow Talk).

Statistical processing of the results was carried out using modern packages of Microsoft Excel 2010 application programs using generally accepted methods (Kramarenko S. S. et al., 2019).

Research results. Our analyses of corn silage in the conditions of southern Ukraine prove that its basic quality indicators, such as the content of dry matter (DM) was 36.1%, the concentration of exchangeable energy (12.8 MJ/kg of DM), the content of crude protein (7.7 %/kg of DM), the *pH* level (4.0), the level of digestibility of organic matter from the total composition of dry matter (75.3%), the content of starch (35.6%) are beyond the existing normative indicators, because due to hot weather conditions silage often has to be collected during a sub-optimal maturity phase. Considering this, any winter crop can be a more optimal crop for ensiling in the conditions of southern Ukraine. We got positive results on winter rye. Thus, rye silage is to a certain extent inferior to corn silage in terms of dry matter content by 12.8%, but in terms of crude protein it exceeds it by 5.0%. Regarding the level of digestion of organic substances, the advantage is on the side of corn silage (by 6.3%). Corn silage is characterized by in-

creased exchangeable energy by 0.8 MJ/kg of dry matter due to the content of 35.6% starch, which is absent in rye silage.

According to such a critically important indicator as neutral detergent fiber (NDF) for ruminants, rye silage (51.9%/kg of DM) has a significant advantage of 17.9% compared to corn silage. In addition, the digestible NDF (77.4%/kg of DM) in rye silage is 7.4% higher than in corn silage.

The use of wet beer groats and rye silage in the feeding of dairy cows makes it possible to optimize the indicators of crude protein content and significantly reformulate the daily dose of protein ingredients of compound feed from 130 g/l to 90 g/l of milk, to ensure the dry matter content of feed rations at the level of 35.0%, increase the level of consumption of the ration with a better appetite.

The strength of feeding rations based on the use of wet beer groats and rye silage is the absence of starch in their composition, which, when formulating a fully mixed ration, allows to ensure the content of total starch at the level of 20.0% of the dry matter of the ration and even along with the increased sugar content in rye silage compared to corn, such rations have an appropriate total content of starch + sugar, which prevents the phenomenon of acidosis.

The use of special modern computer programs for drawing up rations for feeding dairy cows provides the correct approach to the formation of rations based on the concentration of exchangeable energy and crude protein, even when using different components, but due to the different content of fiber (both NDF and physically effective NDF), which effects on the fat content in the milk and on the health status of the cow. With an increase in the content of NDF in the feed rations of the cows of the experimental groups, the fat content in their milk increases in direct proportion. Taking into account the ratio of fat and protein in the milk of the cows of the experimental groups in our research, the optimal ratio of fat to protein was established in the cows of IV experimental group (1.2 : 1).

The analysis of the obtained results according to the indicators that allow to evaluate the relative number of animals chewing the cud in a calm state, the state of rumen filling, the consistency of manure, the degree of digestion of nutrients and the fatness of cows in the dynamics of the early, middle and late phases of lactation (Table 1) indicates the fact that no significant difference was found in the vast majority of the considered cases, only tendencies towards one or another advantage were noted, which to a certain extent indicates that the feed rations of the cows of all experimental groups were relatively correctly balanced. Thus, with regard to the indicator of the relative number of animals chewing the cud in a calm state during early lactation, a reliable advantage was established in cows of III and IV research groups, respectively, by 20.0% ($p < 0.01$) and 30.0% ($p < 0.001$) compared to animals of the control group. In general, a regularity can be observed that with an increase in the level of NDF in the dry matter of the ration, this indicator increases in direct proportion. According to this indicator, a similar pattern continues to be observed in the mid-lactation phase, but the difference between the groups is statistically improbable. It remains in the phase of late lactation, but a significant difference of 20.0% ($p < 0.01$) compared to animals of the control group is noted only in cows of the IV experimental group. In general, we note that under the existing standard, according to this indicator, 50.0-70.0% of the total number of cows in a calm state should chew the cud. This is exactly what happened in the vast majority of animals in the experimental groups. The exceptions were only the cows of the control and I experimental groups in the early lactation phase, when the corresponding indicator was 10.0% less than the lower limit of the norm, and the cows of the IV exper-

imental group in the late lactation phase, which exceeded the upper limit of the norm by 10.0%.

Table 1

Assessment of rumen fullness, manure consistency, feed digestion and fatness of cows of experimental groups (n=10), $\overline{X} \pm S_{\overline{X}}$

Indicator	Group of cows				
	C	E-I	E-II	E-III	E-IV
Early lactation					
Animals chewing the cud, %	40.0 ± 3.09	40.0 ± 6.90	50.0 ± 4.36	60.0 ± 4.88 **	70.0 ± 3.09 ***
Rumen fullness, points	2.8 ± 0.20	3.0 ± 0.15	3.1 ± 0.28	3.2± 0.29	3.4 ± 0.27
Manure consistency, points	2.9± 0.18	3.0 ± 0.15	3.0 ± 0.15	3.2 ± 0.13	3.2 ± 0.20
Manure assessment (digestion), points	1.5 ± 0.27	1.3 ± 0.21	1.2 ± 0.13	1.3 ± 0.21	1.2 ± 0.13
Fatness, points	2.6 ± 0.27	2.7 ± 0.26	2.8 ± 0.20	2.7 ± 0.15	2.9 ± 0.18
Mid-lactation					
Animals chewing the cud, %	60.0 ± 2.18	50.0 ± 9.0	60.0 ± 6.55	60.0 ± 8.16	70.0 ± 4.36
Rumen fullness, points	3.0 ± 0.15	3.0 ± 0.15	3.2 ± 0.25	3.3 ± 0.30	3.3 ± 0.26
Manure consistency, points	3.0 ± 0.15	3.0 ± 0.26	3.0 ± 0.15	3.1 ± 0.10	3.2 ± 0.15
Manure assessment (digestion), points	1.2 ± 0.13	1.2 ± 0.13	1.1 ± 0.10	1.2 ± 0.13	1.3 ± 0.15
Fatness, points	3.0 ± 0.15	2.9 ± 0.18	3.0 ±0.15	3.0 ± 0.15	3.0 ± 0.15
Late lactation					
Animals chewing the cud, %	60.0 ± 3.78	60.0 ± 5.77	60.0 ± 7.24	60.0 ± 5.35	80.0 ± 3.78**
Rumen fullness, points	3.4 ± 0.27	3.4 ± 0.31	3.7 ± 0.30	3.6 ± 0.34	3.8 ± 0.25
Manure consistency, points	3.2 ± 0.25	3.2 ± 0.29	3.2 ± 0.20	3.2 ± 0.13	3.5 ± 0.17
Manure assessment (digestion), points	1.1 ± 0.10	1.2 ± 0.13	1.1 ± 0.10	1.1 ± 0.10	1.1 ± 0.10
Fatness, points	3.6 ± 0.16	3.3 ± 0.15	3.5 ±0.17	3.3 ± 0.15	3.2 ± 0.13

*Note. **- probability p<0.01; ***–p<0.001*

The assessment of the degree of indicators of rumen fullness and manure consistency in the different phases of lactation proves that the corresponding indicators were within the existing norms of 3.0 points each, respectively. The average values of these indicators below the existing norm were established only in animals of the control group in the period of early and middle lactation. In addition, with an increase in the level of NDF in the dry matter of the feeding rations of the cows of the experimental groups, these indicators were characterized by a tendency to increase.

The assessment of manure by the level of digestion in the different phases of lactation proves that this indicator was completely within or approaching the existing norm

of 1.0 points. The average values for this indicator deviated more strongly from the existing norm, again in animals of the control group in the period of early and mid-lactation, but again, with an increase in the level of NDF in the dry matter of the rations of the cows of the experimental groups, this indicator was marked by a tendency to improve as it approached the optimal normative value.

The analysis of the assessment of the fatness index in the section of different phases of lactation proves that the indicated average indicator was completely within the recommended limits or approached the existing norm 3.0 points. The average indicators of fatness in points deviated more significantly from the existing standard in animals of the control group in the period of early lactation (below by 0.4 points from the standard) and in the period of late lactation (higher by 0.6 points from the standard), but also with an increase in the level NDF in the dry matter of the feed rations of the cows of the experimental groups, this indicator was characterized by a tendency to improve as it approached the optimal regulatory value. Animals of I, III, and IV experimental groups in all periods of lactation had the most close-to-normative indicators of total fatness. Regarding the animals of II experimental group, a similar situation was noted in the phase of early and mid-lactation, however, for the cows of this group in the period of late lactation, the fat content had a tendency to prevail over the animals of the other experimental groups and approached the level of the maximum indicator of the control group.

In general, it should be outlined that the average scores of the animals of the control and experimental groups were within the norm, only individual representatives in each of the groups provided a tendency towards one or another deviation from the desired norm 3.0 points.

Analysis of the influence of the use of rye silage and wet beer groats in the rations of dairy cows on the health status, reproductive capacity and their preservation (Table 2) proves that the rations of the cows of the experimental groups are balanced according to basic indicators, but metabolic diseases in the different form occurred in the animals of the control, I, II, IV experimental groups.

We can note that with an increase in the NDF level to 35.6% and more along with the decrease in the content of starch in the dry matter of the ration, no signs of subclinical acidosis were detected in the animals of II-IV research groups, however, the problems with energy deficit are relevant, that is, to solve this issue it is worth thinking about using special ingredients. The mentioned metabolic disorders were not detected only in the animals of III research group.

The reproductive capacity of cows was evaluated based on the absence of heat on the 28th, 49th, and 70th day after calving. Representatives of the control, I and II research groups in the period on the 28th and 49th day after calving had slightly worse indicators compared to the counterparts of III and IV research groups. Thus, 100.0% of representatives of II-IV experimental groups had signs of sexual desire during the 70th day after calving.

Increased indicators of the duration of the service period were found in the animals of the control, I, II experimental groups. The smallest and, accordingly, the most desired indicator was found in the cows of III experimental group, which had fertile insemination 26.9 days or 26.1% earlier than the animals of the control group ($p < 0.05$), and in the cows of IV experimental group this advantage was 23.9 days or 23.2% ($p < 0.05$). In addition, the animals of the control group had a loss of embryos in the first 90 days after fertilization at the level of 10.0%.

Table 2

The influence of the use of rye silage and wet beer groat in the rations of dairy cows on the health status, reproductive capacity and survival of dairy cows (n=10), $\overline{X} \pm S_{\overline{X}}$

Indicator	Group of cows				
	C	E-I	E-II	E-III	E-IV
Hidden rumen acidosis, %	20.0	10.0	-	-	-
Subclinical ketosis, %	10.0	10.0	10.0	-	10.0
Lack of desire on the 28th day after calving, %	40.0	30.0	10.0	20.0	10.0
Lack of desire on the 49th day after calving, %	10.0	10.0	10.0	-	-
Lack of desire on the 70th day after calving, %	10.0	10.0	-	-	-
Service period, days	103.1 ± 9.77	101.0 ± 6.40	99.0 ± 8.62	76.2± 6.24*	79.2 ± 5.63*
Yield of calves per 100 cows, %	94.0	95.0	96.0	102.0	100.0
Loss of embryos in the first 90 days after fertilization, %	10.0	-	-	-	-
Duration of lactation, days	337.0± 12.19	326.0 ± 6.82	324.0± 9.45	301.0± 5.7*	304.0 ± 6.27*
Dry period, days	67.0 ± 4.48	64.5 ± 3.02	63.5 ± 3.66	63.0± 2.13	64.0 ± 3.06
Intercalving interval, days	404.0 ± 6.41	390.5 ± 9.26	387.5± 12.25	364.0± 8.63 *	368.0 ± 8.86
Barrenness, %	10.0	-	-	-	-
Infertility, days/ head.	23.1	21.0	19		
Infertility, days/group	231	210	190	-	-
Rate of elimination of cows, %	20.0	20.0	10.0	-	-

*Note. * – probability p<0.05*

The differentiated duration of the service period also affects the yield rate of calves per 100 cows. Thus, in our studies, the yield of calves in cows of all experimental groups was at a fairly high level of 94.0-102.0%, but the most optimal data for this indicator were in cows of III and IV experimental groups, which exceeded the control group by 8.0, respectively and 6.0%.

The average indicator of the optimal duration of the intercalving period of 365 days is not always reached even in the leading herds of European countries with developed dairy farming. It is worth noting that this indicator as a whole for all experimental groups was in the range from 364.0 to 404.0 days, which, accordingly, roughly reflects the general picture regarding the duration of the intercalving period in average domestic industrial-type milk production farms. It should be noted that in all the cows in the experimental groups there is a tendency to advantage in terms of this indicator over the analogues of the control group. Thus, the duration of the intercalving period of cows of I, II, III, IV research groups was shorter by 13.5 days or by 3.3%, respectively; by 16.5

or 4.1%; by 40.0 or 9.9% ($p < 0.05$); by 36.0 or by 8.9% for the duration of the intercalving period of representatives of the control group.

Thus, for the duration of the service period of less than 80 days, economically undesirable days of infertility are completely absent only in animals of III and IV experimental groups, while the analogues of the control group had 231 days of infertility per group, and in the animals of II experimental group, their number decreased by 9.0%, in animals of III experimental group – by 18.0% compared to the control.

During the year, in conditions of intensive milk production technology, the culling rate is on average 30.0-35.0%. In our studies, the rate of culling of cows was significantly lower and amounted to only 20.0% in animals of the control and I experimental groups, and 10.0% in II experimental group. It is interesting that in III and IV experimental groups, a 100.0% level of cow survival was observed. This confirms our assumption that the use of rye as the basis of the ration, and hence the decrease in the level of starch, contributes to a better level of preservation of dairy cows and increases the necessary productive longevity of the dairy herd.

The analysis of the economic feasibility of using typical and innovative feeding rations for cows in different phases of lactation (Table 3) proves the economic advantage of feeding all experimental groups, where due to the reduction of the daily cost of the feed ration, the cost of feeding decreased from UAH 3340.0 to UAH 9110, or from 10.1% to 21.2% compared to the control group. This, in turn, contributed to the reduction of the total cost of production, which consisted of feed costs and 40.0% of overhead costs, and due to the increased milk yield of the cows of the experimental groups, it led to a decrease in the cost of production of 1 liter of milk by 0.86–1,81 UAH or by 11.3–23.7% compared to animals of the control group.

Table 3

Economic efficiency of using typical and innovative feeding rations of cows in different phases of lactation

Ingridients, kg	Group of cows				
	C	E-I	E-II	E-III	E-IV
Early lactation					
Cost of ration, UAH	168.8	147.5	141.1	137.5	137.7
Savings, UAH	-	21.3	21.7	31.3	31.1
Mid-lactation					
Cost of ration, UAH	151.9	138.4	132.9	128.7	121.0
Savings, UAH	-	13.5	19.0	23.2	30.9
Late lactation					
Cost of ration, UAH	109.6	101.0	90.6	86.9	80.5
Savings, UAH	-	8.6	19.0	22.7	29.1
305 days before lactation					
Feed costs, UAH	43030.0	38690.0	36460.0	35310.0	33920
Savings, UAH	-	4340.0	6570.0	7720.0	9110.0
Savings, %	-	10.1	15.3	18.0	21.2
Full cost of production, UAH	60242.0	54166.0	51044.0	49434.0	47488.0
Yield per lactation, l	7898.0	7993.0	8124.0	8119.0	8156.0
Cost of 1 liter of milk, UAH	7.63	6.77	6.28	6.08	5.82
Savings, UAH	-	0.86	1.35	1.55	1.81
Savings, %	-	11.3	17.7	20.3	23.7

The economic calculation of the efficiency of using rye silage and wet beer groat in the recommended feed rations of dairy cows (Table 4) has a positive effect on the formation of the price of milk (40.0% for fat content, 60.0% for protein content), because with the price policy UAH 10.0 for 1 liter of milk with a fat content of 3.7% and a protein content of 3.3% with a slight but present difference in milk yield between the control and I-IV research groups due to the different content of fat and protein, only the milk of cows of III and IV experimental groups, respectively, corresponded to the declared price of UAH 10.0 or exceeded it by 3.6%. In general, we note that the difference between milk yield, milk quality, which affects the price policy for 1 liter of milk, received a significant difference in the monetary equivalent for commercial milk from cows of the experimental groups, from UAH 2914.52/head (I experimental group – minimal increase) up to UAH 9,556.10/head (IV experimental group – maximum increase) for 305 days of lactation.

Table 4

Economic efficiency of using recommended feeding rations for dairy cows to determine the price of milk

Ingredients, kg	Group of cows				
	C	E-I	E-II	E-III	E-IV
Yield per lactation, l/ head	7898.0	7993.0	8124.0	8119.0	8156.0
Milk fat percentage	3.53	3.66	3.74	3.82	3.99
Milk protein percentage	3.10	3.17	3.18	3.23	3.33
Payment for fat, UAH	3.82	3.96	4.04	4.13	4.31
Payment for protein, UAH	5.64	5.76	5.78	5.87	6.05
Payment for 1 liter of milk. UAH	9.46	9.72	9.82	10.00	10.36
Commercial milk, l/ head	7658.0	7753.0	7884.0	7879.0	7916.0
Commodity milk, UAH/ head	72444.70	75359.20	77420.90	78790.00	82000.8
Additional funds, UAH/ head	-	2914.52	4976.20	6345.30	9556.10

The analysis of dairy cattle conservation indicators per 100 heads in farm conditions was within the technological standard (70.0% or more) in all experimental groups, however, conservation in the conditions of the control and I, II experimental groups exceeded the so-called minimum limit of the technological standard by 10.0%, 10.0% and 20.0%, respectively, and during the period when the experiment continued, not a single head from the III and IV research groups was lost (the level of preservation is 100.0%), which, in our opinion, is due to optimization of the level of starch in the dry matter of the ration due to the use of ingredients that do not contain it such as rye silage and wet beer groats, which in turn is good for preventing metabolic disorders in dairy cattle.

Discussion. Our studies carried out in dairy farming using rye silage are consistent with other researchers (Miedaner T. et al., 2019). Thus. there is a study where rye together with triticale is preferred by domestic researchers in the conditions of southern Ukraine as a zone of risky land use (Podobed L. Y., 2012). There are studies

recommending the use of rye-alfalfa silage (Syrovatko K. M., 2019) or triticale hay (Kotets H. I., 2016) as protein-rich ration ingredients.

Cereal crops can be alternative feed for small dairy farms in limited climatic conditions. given their short growing season (Burgess P. L. et al., 1973). The studies conducted in the conditions of Mexico where the climate is rather arid. evaluated the inclusion of silage from rye (Secale cereale L.) or triticale (Triticosecale spp.) when feeding 10 kg of silage of these species in dry matter per cow per day. In addition, the cows had access to grazing for 8 hours a day with the grass Cenchrus clandestine (Hochst. ex Chiov) and received a concentrate of 3.6 kg in terms of dry matter. Both rye and triticale have been found to be good alternative forage crops (Vega-García Jesús I. et al., 2023). In our studies, the maximum amount of rye silage for animals of IV research group was 10.4 kg. which is in principle consistent with the above-mentioned studies.

According to some research data (Burgess P. L. et al., 1973; Herbstritt S., 2022). dairy cows are fed about 10.0-12.0 kg of rye silage in physical weight in the daily ration, which does not agree with our results, where cows of IV experimental groups were fed with an increased amount of rye silage 45.0 kg/head, which is equivalent to 10.4 kg of dry matter. Provided that there were practically no other types of silage and haylage in the ration, and a cow should receive 12.0 kg of dry matter per day from coarse and juicy fodder, that is why the animals ate such an increased amount of rye silage. In addition, rye silage was fed in combination with wet beer groats, which further improved the palatability of the ration and increased appetite, hence the consumption, which is consistent with the recommendations of Garcia A., 2021. Regarding the feeding of increased amounts of rye silage to dairy cows, we see a difference from the results of other authors, which ensures the scientific novelty of our research on the one hand, but on the other hand, it should be considered as a forced measure, since it becomes increasingly difficult to grow corn for silage in the conditions of southern Ukraine due to frequent droughts.

There is information on improving the attractiveness of ruminant rations after adding wet beer groats to it (Perera A.N.F. et al., 2006). because wet beer groats, like other distillates, provide the ration of dairy cows not only with useful nutrients, but also reduce feed costs (Chase L. E., 2006), which is generally consistent with our research and recommendations.

Replacing corn silage with silage from winter crops is quite possible, which, accordingly, makes it possible to use the moisture of autumn and winter precipitation, and it also optimizes crop rotation, peak loads during autumn work, but the use of silage from winter crops is not a reason to abandon corn silage, it is only an opportunity to prevent risks and to form a suitable fodder base in the farm due to high temperatures and moisture deficit in summer, which has been observed recently, as a rule (*Agroexpert*, 2013).

It is recommended that it is better to prepare rye hay or silage with the use of specialized preservatives based on hybrid varieties and to observe the optimal collection phase – the flag leaf phase. Winter rye has recently become very popular among dairy farmers of the world due to its high yield, quite variable sowing dates, unpretentiousness to soils and early silage harvesting dates (O. Tytarenko, 2020), which is consistent with our results and recommendations.

Rye hay, in case it is collected early (timely and optimally), provides a high level of digestibility of rationary fiber, the content of exchangeable energy and crude protein, which makes it a rather promising ingredient of a fully mixed ration of high-yielding dairy cows, since rye hay complements basic bulk fodder (corn silage, alfalfa

hay), which are suitable for feeding the entire herd, and in the case of low quality, will also compensate for these shortcomings. What's more, if specialized hybrid rye varieties are used, the crude protein content in it even reaches 18.0-19.0% in dry matter, which corresponds to quite nutritious fodder grasses, while the NDF reaches 70.0%. In addition, the level of digestibility of NDF exceeds the similar indicator of the highest quality samples of corn silage (Vysotskyi I., 2016).

In the north-central part of the United States (the state of Wisconsin), it is practiced to sow corn for silage after harvesting rye for silage. Thus, the rye harvested for silage decreased preplant and in-season soil nitrate, and total yield equalled or exceeded corn silage yield despite a 13.0% decrease in corn silage yield (West J. R. et al., 2020).

As feed costs increase, cattle farmers are constantly looking for alternative options to use effective feed ingredients, so many farmers today prefer cereal crops, the most popular of which is rye, which has gained popularity in cattle feed rations in the United States because rye is a versatile and high-yielding crop (DeYoung J., 2022).

The results obtained by us regarding the formation of rations with such a principle that the dry matter from forage accounts for at least 60.0% of the dry matter of the ration, and the content of rationary fiber was more than 34.0% of the dry matter of the ration, which ensures a "healthy ration", and hence the prevention of the problem of rumen acidosis, which is consistent with other researchers (Drackley J.K. et al., 2014; Galbat S.A. et al., 2020).

The results we obtained regarding the difficulty of growing corn both for silage and for grain in the conditions of southern and other regions of Ukraine (Syrovatko K. M., 2019; Borshch O. O. et al., 2021) are consistent with the results of global calculations, which state that under the conditions of global warming, in the near future there will be more and more areas unsuitable for growing corn and other spring cereals (Ramirez-Cabral N. Y. Z. et al., 2017).

The obtained results regarding the influence of the use of rye silage in the feeding rations of dairy cows on the health status, reproductive capacity and their preservation are consistent with the work of other authors (Maltz E. et al., 2020; Galbat S.A. et al., 2020; Jaramillo-López E. et al., 2017).

Conclusions:

1. Positive effect of feeding rations of cows of the III, IV experimental groups using rye silage in combination with wet beer groats on the specific gravity of animals chewing the cud during rest by 20.0% ($p<0.01$) and 30.0% ($p<0.001$), respectively, during early lactation. In addition, a tendency to advantage in terms of rumen fullness, manure consistency, feed digestibility, cow fattening, health status, reproductive capacity, and animal survival was established compared to the use of corn silage and alfalfa hay as basic fodder for animals of the control group in the periods of medium and late lactation.

2. The tendency to advantage in terms of milk yield for 305 days of lactation was established in cows of I, II, III, IV research groups by 95, 226, 221, 258 l. Respectively, compared to animals of the control group, whose milk yield was 7.898 l, and the use of cheaper feeding rations for the animals of the research groups made it possible to reduce the production cost of 1 liter of milk by 11.3-23.7%.

3. The use of the correct approach to the formation of rations based on energy concentration, even with the use of different components in all experimental groups. ensured a generally good level of milk yield in 305 days of lactation (7898-8156 l), however, due to the different content of fiber in the dry matter of the ration, the content of fat and protein in milk turned out to be different (from 3.53% in the control group to 3.66-3.99% in the experimental groups), which along with the differentiated content of

protein in milk and under the condition of forming a price policy for milk of 40.0% fat, 60.0% protein, allowed to receive additional funds in the amount of 2914.52; 4976.20; 6345.30; and UAH 9.556.10, respectively, for cows of I, II, III, IV experimental groups relative to the animals of I control group.

Recommendations for production

In connection with the difficulties of agrotechnical cultivation of corn for silage in the modern conditions of the south of Ukraine as a zone of risky land use, as well as due to the negative effects of global warming recently, we recommend partially switching to the cultivation of fodder crops for silage that use autumn-winter moisture, such as winter rye or triticale, which will successfully complement corn silage and alfalfa hay.

For better eating of rye silage, supplement the daily feeding ration of dairy cows with wet beer groats in the amount of 6.0-8.0 kg per head.

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STUDY OF THE INFLUENCE OF BODY CONDITION ON THE DYNAMICS OF SEX HORMONES IN THE BLOOD OF BITCHES

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The physiological mechanisms of regulation of the reproductive function of bitches are well studied, however, the issue of individual characteristics of the body on the dynamics of sex hormones in the blood remained out of the attention of researchers. Therefore, we set ourselves the goal of studying the influence of body condition on the dynamics of sex hormones in the blood of bitches. The experiment was carried out on 15 bitches of the bull terrier breed with different body conditions according to the Body Condition Scoring scale. Three groups of animals were formed for the experiment: the control group was the ideal weight (the average BCS score was 5 points); I research group – low weight (average BCS score – 3.6 points); Group II – overweight (average BCS score – 6.6 points). For the first time, the dynamics of the content of sex hormones (progesterone, luteinizing hormone, follicle-stimulating hormone, estadiol) in the blood of bull terrier bitches with different body conditions was studied. New scientific data on the breed features of humoral regulation of the sexual cycle in the body of bitches have been obtained. The results of the studies indicate that factors such as overweight and low weight of bitches have an impact on the dynamics of sex hormones in the blood plasma of bitches during the sexual cycle. In particular, a significant effect of excess weight on the level of luteinizing hormone (P≤0.05) and progesterone (P≤0.05) at the stage of diestrus, on the level of progesterone (P≤0.05) at the stage of estrus was established. Low bitch weights were accompanied by higher levels of follicle-stimulating hormone at the proestrus stage (P≤0.05). Thus, our findings highlight that the characteristics of the corpus luteum of bitches and the synthesis of progesterone are influenced by many factors that have received little scientific attention to date.

*Keywords***.** *bitches, sexual cycle, progesterone, luteinizing hormone, follicle-stimulating hormone, estadiol.*

ДОСЛІДЖЕННЯ ВПЛИВУ СТАНУ ТІЛА НА ДИНАМІКУ СТАТЕВИХ ГОРМОНІВ В КРОВІ СУК

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Фізіологічні механізми регуляції репродуктивної функції сук вивчені досить добре, однак, питання індивідуальних особливостей організму на динаміку статевих гормонів у крові залишилось поза увагою дослідників. Тому, ми поставили перед собою за мету вивчити вплив стану тіла на динаміку статевих гормонів в крові сук. Експеримент проведено на 15 суках породи бультер'єр з різним станом тіла відповідно до шкали Body Condition Scoring. Для експерименту було сформовано три групи тварин: контрольна – ідеальна вага (середня оцінка BCS – 5 ба-

лів); І дослідна група – низька вага (середня оцінка BCS – 3,6 бала); ІІ дослідна група – надмірна вага (середня оцінка BCS – 6,6 бала). Уперше досліджено динаміку вмісту статевих гормонів (прогестерону, лютеїнізуючого гормону, фолікулостимулюючого гормону, естадіолу) у крові сук породи бультер'єр з різним станом тіла. Отримані нові наукові дані щодо породних особливостей гуморальної регуляції статевого циклу в організмі сук. Отримані результати досліджень свідчать, що такі фактори, як надмірна вага і низька вага сук мають вплив на динаміку статевих гормонів в плазмі крові сук потягом статевого циклу. Зокрема, встановлено достовірний вплив надмірної ваги на рівень лютеїнізуючого гомону (P≤0,05) та прогестерону (P≤0,05) на стадії діеструса, на рівень прогестерону (P≤0,05) на стадії еструса. Низька вага сук супроводжувалась більшим рівнем фолікулостимулюючого гормону на стадії проеструса (P≤0,05). Таким чином, наші висновки підкреслюють, що на характеристики жовтого тіла сук та синтез прогестерону впливають багато факторів, яким на сьогоднішній день приділяється мало наукової уваги.

Ключові слова. суки, статевий цикл, прогестерон, лютеїнізуючий гормон, фолікулостимулюючий гормон, естадіол.

Introduction. The domestic dog is the most common pet that accompanies people approx. 15,000 years. And today there is about 343 miscellaneous dog breeds (Alvares et al., 2019). It makes dogs the most frequent patients of veterinarians. The problem of canine reproductive medicine today is becoming more and more relevant due to the fact that the available therapeutic approaches are based mainly on clinical experience and empirical data, rather than on an understanding of basic physiological processes that are not yet fully understood (Kowalewski, 2023). The dog is one of the most important laboratory animals and is one of the best laboratory animal models. They are models for investigating comparative aspects of reproductive physiology in other mammals and humans (Kowalewski, 2018).

Compared to other types of pets, the sexual cycle is relatively long. The sexual cycle of bitches is aseasonal and monoestrous with a mandatory anestestrus between them.

A peculiarity of this type of animal is that the corpus luteum (Corpora lutea; CL) is the only major source of circulating steroids in females, regardless of their reproductive status. The dog is the only type of pet that does not produce steroids in the placenta, which emphasizes the central role CL in the control of the reproduction of dogs (Nogueira Aires et al., 2022). The phases of the sexual cycle of bitches are always separated by a resting phase (anestrus). And the biggest difference between dogs and other mammals is the absence of the antiluteolytic principle in the absence of pregnancy, which leads to physiological pseudopregnancy. Pseudopregnancy can last at least as long as or even longer than the time span of a normal pregnancy (Tsuchida et al., 2022).

Unlike other pets, in dogs, the resumption of meiosis and the completion of chromosome division is stimulated by the preovulatory surge of LH and occurs before ovulation. Ovulation of immature eggs occurs, which require another 2-3 days of oviductive maturation, only then acquiring the stage of development of fertile secondary eggs (Bigler et al., 2023). Thus, unlike other types of pets, in which eggs ovulate as secondary oocytes, a dog ovulates primary eggs (Balogh et al., 2018).

Also, the specific features of the reproductive system are the absence of an increase in estrogen content during pregnancy and before birth of puppies. And luteal regression occurs despite an increase in pituitary hormones (PRL, LH) (Pereira et al.,

2021). Finally, from an endocrinological point of view, fetal placental relaxin is the only reliable marker of pregnancy in a dog, since gestational levels of sex hormones are elevated compared to pseudo-pregnancy, they cannot be used to detect pregnancy, since elevated levels of P4 can also be observed in pseudo-pregnancies, in addition to this, significant individual variations are also observed (Bonfim Neto et al., 2022).

Nowadays, scientific interest in the study of the influence of body weight of bitches on the humoral component of the sexual cycle is mainly associated with the comparison of breeds of different body sizes, which obviously does not provide complete information about the physiological characteristics of the sexual cycle, since late breeds sometimes differ quite in their metabolism and physiological differences. It has been found that the weight of the bitch also affects the weight of the average corpus luteum (CL) and DNA content ($P < 0.001$), bitches heavier than 20 kg had more and heavier CL, resulting in more total luteal tissue (Marinelli et al., 2009). The relationship between body size and total luteal mass among other species is well known, and the size of mature CL is relatively constant within a species under physiological circumstances (Reynolds & Redmer, 1999).

The physiological mechanisms that regulate the function of the corpus luteum are of increased scientific interest. However, although the function of individual, predominantly luteotrophic, factors has been characterized, a general understanding of the physiology of CL in dogs remains poorly understood (Kowalewski, 2018). Therefore, we set ourselves the goal of studying the influence of the state of the body on the dynamics of sex hormones in the blood of bitches, which will allow us to obtain basic information about the peculiarities of the regulation of the sexual cycle in bitches and develop methods for their correction.

Materials and methods. The experiment was carried out on 15 females (Canis familiaris) of the Bull Terrier breed with different body conditions. At the time of the research, all animals were free of infectious and invasive diseases. The health status of the animals was assessed through clinical examination and laboratory tests.

The body condition of all bitches was assessed on the Body Condition Scoring scale. BCS is a quantitative tool widely used by veterinarians to assess an animal's body condition, fat accumulation, and nutritional status. The BCS scale ranges from 1 (depleted) to 9 (dangerously obese). The ideal body score is usually 4 or 5 with a physiological amount of muscle and fat (Body Condition Scoring (BCS) Charts, 2023). It should be mentioned that only healthy bitches without signs of hypo- or hypertrophy were selected for groups of animals. And the deviations of the body structure of animals in the experimental groups were within physiological limits. Three groups of animals were formed for the experiment: the control group was the ideal weight (the average BCS score was 5 points); I research group – low weight (average BCS score – 3.6 points); group II – overweight (average BCS score – 6.6 points).

The terms of fertility of bitches were determined using folliculometry, vaginal cytology and analysis of the level of progesterone in the blood. Insemination of bitches was carried out by various methods (both natural and artificial). Pregnancy screening was performed on (or on the 21st-28th day after ovulation). The material for the studies was blood samples of bitches taken from the jugular vein at -3, -1, 0, 2, 4, 9, 23-30, 35-40, 55-60 and 120-150 days after a surge in luteinizing hormone (LH). In the blood plasma of bitches, the content of estradiol (Dog E2 ELISA Kit, ICNE2KT, Innovative Research, USA), follicle-stimulating hormone (Dog Follicle Stimulating Hormone (FSH) ELISA Kit, Abbexa LTD, Great Britain), luteinizing hormone (Dog Luteinizing Hormone (LH) ELISA Kit, Abbexa LTD, UK) and progesterone (Progesterone –

ELISA, HEMA, Ukraine). Measurements were carried out on a universal reader for the ELx800 microplate (Bio-Tek Instruments, USA).

The experiment was carried out in compliance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.06 "On the Protection of Animals from Cruel Treatment" and is fully consistent with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986).

Results. The hypothalamic-pituitary-gonadal (HPG) axis primarily controls and regulates mammalian reproduction. Anatomically, the HPG axis consists of:

- the hypothalamus (especially the infundibular nucleus, a homolog of the human arcuate nucleus, where neurons producing KNDy and GnRH are located);
- the anterior part of the pituitary gland, where gonadotropes secrete luteinizing and follicle-stimulating hormones;
- gonads, which are responsible for the production of both sex steroids and gametes, under the influence of sex hormones.

As in other endocrine systems, the HPG axis is regulated by forward and reverse feedback (Tena-Sempere, 2005). Hormones of various origins (pituitary, placenta, and ovary) are involved in the control of the sexual cycle of dogs (Conley et al., 2023; Gobello, 2007).

FSH acts on the appropriate mammalian target organs, namely the testes, and ovaries, to exert its biological functions through peripheral circulation (Wang et al., 2021). Since FSH is a crucial regulator of the hypothalamic-pituitary-gonadal system, it plays an indispensable role in reproductive activity. Some reproductive disorders are associated with impaired secretion of FSH, and its concentration was lower in polycystic ovary syndrome (BAIRD et al., 1977). A lack of FSH can cause impaired spermatogenesis and infertility (Rougier et al., 2019). In females, FSH stimulates follicular growth and development and increases oxygen uptake by parietal granulosa cells to promote the synthesis of the corresponding protein (Widayati & Pangestu, 2020).

Studies have shown an increase in the level of FSH in the blood plasma of bitches depending on the body condition from the third to the first day up to a surge of LH of 2.1–2.4 times ($P \leq 0.001$). It should be noted that three days before the LH surge, the FSH content in the blood plasma of bitches of the first experimental group (with low weight) was 1.57 (1.28–2.00) ng/ml, which is significantly higher by 31.3% ($P \leq 0.05$) than in the bitches of the control group (Fig. 1).

The level of FSH in bitches by the second day after the LH surge continues to increase, reaches its maximum, and in the bitches of the control group is 13.3 (11.12–15.27) ng/ml. In overweight bitches (II experimental group), the level of FSH in the blood on the day of the LH surge (day 0) and two days after it is 32.1% ($P \leq 0.05$) and 31.9% ($P \leq 0$, respectively, 05), while in the females of the II experimental group only the corresponding tendency was noted. It is known that the sensitivity of the hypothalamic-pituitary-gonadal axis is relatively reduced in overweight individuals (Kim et al., 2015; Pasquali, 2006), among overweight women, a lower steady-state FSH level was often accompanied by a low E2 level. De Pergola (2006) established a negative correlation of body mass index with FSH and LH levels ($p < 0.001$ and $p < 0.01$, respectively) (De Pergola et al., 2006). However, a significantly lower level of FSH in overweight bull terrier bitches was found only within two days of the LH surge.

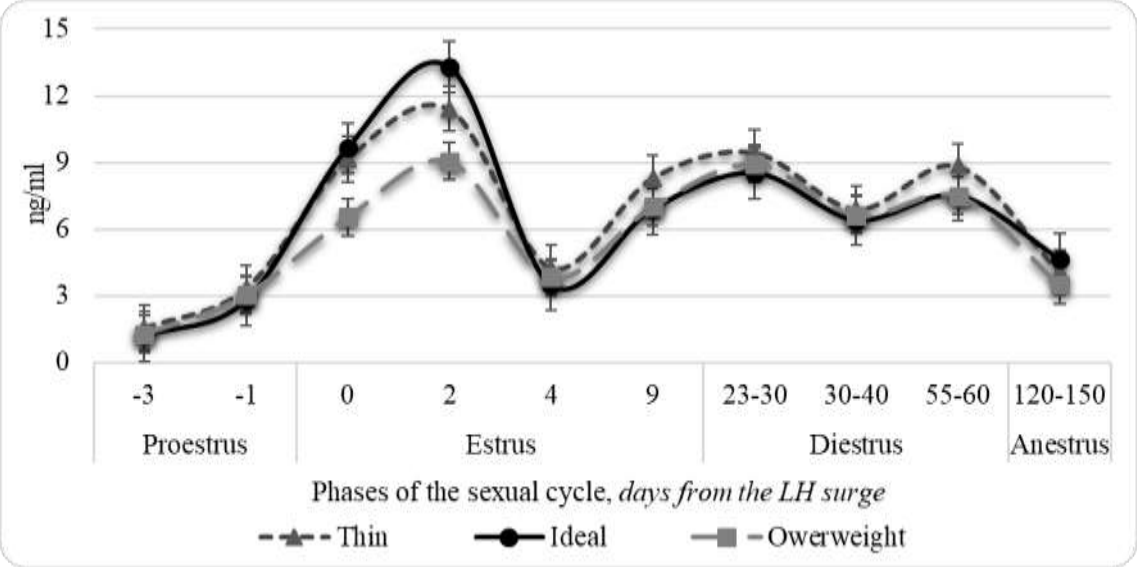

Fig. 1. **Dynamics of follicle-stimulating hormone content in the blood plasma of pregnant bitches of different body condition (n= 5; ng/ml).**

From the second to the fourth day after the LH surge, the level of FSH in the blood plasma of all experimental groups decreases by 2.4-2.7 times (P≤0.001) and ceases to differ significantly and reaches its minimum value at the level of (3.88, 2.5-6.6) ng/ml. Subsequently, by the 20-30th day after the LH surge, the level of FSH in the blood plasma of bitches increases by 2.2-2.5 times (P≤0.001), After that, by 55-60 days, it is restored to the previous values. Consequently, during pregnancy, the level of FSH in the blood plasma of bitches decreases slightly and increases before delivery. It should be noted that in pregnant bitches of different body conditions there are no significant differences in the content of the level of FSH in the blood, however, we note a tendency towards its higher level in bitches with low body weight (by 7.8-17.9%).

Estrogens are steroid hormones responsible for the growth and regulation of the female reproductive system and secondary sexual characteristics. Estradiol (E2) is the primary and most active hormone from the group of estrogens. Estrogens, mainly E2, are produced by the granulosa cells of the developing follicle and negatively affect LH production at the onset of the menstrual cycle (Holesh et al., 2023). However, once E2 levels reach a critical level, as eggs mature in the ovary in preparation for ovulation, estrogen begins to provide positive feedback on LH production, resulting in a surge. In addition to reproduction, E2 has many other effects that are important to the body (Tsutsumi & Webster, 2009). E2 has an inverse effect on the secretion of gonadotropic hormones during most stages of the estrous cycle (Kowalewski et al., 2011). The general pattern of E2 secretion roughly corresponds to the pattern of P4 secretion. It decreases towards the end of luteal life, indicating its source within the CL; no prenatal increase in E2 is observed (Kowalewski, 2017).

Kowalewski (2017) notes that pWith proestrus, E2 levels in bitches increase from initial low values of 5-10 pg/mL to preovulatory concentrations of 45-120 pg/mL (Kowalewski, 2017). Three days before the LH surge, the E2 content in the bitches of the control group was 45.1 (29.4–70.7) pg/ml, and before the LH surge, it increased by 1.4-1.6 times (P≤0.01), although the maximum peaks were shown one day before the LH surge (Fig. 2). Subsequently, during estrus, the content of the hormone in the blood plasma of bitches gradually decreases to values of 11.64 (8.9–19.7) pg/ml. At the beginning of diestrus, the dominance of P4 over E2 is noticeable (Feldman et al., 2014),

however, luteal formation and an increase in P4 levels have been associated with a new increase in E2 starting around day 10, in both pregnant and pseudo-pregnant dogs (Kowalewski, 2018). An increase in E2 levels in bitches during estrus to values of 34.6 (19.9-35.3), but preovulatory concentrations are not achieved. By the time of anestrus, there is a decrease in the level of E2 in the blood of bitches as a result of luteal regression. It should be outlined that no significant differences in the concentration of E2 in the blood of bitches with different body conditions were found during the experiment.

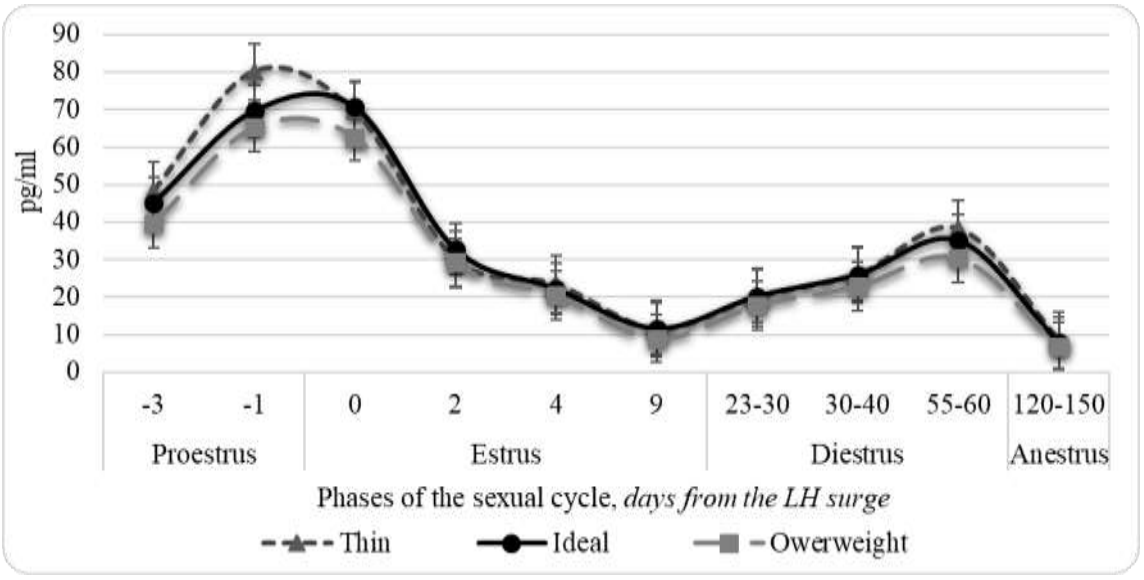

Fig. 2. **Dynamics of oestradiol content in blood plasma of pregnant bitches of different builds (n= 5; pg/ml).**

Luteinizing hormone (LH) is a glycoprotein hormone with a molecular weight of 28 kDa (Ezcurra & Humaidan, 2014) that is part of the HPG axis. Degradation and excretion remove LH and LHRH from the blood (Melnyk et al., 1976). LH release is stimulated by GnRH and inhibited by estrogens in women and testosterone in men. LH performs different functions that differ depending on the sex, but the common one is to promote the maturation of germ cells. In females, the hormone stimulates the synthesis of steroid hormones by the ovaries (Ilahi & Ilahi, 2022) and, to some extent, regulates the sexual cycle, playing a role in both ovulation and egg implantation (Kumar & Sait, 2011).

Thus, the surge of LH in bitches of the control group is characterized by an increase in the concentration of the hormone during the day (3.2-3.8 times; $P \leq 0.001$) from 3.56 (2.2–4.3) ng/ml to 13.6 (10.7–20.0) ng/ml (Fig. 3). In overweight bitches (II experimental group), the concentration of LH in the blood during the day before the surge increases only 3.1 times (against 3.7-3.8 times in the bitches of the control and I experimental groups), as a result of which the level of the hormone on the day of the surge was less by 38.5 % than in the bitches of the control group, however, due to the high variability of indicators, these differences have the character of a trend.

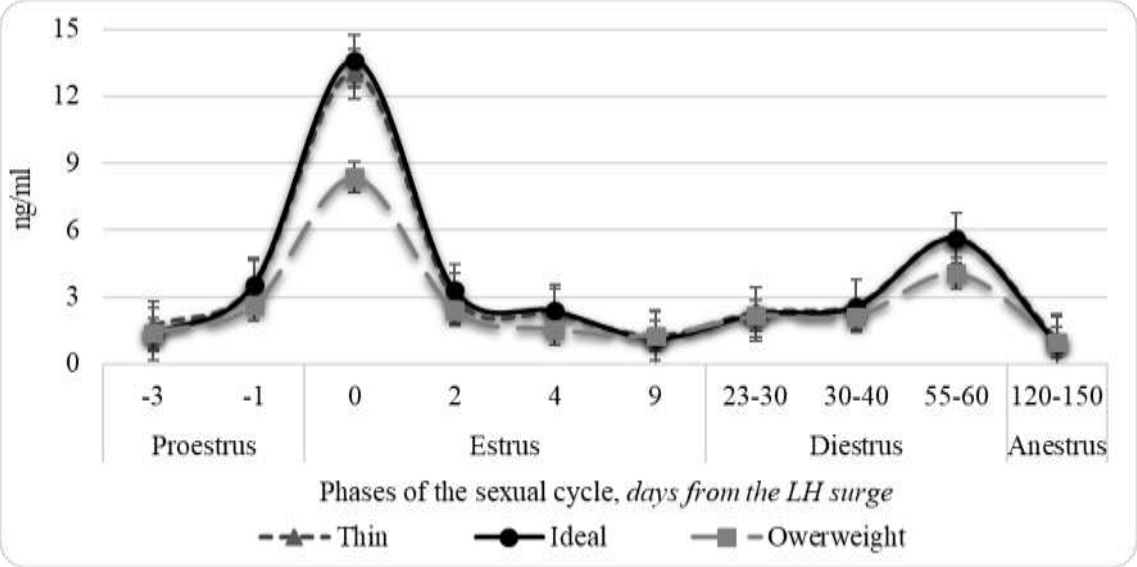

Fig. 3. **Dynamics of luteinizing hormone content in the blood plasma of pregnant bitches of different complexion (n= 5; ng/ml).**

Luz (2006) investigated mixed breeds and found significant fluctuations in individual variations in plasma P4 levels (Luz et al., 2006), however, in the study of P4 levels in the group of beagle bitches during the sexual cycle, these deviations were significantly reduced (Marinelli et al., 2009). In our studies, the established variability of this indicator was less than in these works, obviously due to the selection of groups of analogues by age and, most importantly, by breed. It is known that the size of the animal (the size of the breed) significantly affects the dynamics of sex hormones in their blood (Luz et al., 2006).

Endocrinologically, estrus begins 0-1 day from the LH surge (Kowalewski, 2018). Later, by the second day after the surge, the concentration of LH in bitches decreases by 3.4-4.3 times ($P \leq 0.001$), returning to previous values. During estrus flush, LH in bitches gradually decreases to 1.15 (0.8–1.5) ng/ml. And the subsequent increase in the diestrus stage is associated with pregnancy (or pseudopregnancy) of bitches. In particular, from the 9th to the 23-30th day after the surge, the level of LH in the blood of bitches increases by 1.71-1.94 times ($P \leq 0.001$), and to a lesser extent in overweight bitches. The LH content reaches its highest values at the stage of diestrus on the 55-60th day – 5.1 (3.1-7.0) ng/ml, and it was found that the content of the hormone in the blood of bitches was significantly lower (by 27.9%; $P \leq 0.001$) overweight.

At the stage of anestrus, the level of LH in the blood of bitches returns to baseline values of 0.98 (0.3-1.4) ng/ml. The researchers note that in the anestrus phase, the level of LH in the blood of bitches is quite low (<1 ng/ml) with small pulse intervals (4–24 cph) (Concannon et al., 2009).

Progesterone (P4) is a steroid hormone that prepares the endometrium to implant a fertilized egg in the uterus and maintain pregnancy. If the fertilized egg implants, the corpus luteum secretes progesterone early in pregnancy until the placenta develops and produces progesterone for the rest of the pregnancy (Holesh et al., 2023). Progesterone levels are widely used as a clinical biomarker in female reproductive management (Conley et al., 2023). The degree of variation in circulating levels of P4 among bitches is related to the multiple and variable number of ovulations and corpora lutea (Concannon P. W. et al., 1978). In many species, plasma concentrations of progesterone increase with the rate of ovulation and the number of CLs (Knox et al., 2003). Adult bitches have a higher efficiency of P4 synthesis than young bitches, suggesting that

luteal endocrine activity changes from young age to adulthood during the maturation process (Marinelli et al., 2009b).

The production of progesterone is influenced by many factors that have received little scientific attention to date. Due to the high variability among and within breeds, much of the literature on canine reproductive endocrinology has been obtained with homogeneous experimental groups of beagles (Marinelli et al., 2009). Although this approach has led to important advances in the understanding of basic physiological mechanisms, more specific characteristics of the species, such as variability in body size, have not yet been given the attention they deserve.

Proestrus is characterized by low levels of P4 in the blood of bitches, although these values begin to increase (to mean values of 1.4-1.7 ng/ml) compared to those at the stage of anestrus (mean values of 0.31-0.35 ng/ml). From a biological point of view, the onset of discourse is defined as the point of ovulation that induces the formation of CL. In bitches, the leading role at the stage of the sexual cycle – diestrus – is played by the secretion of P4. At the beginning of the diestrus, the dominance of P4 over E2 is established. (Kowalewski, 2017). At this time, the level of the hormone in the blood of the control group of bitches was 7 (5.11–9.1) ng/ml and does not differ significantly from the indicators of bitches of the experimental groups. During estrus, the level of P4 in the blood of bitches increases 1.5-1.7 times (P≤0.001), depending on the condition of the body.

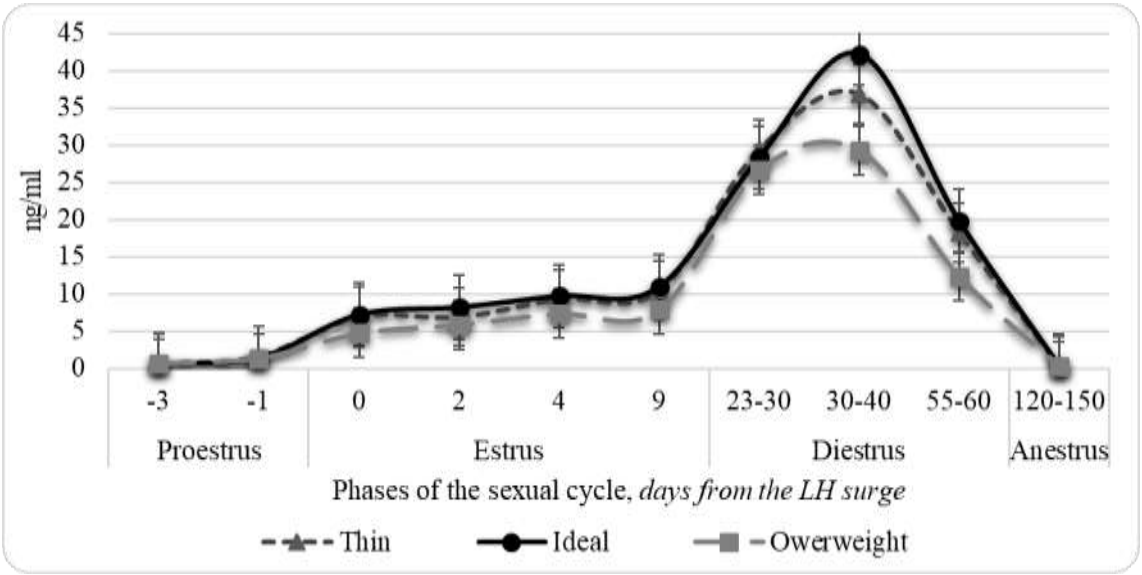

Fig. 4. **Dynamics of progesterone content in blood plasma of pregnant bitches of different complexion (n= 5; ng/ml)**

Unlike other domestic animals, in fully developed canine CLs, both morphologically and functionally, only one population of steroidogenic cells can be identified, which are activated at the onset of diestrus. As a consequence, P4 concentrations rise rapidly over 15 to 30 days, when CL is fully formed and maximum P4 concentrations are usually 30 to 35 ng/mL (up to 80 ng/mL or higher) (Kowalewski, 2018). Such effects on the reproductive system of high circulating concentrations of P4 are not observed in other domestic animal species. While early luteal phase develops CL, at least to some extent, gonadotropin-independent, and prostaglandins, in particular PGE2, are among the most important regulatory factors (Kowalewski, 2018).

The presence of a local mechanism that supports progesterone secretion after implantation still needs more research (Marinelli et al., 2009). Gudermuth (1998), by

measuring the concentration of fecal progesterone, found that the greatest increase in progesterone production during pregnancy occurs between 26 and 45 days after ovulation (Gudermuth et al., 1998). An increase of 2.6-3.4 times (P≤0.001) was found in the blood of bitches up to 20-30 days after a surge of LH level in the blood of bitches depending on the body condition with a peak on the 30-40th day after a surge of LH with an indicator of 36.9 (30.1–50.6) ng/ml. Subsequently, by the end of diestrus, the level of the hormone decreases by 2-2.4 times (P≤0.001). Excess weight negatively affects the content of P4 in the blood plasma of pregnant bitches in the second half of pregnancy. In particular, on the 30-40th and 55-60th day after the LH surge, the hormone content in the blood plasma was significantly less by 30.7% (P≤0.05) and 7.2% (P≤0.05), respectively, from the indicators of the bitches of the control group.

Diestrus ends when baseline P4 levels drop below <1 ng/mL and the cycle enters the anestrus stage. This can last up to 3 months after ovulation (Kowalewski, 2018). In bull terrier bitches in the anestrus stage, the level of P4 in the blood plasma did not depend on the condition of the body and ranged from 0.27-0.5 ng/ml.

Discussion. To date, no molecular-genetic bases have been established that provoke proestrus (Kowalewski, 2018). However, it is known that at the end of the anesterus, gonadotropin-releasing hormone (GnRH) impulses are amplified, followed by an increase in FSH release (Concannon, 2009).

For the first time, the phenomenon of preovulatory luteinization in bitches was described by von Bischoff in 1845 (Bischoff, 1845), the author notes that the difference between this process and that of other domestic animal species. Preovulatory luteinization is characterized by an increase in P4 levels 6 days before the LH surge (up to 0.6 to 1.0 ng/ml). During the LH surge, the P4 level rises, reaching a level of 5-10 ng/mL at the time of ovulation (Kowalewski, 2018). At the same time as the E2 level decreases, the P4 level increases, creating the conditions for an LH surge (Concannon et al., 2009). A surge of LH is considered to be when its level exceeds its basal levels by more than two times (Concannon, 2011). The surge is characterized by an initial increase in LH levels within 12 to 36 hours (Kowalewski, 2017).

The researchers point out that none of the progesterone parameters were influenced by the age or weight of the animal (Marinelli et al., 2009). However, we found that 4 and 9 days after the LH surge, the content of P4 in the blood of overweight bitches (II experimental group was at a significantly lower level, respectively, in bitches of the control group by 24.4% (P≤0.05) and 28.7% (P≤0.05).

Thus, excessive female body weight affects progesterone levels on days 4, 9, 35–40, and 55–60 after the LH surge, follicle-stimulating hormone levels on the day of the surge, and day two after the surge LH and the level of luteinizing hormone on the 55th–60th day after the LH surge (p≤0.05). The effect is characterized by a lower level (p≤0.05) of sex hormones in the blood of overweight bitches in the indicated periods of the sexual cycle. The low weight of bitches affects the level of follicle-stimulating hormone three days before the LH surge (p≤0.05).

Conclusions. For the first time, the dynamics of the content of sex hormones in the blood of bitches of the bull terrier breed with different body conditions was studied. The breed features of the humoral regulation of the sexual cycle in the body of bitches have been established and described. The results of the studies indicate that factors such as overweight and low weight of bitches have an impact on the dynamics of sex hormones in the blood plasma of bitches during the sexual cycle. A significant effect of overweight on the level of luteinizing hormone (P≤0, 05) and progesterone (P≤0.05) at the diestrus stage, at the level of progesterone (P≤0.05) at the estrus stage are determined. Low bitch weights were accompanied by higher levels of follicle-

stimulating hormone at the proestrus stage (P≤0.05). Thus, our findings highlight that the characteristics of the corpus luteum of bitches and the synthesis of progesterone are influenced by many factors that have received little scientific attention to date.

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None.

Conflict of interest

The authors declare no conflict of interest.

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MOISTURE SUPPLY AND WEED INFESTATION OF SPRING BARLEY CROPS (*HORDEUM VULGARE* L.) DEPENDING ON THE BASIC TILLAGE SYSTEM

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Spring barley (Hordeum vulgare L.) is one of the most common crops in the world of agriculture. Field research was conducted at the Poltava State Agricultural Experimental Station. M. I. Vavilov during 2021–2022. The research results show that the smallest number of weeds in crop crops (32.8 pcs./m2) was counted during tillage using Mini-till technology. When tillage, according to classical technology, there was an increase in the number of weeds by 2.4 pcs/m2 compared to the Mini-till system. The maximum abundance of weeds was observed on the No-till variant, which is 5.1 pcs/m2 more compared to Mini-till tillage. A similar level of weed infestation of crops was observed with shallow tillage (37.4 pcs./ m2). A decrease in the number of weeds was achieved on average during the growing season of spring barley by 7.1%, compared to No-till technology when tillage according to the classical scheme, which provided for plowing to a depth of 20-22 cm. Studies have revealed that the most negligible mass of raw weed plants in an air-dry state was formed with shallow primary tillage. Compared to other experiment variants, their mass was less, by 4.7–20.7 and 4.6–20.3 %, respectively. Studies have found that regardless of the technology of primary tillage, annual monocotyledonous and dicotyledonous species prevailed in the structure of biological groups of weeds. As for perennial weeds, there was an increase in their share by 2.9–3.8 times compared to other variants of the experiment, only with shallow tillage. It was found that the largest reserves of available moisture in a meter layer of soil both at the time of sowing and harvesting spring barley were formed on the variants of non-flange tillage, which were more extensive, respectively, by 5.7–13.3 and 4.1–4.7 mm, compared to the variant of the classic primary tillage.

Key words: spring barley (Hordeum vulgare L.), classic cultivation, shallow tillage, Mini-till, No-till, available moisture, total water consumption, weeds.

ВОЛОГОЗАБЕЗПЕЧЕНІСТЬ ТА ЗАБУР'ЯНЕНІСТЬ ПОСІВІВ ЯЧМЕНЮ ЯРОГО (*HORDEUM VULGARE* L.) ЗАЛЕЖНО ВІД СИСТЕМИ ОСНОВНОГО ОБРОБІТКУ ҐРУНТУ

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Ячмінь ярий (Hordeum vulgare L.) входить до складу найбільш розповсюджених сільськогосподарських культур світового землеробства. Польові дослідження проведено на Полтавській державній сільськогосподарській дослідній станції ім. М. І. Вавилова впродовж 2021–2022 рр. Результати досліджень свід-

чать, що найменшу кількість бур'янів у посівах культури (32,8 шт./м^2) нараховували за обробітку ґрунту по технології Mini-till. За обробітку ґрунту за класичною технологією відзначено збільшення кількості бур'янів на 2,4 шт./м^2, порівняно з системою Mini-till. Максимальну рясність бур'янів спостерігали на варіанті No-till, що на5,1 шт./м^2 більше відносно обробітку ґрунту за технологією Mini-till. Подібний рівень забур'яненості посівів культури відзначено за мілкого обробітку ґрунту (37,4 шт./м^2). За обробітку ґрунту за класичною схемою, яка передбачала оранку на глибину 20–22 см, досягнуто зниження чисельності бур'янів у середньому за вегетаційний період ячменю ярого на 7,1 %, порівняно з технологією No-till. Дослідженнями виявлено, що найменша маса як сирих рослин бур'янів, так і в повітряно-сухому стані формувалася за мілкого основного обробітку ґрунту. Порівняно з іншими варіантами досліду їх маса була меншою, відповідно на 4,7–20,7 і 4,6–20,3 %. Дослідженнями встановлено, що не залежно від технології основного обробітку ґрунту у структурі біологічних груп бур'янів переважали однорічні однодольні та дводольні види. Що стосується багаторічних бур'янів, то відзначено збільшення їх частки у 2,9–3,8 разу, порівняно з іншими варіантами досліду, лише за проведення мілкого обробітку ґрунту. Встановлено, що найбільші запаси доступної вологи в метровому шарі ґрунту як на час сівби, так і збирання ячменю ярого формувалися на варіантах безполицевого обробітку ґрунту, які були більшими, відповідно на 5,7–13,3 і 4,1–4,7 мм, порівняно із варіантом класичного основного обробітку ґрунту.

Ключові слова: ячмінь ярий (Hordeum vulgare L.), класичний обробіток, мілкий обробіток, Mini-till, No-till, доступна волога, сумарне водоспоживання, бур'яни.

Spring barley (*Hordeum vulgare* L.) is one of the most widespread crops in world agriculture, widely cultivated since prehistoric times. In terms of cultivation area, this crop ranks fourth in the world's crop structure after wheat, rice, and corn. The sown area of barley in the world is about 55-60 million hectares, from which 135-150 million tons of grain are obtained. It is predominantly used for grain forage and brewing purposes. It should be noted that over the past 20 years, the total world production of barley has decreased by 20%, and the area under crops has decreased by 30%. It is well known that spring barley is considered the leading grain fodder crop in Ukraine and ranks third after winter wheat and corn in terms of sown area. The area under spring barley has sharply decreased recently, from 4317 thousand hectares in 2010 to 763 thousand hectares in 2023, i.e., by 82.3%. In 2023, the share of spring barley in the structure of sown areas of cereals and leguminous crops is 10.2% (Hanhur V. V., et al., 2021; Romaniuk V. I., 2019).

In the context of the functioning of the market economy in Ukraine, when the formation of selling prices for grain occurs under the influence of supply and demand and is characterized by their instability during the marketing year, increasing the profitability of spring barley cultivation is possible both by increasing grain productivity and by reducing production costs (Korotkova I., et al., 2021).

The world experience of agriculture shows that the leading countries in grain production have long since moved from classical to resource-saving technologies, contributing to savings in production costs from 30 to 80% (Wacławowicz R., et al., 2023). A scientifically grounded approach to the introduction of resource-saving technologies by specific meteorological conditions, the type of soil and its provision with the main elements of mineral nutrition, and the biological characteristics of spring barley varie-

ties will make it possible to grow relatively high yields of this crop in different soil and climatic zones of Ukraine.

The formation of spring barley grain productivity occurs under the influence of several factors, including predecessors, the system of primary and pre-sowing tillage, a rational system of mineral nutrition of plants, effective measures to control the spread of pests, diseases and weeds in crops, varietal properties and technological features, which ensures effective regulation of plant growth and development processes (Hanhur V., et al., 2020; Korotkova I., et al., 2021; Chauhan, B.S., 2020). In this regard, there is a need to find effective measures to increase the grain productivity of spring barley under specific soil and climatic conditions of its cultivation.

Among the reasons for the low productivity of arable land in Ukraine, the key problem is the presence of segetal vegetation in agrophytocenoses. The negative effect of weeds primarily leads to a decrease in the economic and energy indicators of the efficiency of the crop rotation and tillage system, technological methods of applying fertilizers, plant protection preparations, the introduction of the latest achievements in breeding and seed production (Gharde Y., et al., 2018). Due to the increased competitiveness of weeds over cultivated plants, the shortfall in the yield of grain crops, depending on their composition and the degree of favorable weather conditions of the growing season, can vary from 5 to 30% or more (Wacławowicz R., et al., 2023; Kadziene G., Suproniene S., et al., 2020).

Although spring barley belongs to the group of medium-competitive crops, it requires effective measures to reduce the number of weeds in crops to an economically acceptable level. A significant number of weeds in crops, especially at the initial stages of vegetation, decreases grain yield by 25–40% or more (Chernelivska O. O., et al., 2018; Chauhan B. S., et al., 2017).

Mechanical tillage is among the most common methods of regulating the abundance of the weed component in agrophytocenoses. In the scientific circles of Ukraine at present, there is no unanimous opinion on the most reliable methods, optimal measures, depth, and tools for tillage that will ensure effective control of weeds in agrocenoses. This is caused by the duration and nature of weather conditions, regional features of field crop cultivation technologies, the set, and ratio of crops in crop rotations, etc., which have a direct or indirect impact on the formation of a specific group of weeds for a particular agricultural landscape, which requires a differentiated approach to the selection of measures and means of regulating their number in crops (Nikoli´c N., et al., 2021; Travlos I., et al., 2020).

The basis of modern tillage technologies should be the principle of minimization, which reduces the mechanical load on the soil and helps to strengthen its resistance to the manifestation of water and wind erosion, improving the conditions for the reproduction of soil fertility. The most common ways to minimize tillage include the replacement of plough tillage measures with non-flange tillage, as well as reducing the depth of loosening, the using wide-span multi-operation combined units (Kachmar O. Y., et al., 2020; Shevchenko M. S., et al., 2020).

The results of the research, obtained in the conditions of the southern Forest-Steppe of Ukraine, indicate that the most pronounced negative impact of the presence of weeds in crops on the productivity of spring barley was observed at the time of the onset of the earing phase of the crop. According to the average three-year data, it was found that each vegetative weed plant led to a shortfall of 5.73 kg/ha of barley grain (Koval H. V., et al., 2018).

Experiments conducted in the conditions of the Northern Steppe revealed that tillage for spring barley using minimal technology led to an increase in the abundance

of weeds, especially ragweed, compared to traditional and non-molded cultivation (Shevchenko M. S., et al., 2019; Syromiatnykov Yu. M., 2020).

In the experiments of O.O. Chernelivska and co-authors, it was noted that the use of herbicides provided a reduction in weed infestation of spring barley crops by 92.9—98.3 %, but the effectiveness of the use of preparations was excellent in different tillage technologies. Thus, primary tillage to a depth of 10–12 cm increased plant protection measures' effectiveness by 5.4% relative to No-till technology (Chernelivska O.O., et al., 2018). According to the data obtained in the conditions of the Forest-Steppe of Ukraine, a twofold increase in the number of weeds in spring barley crops under no-tillage was detected compared to moldboard tillage. However, their air-dry mass did not undergo significant changes. The authors justify this pattern by the increased interspecific competition of segetal vegetation for an increase in its abundance (Ptashnik M. M., et al., 2021). In the conditions of the Right-Bank Forest-Steppe of Ukraine, a decrease in the abundance of weeds by 18.9% was observed in the case of replacing moldboard tillage in crop rotation with moldboard-free tillage. With systematic tillage with tools with chisel and disc working bodies, the weed infestation of crops increased by 1.52 and 1.67 times, respectively (Prymak I. D., et al., 2018).

Spring barley is a relatively demanding crop for moisture conditions during the growing season. It is well known that the methods of basic tillage and the depth of loosening significantly impact the formation of moisture reserves. Thus, according to the results of research in the conditions of the Northern Steppe, it was noted that during chisel and shallow disc tillage, 11.9 and 5.8 % more moisture accumulated, respectively, compared to moldboard cultivation for spring barley (Tsyliuryk O. I., et al., 2014). A similar pattern was observed in the conditions of the Forest-Steppe zone (Davydenko H. A., et al., 2013).

According to experimental data obtained in the conditions of the province of Alberta (Canada), there was an increase in the moisture content in the soil by No-till, compared to traditional tillage (Nyborg M., et al., 1989).

Thus, the analysis of the sources of scientific literature testifies to the significant importance of the primary tillage methods in the regulation of moisture supply and weed infestation of spring barley crops.

The analysis of the sources of scientific literature testifies to the different views of scientists regarding the effectiveness of primary tillage methods in the regulation of moisture supply and weed infestation of spring barley crops. In the context of permanent climate change, in particular, the increase in its aridity, the role of tillage in creating better conditions for accumulating and reducing unproductive moisture consumption, and the harmful effects of segetal vegetation are only increasing. Therefore, it is essential to scientifically substantiate the impact of minimum tillage, exceptionally shallow, mini-till, and no-till, on the formation of productive moisture reserves and quantitative and species composition of weeds in spring barley crops.

The purpose of the research is to find out the influence of different systems of primary tillage on the moisture supply of plants and their abundance, as well as the species composition of weeds in spring barley crops.

Materials and methods. Field research was carried out on the experimental field of the Poltava DSGDS. During 2021–2022, a long-term stationary experiment was laid down in 2008. It is characterized by the following agrochemical indicators: humus content in the soil layer 0–20 cm 4.1 %; easily hydrolyzed nitrogen – 7.1 mg/100 g of soil (according to Tyurin and Kononova); P2O5 in acetic acid extract – 12.8 mg/100 g of soil (according to Chirikov); exchangeable potassium – 17.3 mg/100 g of soil (ac-

cording to Maslova). The soil solution has a slightly acidic reaction (pH of the salt extract is 6.2).

The research site belongs to the temperate continental climate zone. An ambiguous manifestation of weather conditions characterized the years of the study during the growing season of barley compared to the average long-term data. Thus, the hydrothermal coefficient in May, June, and July 2021 was 0.87, 0.64, and 0.51, respectively, with long-term average values of 1.12, 0.93, and 0.67 units. In 2022, there was also a deviation of this indicator by month. The hydrothermal coefficient was 1.42 and 1.17 in May and June, respectively, 26.8 and 25.8% higher than the long-term average. Along with this, in July, this figure was lower than the norm by 0.27 units.

The scheme of the field experiment included the following options: 1. Classical tillage (plowing to a depth of 20-22 cm), (control). 2. Shallow tillage (shallow tillage to a depth of 10-12 cm). 3. Mini-till (stubble plowing to a 6-8 cm depth + direct sowing). 4. No-till (direct sowing).

The sown area of the plot was 972 m2, and the accounting area was 200 m2. The experiment was repeated four times. Variants and repetitions in the experimental area are placed systematically. The predecessor of spring barley was grain corn. Spring barley was grown against the background of applying mineral fertilizers in a dose of N48P48K48. Sowing of the crop, on all tillage options, was carried out with a Great Plains ADC 2220 direct seeder.

According to the variants of the experiment, weed counting was carried out by the quantitative-weight method in the phase of mass tillering and before harvesting spring barley (Yeshchenko V. O., et al., 2014). Atlases-determinants were used To determine the species composition of segetal vegetation in spring barley crops (Veselovskyi I. V., et al., 1993).

Research results. In recent years, in the Left-Bank Forest-Steppe of Ukraine, the main factor limiting the maximum return from the implemented agrotechnical measures and the realization of the genetic potential of grain productivity of spring barley is the level of provision of crops with affordable moisture. However, significant climate changes observed in recent years are due to an increase in air temperature and a decrease in precipitation and its uneven distribution during the growing season. This, in turn, directly impacts the formation of the soil water regime and the provision of crops with moisture.

The importance of moisture in the ontogeny of crops is hugely multifaceted. It participates in the process of photosynthesis, ensures the maintenance of turgor in cells, prevents overheating of the plant organism, affects the formation of the root system and its distribution along the soil profile, and, therefore, the water consumption of plants. Moisture in the soil has a direct impact on its most critical processes. It determines the biological activity of the soil environment and the nature of nutrient, air, and thermal regimes (Netis I. T., et al., 2012).

In the experiment, using the thermostat-weight method, we determined the moisture supply of a meter layer of soil in two terms - at the time of sowing and before harvesting spring barley. The research results show that, on average, for 2021–2022, the largest reserves of available moisture in a meter layer of soil at the time of sowing spring barley were contained in the variant with the No-till tillage system. Thus, against the background mentioned above, the content of available moisture in the layer 0–100 cm was 190.1 mm, which is 13.3 mm or 7.5% compared to the variant of classical tillage (Table 1). The improvement of the process of moisture accumulation using No-till technology was facilitated by the plant residues of the previous crop left on the soil sur-

face, which, during the winter, ensured the retention and more even distribution of snow in the field.

Table 1

Influence of tillage methods on the content of productive moisture under spring barley, average for 2021–2022

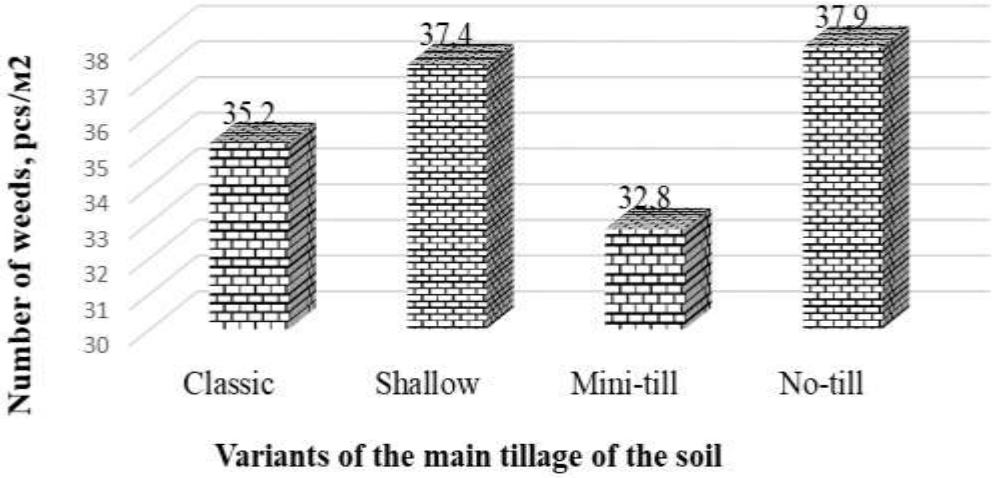
Basic tillage options	Available moisture content in 0–100 cm soil layer, mm		Total moisture consumption per unit of dry matter, m^3/t
	at the time of sowing	at the time of assembly	
Classic (control)	176,8	73,6	717
Shallow	175,9	77,7	720
Mini-till	182,5	78,0	731
No-till	190,1	78,3	779

When carrying out shallow tillage to a depth of 10-12 cm, the content of available moisture was almost at the level of classical tillage; the difference between these options was only 0.9 mm. Comparatively large reserves of productive moisture in the soil layer of 0–100 cm were formed against the background of surface tillage to a depth of 6–8 cm in the Mini-till system. Thus, the content of available moisture in this variant of primary tillage was 5.7 mm higher compared to the background of classical tillage. However, at the same time, it was inferior to No-till technology by 7.6 mm. The determination of soil moisture before harvesting spring barley shows that until this period, the previously identified trend regarding the difference in the reserves of productive moisture in a meter layer of soil depending on the methods of soil cultivation has been preserved. The largest reserves of available moisture were contained using No-till technology, where the difference compared to classical tillage, which involved plowing by 20-22 cm, was 4.7 mm or 6.4%.

One of the ways to substantiate the efficiency of using moisture for the formation of a unit of dry matter of the main barley product is the calculation of its total costs. Thus, in the experiment, the yield of barley grain according to the options of primary tillage, in terms of dehydrated matter, was classic – 3.78; shallow – 3.70; mini-till – 3,72; no-till – 3.61 t/ha.

Calculations show that the total moisture consumption per unit of dry matter was 62.0 m3/t lower compared to the background, where spring barley was grown using No-till technology. It should be noted that in the case of shallow tillage for barley to a depth of 10-12 cm or cultivation of the crop against the background of Mini-till. The total amount of moisture consumed by crops to form a unit of dry matter was almost the same and exceeded the control by only 0.4–1.9%.

The analysis of the obtained results made it possible to identify differences in the abundance of the weed component in spring barley crops on average during the crop's growing season in different variants of basic tillage. Thus, on average, over the years of research (2021–2022), the smallest number of weeds in spring barley crops was counted when tillage using Mini-till technology, where it was 32.8 pcs/m^2 (Fig. 1).



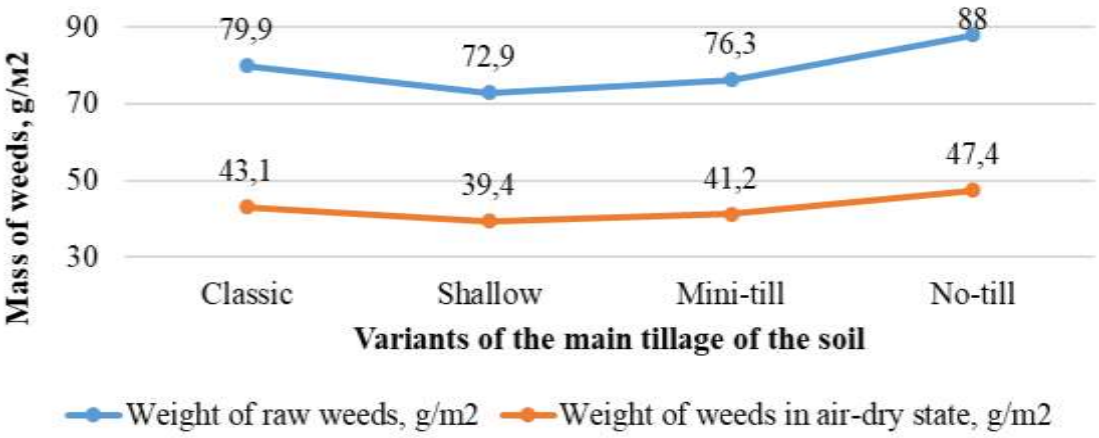
R&D $_{0.5}$ = 6.3 pcs/m^2

Rice. 1. Abundance of weeds in spring barley crops depending on tillage technology, average for 2021–2022

During the classic tillage for barley, the number of weeds increased by 2.4 pcs/m^2 compared to the Mini-till system. The maximum abundance of weeds was observed in the No-till technology variant, where their number was higher by 5.1 pcs/m^2 relative to soil cultivation using Mini-till technology and by 2.7 pcs./m^2 relative to the variant with classic primary tillage. With shallow tillage, where only the soil was loosened to a depth of 10-12 cm with a heavy disc harrow, the level of weed infestation of crops (37.4 pcs./m^2) was almost at the level of the No-till version. The difference between the variants of primary tillage mentioned above, in terms of the abundance of weed plants, was only 0.5 pcs./m^2 or 1.3%.

Studies conducted in irrigated agriculture show that the nature of competitive relations in field crops is significantly influenced by the number of weed plants per unit area and the degree of development of their vegetative mass (Vozhehova R.A. et al., 2020). As for the weight of weed plants in the damp and air-dry state, it should be noted that the highest values of these indicators were both on the variant with the highest number of them in spring barley crops, in particular the No-till system (88.0 and 47.4 g/m2, respectively), and against the background with the most favorable conditions for the formation of vegetative mass of segetal vegetation, in particular under classical primary tillage (79.9 and 43.1 g/m^2, respectively) and Mini-till technology (76.3 and 41.2 g/m^2, respectively) (Fig. 2).

The most negligible mass of both raw weed plants and in the air-dry state was characterized by the version of the experiment where shallow primary tillage was carried out. Compared to other variants of the experiment, the wet weight of weed plants in the air-dry state decreased by 3.4–15.1 and 1.8–8.0 g/m^2 or 4.7–20.7 and 4.6–20.3 %.



R&D $_{0.5}$ = 10.7 g/m2 (for the mass of raw weeds); R&D 0.5 = 5.4 g/m2 (for the mass of weeds in an air-dry state).

Rice. 2. **Weight of weeds in spring barley crops depending on tillage technology, average for 2021–2022**

The variants of the experiment also differed in the effect on the ratio of biological groups of weeds to their total number in spring barley crops (Table 2).

Table 2

Structure of biological groups of weeds in spring barley crops depending on the tillage system, average for 2021–2022

№	Basic tillage options	Total weeds, pcs./m²	Including, %		
			Perennial	Annual monocots	Annual dicotyledonous
1.	Classic (control)	35,2	4,8	60,1	35,1
2.	Shallow	37,4	14,2	49,2	36,6
3.	Mini-till	32,8	4,8	55,9	39,4
4.	No-till	37,9	3,7	74,0	22,3
	NIR $_{0.5}$	6,3	–	–	–

Thus, the No-till variant of primary tillage characterized the maximum proportion of annual monocotyledonous weeds. Annual dicotyledonous species were relatively evenly represented in the structure of biological groups of weeds, except the No-till variant of primary tillage, where their share was the smallest. The most significant percentage of perennial species of segetal vegetation accounted for shallow tillage, 14.2%, and for other variants of the experiment, this figure was 3.7–4.8 %.

In the agrophytocenosis of spring barley, the juvenile type of weeds with the dominance of cereal weeds prevailed. The most common cereal weeds in the years of research were chicken millet (*Echinochloa crus-gali L.*), gray mouse (*Setaria glauka L.*). Annual dicotyledonous species were represented mainly by weeds such as white quinoa (*Chenopodium album L.*), Amaranthus retroflexus L., *Poligonum* convolvulus *L.* The most common perennial weeds were field bindweed (Convolvulus arvensis L.) and pink thistle (*Cirsium arvense L.*).

Discussion. Thus, the research results show that with the use of spring tillless primary tillage or No-till in the technology of growing barley, conditions are created to improve the moisture supply of crops both at the time of sowing and before harvesting, compared to classical tillage. The results obtained are consistent with the data of the experiments of the Kharkiv NAU. V. V. Dokuchaev, according to which it was noted that for the cultivation of spring barley according to the No-till system, the moisture content in the arable layer of soil at the time of harvesting exceeded the control by 6.9 mm or 25.9 % (Syromiatnykov Yu. M. 2020).

As for weed infestation, the research results show that during spring barley cultivation using the Mini-till technology, the least number of weed plants vegetated in the crops. Compared to other experiment variants, their number was lower by 7.3–15.5%. Scientific research has established that the first and most crucial link in the primary tillage system is stubble plowing. With the help of this technological operation, vegetative plants and weeds are destroyed, and the proper conditions for germinating their seeds are formed. With sufficient moisture supply of the sowing layer, stubble plowing provokes the germination of about 40% of fresh weed seeds and significant reserves from previous years (Pavlichenko A.D., 2018).

It should be noted that the highest level of weed infestation of crops was with No-till technology. Tillage ensured a decrease in the number of weeds on average during the growing season of spring barley by 7.1%, compared to No-till technology according to the classical scheme, which provided for plowing to a depth of 20-22 cm. According to O. I. Tsyliuryk and V. P. Shapka (2014), the decrease in the number of weeds against the background of moldboard tillage is due to the movement of seeds of segetal vegetation from the upper to the lower layers, which limits their germination from deeper horizons.

The difference was only 1.3% with shallow tillage, and the abundance of segetal vegetation in the crops was almost at the level of the variant with No-till technology. Studies by V.P. Borona et al. (2009) also indicate an increase in weed infestation of crops by 1.4–2.3 times, with no-till tillage. According to experimental data obtained in Right-Bank Ukraine, it was found that the highest level of weed infestation at the time of harvesting spring cereal crops was formed against the background of carrying out direct sowing. Thus, in the variant mentioned above, the abundance of segetal vegetation was higher by 27.8% compared to the variant where shelf cultivation was carried out to a depth of 20–22 cm and by 19.8%, according to the variant where the technology provided for only disking at 6–8 cm (Behei S. S., et al., 2020). Similar research results were obtained in the experiments of O. M. Khilnitsky and co-authors (2007). S. P. Tanchyk, Y. Mykolenko (2016) believe that the main reason for the increase in the number of weed plants in field crops before harvesting, using No-till technology, is the presence of mulch from plant residues on the soil surface, which prevents the germination of weed seeds, as it leads to a decrease in soil temperature, which in turn inhibits the germination and emergence of seedlings of segetal vegetation at the initial stages of growth. However, with stable warming of the topsoil, the number of weeds in crops increases.

Studies have found that, regardless of the technology of primary tillage, the structure of biological groups of weeds was dominated by annual monocotyledonous and dicotyledonous species. As for perennial weeds, there was an increase in their share by 2.9–3.8 times compared to other variants of the experiment, only with shallow tillage. In the experiments of Y.P. Manko, the dominance of one- and two-year-old weeds during plowing and the increase in weed infestation by perennial species during flat-cut weeds were observed. tillage (Manko Yu. P., 1991). M. M. Ptashnik, S. V. Dud-

nyk, F. Y. Bruhal, N. E. Borys (2021) believe that rational tillage plays a significant role in managing the level of weed infestation of agrocenoses. It should provide a high anti-weed effect when favorable conditions are formed for the growth of the ability of agrocenoses to self-regulate the presence of segetal vegetation in crops with a decrease in its share.

Conclusions:

1. Due to the No-till system, the accumulation of moisture in autumn-winter precipitation improves, and a higher content is formed when sowing the crop.

2. The lowest level of weed infestation of spring barley crops (32.8 pcs./m2) is ensured by Mini-till technology, which involves stubble plowing to a depth of 6-8 cm and direct sowing of the crop.

3. Cultivation of crops using No-till technology led to the formation of the highest level of weed infestation. The increase in the number of weeds, compared to other variants of primary tillage, was 1.3–15.6%.

4. The classical method of tillage for barley, which provided for plowing to a depth of 20-22 cm, ensured a decrease in weed infestation of crops compared to the option of minimum tillage and No-till technology, by 5.9 and 7.1 %, respectively

5. Studies did not reveal an evident influence of primary tillage methods on the structure of biological groups of weeds; annual monocotyledonous and dicotyledonous species dominated in spring barley crops.

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ACTIVITY OF SERUM ENZYMES OF YOUNG PIGS OF DIFFERENT GENOTYPES BY MELANOCORTIN RECEPTOR GENE (MC4R) AND THEIR RELATIONSHIP WITH FATTENING AND MEAT QUALITIES

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The article is devoted to studying the activity of serum enzymes, fattening, and meat qualities of young pigs of different genotypes by the melanocortin receptor gene MC4R, calculating the level of correlation between traits, and economic efficiency of research results. The experimental part of the research was carried out in the agricultural formations of the Dnipropetrovsk region, the Research Center for Biosafety and Environmental Control of Agricultural Resources of the Dnipro State Agrarian and Economic University, the Genetics Laboratory of the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine and the Laboratory of Animal Husbandry of the State Institution "Institute of Grain Crops of the National Academy of Agrarian Sciences". The work was carried out by the research program of the National Academy of Agrarian Sciences of Ukraine No. 30, " Innovative Technologies of Breeding, Industrial and Organic Production of Pig Products," task "To develop a local system of selection and hybridization of pigs using modern genetic methods (DNA markers)". Laboratory studies and the results of control fattening indicate that the biochemical parameters of blood serum (activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) of young pigs of large white breed of the controlled population correspond to the physiological norm of clinically healthy animals, and according to the leading indicators of fattening and meat qualities (age of reaching body weight of 100 kg, days; fat thickness at the level of 6-7 thoracic vertebrae, mm; length of chilled carcass, cm) belongs to class I and class elite. Analysis of the data of control fattening and slaughter shows that young pigs of the second experimental group ($MC4R^{AG}$) are superior to their peers of I ($MC4R^{AA}$) in terms of average daily weight gain, age of body weight100 кг, fat thickness at the level of 6-7 thoracic vertebrae and length of chilled carcass by an average of 4.58%. Several significant associations between interior parameters (aspartate aminotransferase (AST) activity, units/l; alanine aminotransferase (ALT) activity, unit/l), fattening and meat qualities of young pigs of the controlled population is 28.57 %. This indicates the possibility of using interior indicators for early prediction of fattening and meat qualities of young pigs. The maximum increase in additional production was obtained from young pigs of the second experimental group ($MC4R^{AG}$) – +3.24 %, and its cost, which was obtained

from the sale of one head of young pigs of the specified genotype is equal to +261.56 UAH or +6.62 USD.

Keywords: young pigs, genotype, serum biochemical parameters, fattening and meat qualities, correlation, cost of additional products

АКТИВНІСТЬ ФЕРМЕНТІВ СИРОВАТКИ КРОВІ МОЛОДНЯКУ СВИНЕЙ РІЗНИХ ГЕНОТИПІВ ЗА ГЕНОМ РЕЦЕПТОРА МЕЛАНОКОРТИНУ (MC4R) ТА ЇХ ЗВ'ЯЗОК З ВІДГОДІВЕЛЬНИМИ І М'ЯСНИМИ ЯКОСТЯМИ

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Стаття присвячена вивченню активності ферментів сироватки крові, відгодівельних та м'ясних якостей молодняку свиней різних генотипів за геном рецептора меланокортину MC4R, розрахунку рівня кореляційних зв'язків між ознаками та економічної ефективності результатів досліджень. Експериментальну частину досліджень проведено в агроформуваннях Дніпропетровської області, Науково-дослідному центрі біобезпеки та екологічного контролю ресурсів АПК Дніпровського державного аграрно-економічного університету, лабораторії генетики Інституту свинарства і АПВ НААН та лабораторії тваринництва Державної установи «Інститут зернових культур НААН». Роботу виконано згідно програми наукових досліджень Національної академії аграрних наук України № 30 «Інноваційні технології племінного, промислового та органічного виробництва продукції свинарства», завдання «Розробити локальну систему селекції та гібридизації свиней із використанням сучасних генетичних методів (ДНК-маркерів)». Лабораторні дослідження та результати контрольної відгодівлі свідчать, що біохімічні показники сироватки крові (активність аспартатамінотрансферази, аланінамінотрансферази та лужної фосфатази) молодняку свиней великої білої породи підконтрольної популяції відповідають фізіологічній нормі клінічно здорових тварин, а за основними показниками відгодівельних і м'ясних якостей (вік досягнення живої маси 100 кг, діб; товщина шпику на рівні 6-7 грудних хребців, мм; довжина охолодженої туші, см) належить до І класу та класу еліта. Аналіз даних контрольної відгодівлі та забою свідчать, що молодняк свиней ІІ піддослідної групи ($MC4R^{AG}$) переважає ровесників І ($MC4R^{AA}$) за середньодобовим приростом живої маси, віком досягнення живої маси 100 кг, товщиною шпику на рівні 6-7 грудних хребців та довжиною охолодженої туші в середньому на 4,58 %. Кількість достовірних зв'язків між показниками інтер'єру (активність аспартатамінотрансферази (АсАТ), од/л; активність аланінамінотрансферази (АлАТ), од/л), відгодівельними і м'ясними якостями молодняку свиней підконтрольної популяції становить 28,57 %. Зазначене свідчить про можливість використання показників інтер'єру для раннього прогнозування відгодівельними і м'ясними якостями молодняку свиней. Максимальну прибавку додаткової продук-

ції одержано від молодняку свиней II піддослідної групи (MC4R^{AG}) – +3,24 %, а її вартість яку було одержано від реалізації однієї голови молодняку свиней зазначеного генотипу дорівнює +261,56 гривень або +6,62 доларів США.

Ключові слова: молодняк свиней, генотип, біохімічні показники сироватки крові, відгодівельні і м'ясні якості, кореляція, вартість додаткової продукції.

Entry. Topical issues that significantly affect the pace of selection and breeding work in the field of pig breeding include the search for effective methods for assessing the breeding value of animals and selecting highly productive individuals for their further intensive use in breeding reproducers and factories, as well as industrial complexes (Balatsky et al., 2016; Vashchenko, 2019; Albuquerque et al., 2021).

It has been established that such methods for assessing the breeding value of animals and selecting highly productive ones include linear models, DNA markers, and indices of various designs. Thus, Vashchenko P. (2019) notes that linear models for determining the breeding value of pigs by reproductive qualities make it possible at an early stage of ontogeny to determine the most valuable animals for further repair of the herd. The author found that in the herd of pigs of the great white breeds of the factory type "Bagachansky" correlations between the estimates of the breeding value of queens according to the developed models and the productivity of their daughters are significant and 9.9 – 10.5 times more substantial than the links between the productivity of queens and the productivity of their daughters. Long-term research by P. Vashchenko (2019) shows that the BLUP method, which most accurately characterizes their offspring's productivity, is an effective method for assessing boars' breeding value. Thus, the results of the assessment by the method of control fattening of boar offspring and the BLUP method indicate the presence of significant correlations taking into account the length of the body ($r = +0.42 \pm 0.209$, $p \leq 0.05$) and the thickness of the fat ($r = +0.67 \pm 0.170$, $p \leq 0.001$). The relationship between the indicators of boars' productivity and the productivity of their offspring is unreliable and is 1.4-3.5 times lower.

Studies by Verbych I. and Bratkovska G. (Verbych, I. V., & Bratkovska, 2020) show that when comparing productivity indicators with the breeding value indices of large white pigs of the "SE "DG "Pasichna" of the Institute of Feed and Agriculture of Podillya of the National Academy of Agrarian Sciences of Ukraine" of the Khmelnytsky region, a weak correlation was established between the body weight of piglets at the time of their weaning and the breeding value of pigs ($r = 0.21$, $R>0.99$). The coefficient of paired correlation between the indices of fattening and meat qualities of M. D. Berezovsky and the index of B. Tyler is $r = 0.56$ ($P >0.99$).

Researchers (Balatsky et al., 2016) propose using the genetic marker LEPR SNP NM001024587.1, 1987 C>T in marker selection to predict and improve the meat quality of large white pigs of the Ukrainian breed. The results of the authors' research established that the genotype of animals by the genome LEPR has a significant effect on intramuscular fat content in meat ($\eta 2=14.212\%$, $p<0.05$), total moisture ($\eta 2=7.896\%$, $p<0.05$) and dry matter ($\eta 2=7.577\%$, $p<0.05$); The fat content in the meat of pigs with a heterozygous genotype was significantly lower compared to animals with a homozygous genotype.

RNA sequencing transcriptome analysis shows that AL pigs lived an average of 150.6 days during the trial with a mean daily gain (ADG) of 582 g/day, while BI pigs lived an average of 135.2 days during the trial ($p = 0.273$) and ADG 656 g/d ($p = 0.297$) (Albuquerque et al., 2021). At a weight of 150 kg and compared to BI pigs, AL showed higher levels of total plasma protein (69.8 vs. 64.4 g/L, $p<0.05$), urea (6.9 vs. 5.6 mmol/L, $p<0.05$) and total cholesterol (2.66 vs. 2.23 mmol/L, $p<0.05$). The average

thickness of the bacon was also significantly higher in AL (7.9 vs. p<0.001) compared to BI pigs. For LL muscle, AL pigs had a lower loin proportion (3.63 vs. 5.14%, p<0.05) but a higher total IMF (7.3 vs. 5.7 g/100 g, p<0.01) compared to BI pigs. 4,3 см

The relevance of the chosen direction of research is confirmed by the scientific works of the following scientists: Vylleke et al., 2003; Szyndler-Nкdza et al., 2010; Draguljan et al., 2013; Kostyunina et al., 2015; Herrero-Medrano et al., 2015; Tsereniuk et al., 2016; Balatskyi et al., 2018; Martínez-Montes et al., 2018; Bankovska et al., 2020; Matiiuk et al., 2020; Poklukar et al., 2020; Mykhalko et al., 2022.

Blood analysis using biochemical methods is one of the most informative laboratory diagnostic tools, providing essential information about the functional state of various animal organs and systems. A biochemical blood test makes it possible to detect disorders of the internal organs without interfering with them. The biochemical composition of blood depends on various factors, including the conditions of keeping, feeding, age, and physiological state of animals, as well as on their genotype and lineage.

The aim of the work was to investigate the activity of serum enzymes, fattening, and meat qualities of young pigs of different genotypes by the melanocortin receptor MC4R gene to calculate the level of correlation between traits and the economic efficiency of research results.

Materials and methods. The study was carried out in agricultural formations of the Dnipropetrovsk region, the Research Center for Biosafety and Environmental Control of Agricultural Resources of the Dnipro State Agrarian and Economic University, the Genetics Laboratory of the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine and the Laboratory of Animal Husbandry of the State Institution "Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine".The work was carried out by the research program of the National Academy of Agrarian Sciences of Ukraine No. 30 "Innovative technologies for breeding, industrial and organic production of pig products", the task "To develop a local system of selection and hybridization of pigs using modern genetic methods (DNA markers)".

Assessment of young pigs of large white breed (n = 40) for fattening and meat qualities was carried out taking into account the following quantitative characteristics: average daily weight gain, g; age of reaching body weight 100 kg, days; fat thickness at the level of 6-7 thoracic vertebrae, mm; length of chilled carcass cm; length of the bacon half of the chilled half carcass, cm, the most significant (front) width of the bacon half of the carcass, cm; smaller (back) width of the bacon half of the carcass, cm. The length of the chilled carcass (cm) was measured with a measuring tape from the edge of the pubic bone fusion to the anterior surface of the first cervical vertebra; the length of the bacon half of the chilled half-carcass (cm) – from the front edge of the pubic bone to the middle of the front edge of the first rib; the most significant (front) width of the bacon half is at the level of the seventh thoracic vertebra perpendicular to the half of the carcass; the most minor (back) width of the bacon half is at the level of the penultimate lumbar vertebra perpendicular to the half of the carcass (Berezovskyi & Khatko, 2005; Voloshchuk et al., 2017).

The age of reaching a body weight of 100 kg (1, 2) and the multicomponent estimated index of M. D. Berezovsky (3) were calculated according to the following formulas:

If the body weight of the animal is 85-99 kg:

$$Д_{100} = \left[(100\ кг - М_0\ ) \div \quad \frac{М_0 - М_{по}}{Д_0 - Д_{по}} \right] + Д_0, \qquad (1)$$

If the body weight of the animal is 101-115 kg:

$$Д_{100} = Д_0 - \left[(M_0 - 100\ кг) \div \frac{M_0 - M_{no}}{Д_0 - Д_{no}} \right] + Д_0, \quad (2)$$

where: D_{100} is the age of reaching a body weight of 100 kg, days; D_0 is the age at the last weighing, days; D_{no} – age of pre-weighing, days; M_0 – body weight at the last weighing, kg; M_{no} – body weight at pre-weighing, kg (Instruktsiia , 2003);

$$I = 100 - \frac{K \times B \times C}{A^2} \quad (3)$$

where: K – is the age of reaching a body weight of 100 kg, days; A – gross weight gain for the fattening period, kg; B – the number of days of fattening; C – the thickness of the bacon at the level of 6-7 ribs, cm (Vashchenko, 2019).

DNA typing of young pigs by the melanocortin receptor MC4R gene was carried out in the genetics laboratory of the Institute of Pig Breeding and APV of NAAS (Kim et al., 2000; Kim et al., 2004). The bristles of animals of the specified production group were used as a biomaterial.

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in the serum of young pigs of different genotypes by the melanocortin receptor gene MC4R (n=13) was determined by generally accepted methods (Hryban et al., 2001; Vlizlo et al., 2012).

The cost of additional products was calculated using the following data: the purchase price of a unit of production, according to the existing prices in force in Ukraine (UAH); average animal productivity; average mark-up of primary products (%), which is expressed as a percentage per 1 head when applying a new and improved breeding achievement compared to the productivity of animals of primary use; constant coefficient of decrease in the result, which is associated with additional costs for profitable products (0.75); the number of livestock of farm animals of a new or improved breeding achievement, heads (Chernenko, 2016).

Biometric processing of research results was done according to generally accepted methods using the programmable "Data Analysis" module in Microsoft Excel. Statistical errors for the arithmetic mean (4), standard deviation (5), coefficient of variation (6), and correlation coefficient (7) were calculated using the following formulas:

$$S_x = \pm\frac{\sigma}{\sqrt{n}} \quad (4) \qquad S_\sigma = \pm\frac{\sigma}{\sqrt{2_n}} \quad (5) \qquad S_{Cv} = \pm\frac{C_v}{\sqrt{2_n}} \quad (6) \qquad S_r = \frac{1-r^2}{\sqrt{n}} \quad (7)$$

where: n is the sample size; σ is the standard deviation; Cv is the coefficient of variation (Kovalenko et al., 2010, Kramarenko et al., 2019).

The strength of correlations between traits was determined by the Cheddock scale (Sidorova et al., 2003) (Table 1).

Table 1

Cheddock Scale for Gradation of the Strength of the Correlation Relationship between Quantitative Traits

Correlation coefficient value	The Strength of the Correlation
0,1-0,3	Weak
0,3-0,5	Moderate
0,5-0,7	Noticeable
0,7-0,9	High
0,9-0,99	Very high

Research results. The results of laboratory studies indicate that the biochemical parameters of blood serum, namely the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in the serum of young pigs correspond to the physiological norm of clinically healthy animals (Hryban et al., 2001; Vlizlo et al., 2012). Thus, the activity of aspartate aminotransferase (AST) is 59.38±3.761 units/l, alanine aminotransferase (ALT) – 44.38±2.474 units/l, alkaline phosphatase (ALP) – 122.12±8.653 units/l.

It was found that the average daily weight gain of young pigs (n=40) during the period of control fattening is 781.9±6.10 g, the age of reaching body weight is 177.1±0.79 days, the thickness of fat at the level of 6-7 thoracic vertebrae is 20.8±0,35 mm, the length of the chilled carcass is 96.6±0.36 cm, the length of the 100 kg 0,35 mm 0,36 см bacon half of the chilled half-carcass is 85.3±,0,52 cm the estimated index of M. D. Berezovsky is 89.5±0.54 points. Indicators The most significant (front) and most minor (back) widths of the bacon half are 34.2±0.45 and 24.7±0.37 cm, respectively.

The coefficient of variability of biochemical parameters of blood serum, fattening, and meat qualities of young pigs ranges from 1.81 to 25.55 % (Table 2).

Table 2

Indicators of variability of interior traits (n=13), fattening and meat qualities (n=40) of young pigs of large white breed

Indicators, units of measurement	Biometric indicators	
	$\sigma \pm S\sigma$	$Cv \pm Sc_v$, %
Aspartate aminotransferase (AST) activity, U/L	13,56±2,664	22,84±4,487
Alanine aminotransferase (ALT) activity, units/l	8,92±1,752	20,10±3,948
Alkaline phosphatase (ALP) activity, U/L	31,20±6,129	25,55±5,019
Average daily weight gain, g	38,64±4,322	4,94±0,116
Age of reaching body weight 100 kg, days	5,02±0,561	2,84±0,317
The thickness of the bacon at the level of 6-7 thoracic vertebrae, mm	2,25±0,251	10,81±1,209
Length of chilled carcass, cm	1,75±0,195	1,81±0,202
Length of bacon half of chilled half carcass, cm	2,51±0,280	2,95±0,329
The largest (front) width of the bacon half of the chilled carcass, cm	2,33±0,260	6,83±0,763
Smallest (back) width of the bacon half of the chilled carcass, cm	1,88±0,210	7,63±0,853
Evaluation index of M. D. Berezovsky, point	3,54±0,395	3,96±0,442

The results of the study of biochemical parameters of blood serum, fattening, and meat qualities of young pigs of different interbreed differentiation by the melanocortin receptor gene MC4R are given in Tables 3 and 4.

The results of the study of biochemical parameters of blood serum, fattening, and meat qualities of young pigs of different interbreed differentiation by the melanocortin receptor gene MC4R indicate that the difference between the groups in aspartate aminotransferase activity (AST) is 5.98 units/l (td=0.81; p>0.05), alanine aminotransferase activity (ALT) – 6.72 units/l (td=1.44; p>0.05), alkaline phosphatase activity (ALP) – 3.70 units/l (td=0.20; p>0.05) (Table 3).

Table 3

Biochemical parameters of blood serum of young pigs of different intrabreed differentiation by the melanocortin receptor gene MC4R

Indicator (feature), units of measurement	Biometric indicators	Genotype	
		MC4R^{AA}	***MC4R^{AG}***
		group	
		I	II
Aspartate aminotransferase (AST) activity, U/L	*n*	*7*	*6*
	X±Sx	62,14±6,185	56,16±4,020
	σ±Xσ	16,36±4,374	9,84±0,289
	Cv±Sc$_v$, %	26,32±7,037	17,52±5,063
Alanine aminotransferase (ALT) activity, units/l	*X±Sx*	41,28±3,649	48,00±2,886
	σ±Xσ	9,65±2,580	7,07±2,043
	Cv±Sc$_v$, %	23,37±6,248	14,72±4,254
Alkaline phosphatase (ALP) activity, U/L	*X±Sx*	120,41±12,341	124,11±13,217
	σ±Xσ	32,65±8,729	32,37±9,355
	Cv±Sc$_v$, %	27,11±7,248	26,08±7,537

It was found that the maximum indicator of the average daily weight gain (808.1±5.37 g) and the minimum value of the age of reaching body weight (174.9±0.78 days) are characterized by the animals of the second experimental group. The difference between the animals of the II 100 ($MC4R^{AG}$) and I ($MC4R^{AA}$) experimental groups is (td=5.06; p<0.001) and 3.9 days (td=2.76; p<0.05), respectively 47,4 g. (Table 4).

In terms of the thickness of bacon at the level of 6-7 thoracic vertebrae, young pigs of the second experimental group outnumbered their peers I by 1.8 mm (td=2.85; p<0.01), the length of the chilled carcass – (td=3.33; p<0.01), the length of the bacon half of the chilled half-carcass 1,9 см – (td=2.95; p<0.01), the estimated index of M. D. Berezovsky – 2.8 points (td=2.71; p<0.01) 2,6 cm. Studies show that the maximum values "the largest (front) width of the bacon half of the chilled carcass, cm" and "the smallest (back) width of the bacon half of the chilled carcass, cm" are also characterized by young pigs of the second experimental group. Compared to the peers of the first experimental group, the difference in these indicators is 2,2 cm (td=2.71; p<0.01) and 1.3 cm (td=1.88; p>0.05), respectively.

Table 4

Fattening and meat qualities of young pigs of different intrabreed differentiation by the melanocortin receptor gene MC4R

Indicator, units of measurement	Biometric indicators	Genotype	
		MC4R^{AA}	***MC4R^{AG}***
		group	
		I	**II**
Average daily weight gain, g	*n*	*22*	*18*
	X±Sx	760,7±7,66	808,1±5,37
	σ±Xσ	35,93±5,419	22,82±3,803
	Cv±Sc$_v$, %	4,72±0,711	2,82±0,470
Age of reaching body weight 100 kg, days	*X±Sx*	178,8±1,18	174,9±0,78
	σ±Xσ	5,55±0,837	3,32±0,553
	Cv±Sc$_v$, %	3,10±0,467	1,89±0,315
The thickness of the bacon at the level of 6-7 thoracic vertebrae, mm	*X±Sx*	21,6±0,52	19,8±0,36
	σ±Xσ	2,44±0,368	1,54±0,256
	Cv±Sc$_v$, %	11,29±1,702	7,77±1,295
Length of chilled carcass, cm	*n*	*10*	*13*
	X±Sx	95,5±0,34	97,4±0,47
	σ±Xσ	1,08±0,241	1,71±0,335
	Cv±Sc$_v$, %	1,13±0,252	1,75±0,343
Length of bacon half of chilled half carcass, cm	*X±Sx*	83,8±0,61	86,4±0,64
	σ±Xσ	1,93±0,431	2,33±0,457
	Cv±Sc$_v$, %	2,30±0,514	2,69±0,528
The largest (front) width of the bacon half of the chilled carcass, cm	*X±Sx*	33,1±0,63	35,3±0,52
	σ±Xσ	2,30±0,514	1,88±0,369
	Cv±Sc$_v$, %	6,94±1,552	5,32±1,045
Smallest (back) width of the bacon half of the chilled carcass, cm	*X±Sx*	24,0±0,38	25,3±0,59
	σ±Xσ	1,38±0,308	2,14±0,420
	Cv±Sc$_v$, %	5,75±1,286	8,45±1,660
Evaluation index of M. D. Berezovsky, point	*n*	*22*	*18*
	X±Sx	88,5±0,71	91,3±0,75
	σ±Xσ	3,83±0,577	2,73±0,455
	Cv±Sc$_v$, %	4,32±0,651	2,99±0,498

The coefficient of variability (Cv,%) of absolute indicators of fattening and meat qualities in young pigs of different interbreed differentiation according to the MC4R gene ranges from 1.13 (length of chilled carcass in animals of the experimental group I – MC4R^{AA}**)** to 11.29 % (fat thickness at the level of 6-7 thoracic vertebrae in animals of the experimental group I – MC4R^{AA}).

The calculation of the pair correlation coefficient between the biochemical parameters of blood serum, fattening, and meat qualities of young pigs of large white breed shows that this biometric indicator varies from -0.344±0.1421 to +0.402±0.1411 (Table 5).

Table 5

The level of correlation between the biochemical parameters of blood serum, fattening and meat qualities of young pigs of large white breed

Sign		Biometric indicators		The Strength of the correlation
x	***y***	**r±Sr**	**tr**	
Aspartate aminotransferase (AcBP) activity, U/L	1	-0,049±0,1578	0,31	-
	2	+0,212±0,1511	1,40	*Weak*
	3	-0,316±0,1424*	2,22	*Moderate*
	4	+0,073±0,1574	0,46	-
	5	+0,197±0,1521	1,30	*Weak*
	6	-0,058±0,1577	0,37	-
	7	+0,179±0,1532	1,17	*Weak*
Alanine aminotransferase (ALT) activity, units/l	1	+0,281±0,1457	1,93	*Weak*
	2	-0,344±0,1395*	2,47	*Moderate*
	3	+0,091±0,1569	0,58	-
	4	+0,293±0,1446*	2,03	*Weak*
	5	+0,402±0,1327**	3,03	*Moderate*
	6	+0,169±0,1537	1,10	*Weak*
	7	+0,097±0,1567	0,62	-
Alkaline phosphatase (ALP) activity, U/L	1	+0,128±0,1556	0,82	*Weak*
	2	-0,001±0,1582	0,01	-
	3	-0,101±0,1566	0,64	*Weak*
	4	-0,111±0,1563	0,71	*Weak*
	5	+0,194±0,1523	1,27	*Weak*
	6	-0,285±0,1454	1,96	*Weak*
	7	-0,146±0,1549	0,94	*Weak*

*Note: x – biochemical parameters of blood serum; y – fattening and meat qualities of young pigs (total sample); 1 - average daily weight gain, g; 2 - age of reaching body weight 100 kg, days; 3 - the thickness of the bacon at the level of 6-7 thoracic vertebrae, mm; 4 - length of chilled carcass, cm; 5 - length of the bacon half of the chilled half carcass, cm; 6 - the largest (front) width of the bacon half of the carcass, Cm; 7 - the smallest (back) width of the bacon half of the carcass, cm; * - p<0.05; ** - p<0.01*

Significant pairwise correlation coefficients were established between the following pairs of traits: aspartate aminotransferase (AST) activity × fat thickness at the level of 6-7 thoracic vertebrae (r = -0.316, tr = 2.22), alanine aminotransferase activity (ALT) × age of reaching a body weight of 100 kg (r = -0.344, tr = 2.47), alanine aminotransferase activity (ALT) × length of chilled carcass (r = + 0.293, tr = 2.03), alanine aminotransferase (ALT) activity × the length of the bacon half of the chilled half-carcass (r= +0.402, tr=3.03).

The calculation of the economic efficiency of the research results shows that the maximum increase in additional production was obtained from young pigs of the second experimental groups - +3.24 % (Table 6).

The cost of additional products obtained from one head of young pigs of this group is + 261.56 or + 6.62 US dollars.

Table 6

Cost-effectiveness of research results

Group	n	Average daily body weight gain, g	± to the population average	Cost of additional products, UAH / USD / Goal
Total Sample	40	781,9±6,10	-	-
I	22	760,7±7,66	-2,71	-223,65 / -5,66
II	18	808,1±5,37	+3,24	+261,56 / +6,62

*Note: * - the selling price of young pigs at the time of the study was UAH 78.80 or USD 1.99 per 1 kg of body weight.*

Discussion. Enzymes are involved in all biochemical processes of the body, and metabolic disorders caused by various harmful factors lead to changes in the concentration of the corresponding enzymes in biological fluids. Alanine aminotransferase and aspartate aminotransferase are the most important representatives of the group of enzymes (intracellular enzymes) involved in the synthesis and breakdown of amino acids; interconnection of carbohydrate, lipid, and amino acid metabolism pathways; synthesis of some specific compounds, including urea and γ-aminobutyric acid.

That is why we investigated the activity of aminotransferases in the serum of young pigs of different genotypes according to the melanocortin receptor gene (MC4R) and determined their relationship with fattening and meat qualities. Reliable links have been established between the indicators of the interior and the fattening and meat qualities of young pigs of the controlled population.

It was found that animals of the $MC4R^{AG}$ genotype outnumbered their peers of the $MC4R^{AA}$ genotype in terms of fattening and meat qualities by an average of 4.29%. The pairwise correlation coefficients between interior, fattening, and meat qualities in animals of different genotypes ranged from -0.917 to +0.577, indicating their use's effectiveness for early prediction of these groups of traits (Khalak et al., 2021).

The authors (Kirovych et al., 2023) recommend that in order to increase the reproductive capacity of pigs of the pietrain breed, the selection of replacement gilts should be carried out, taking into account polymorphism for the RYR-1 and MC4R genes. When forming parental pairs in order to obtain a high level of productivity for reproductive traits (multiple births of more than 8.00 heads), boars can be carriers of the homozygous genotype GG^{nn} according to the genes MC4R and RYR1, which in turn contributes to the production of offspring with an increased level of meat productivity.

The efficiency of pork meat production, along with reproductive and fattening characteristics, largely depends on the level of slaughter and meat quality. This issue is particularly important when using specialized meat breeds of foreign selection to improve the meat qualities of pig breeds of domestic selection when breeding new interbreed types and lines or obtaining hybrid commercial young animals.

When studying the influence of carriers of different alleles of the MC4R gene on the manifestation of body weight, fattening, and meat traits in young pigs of hybrid origin F_2 (¼BB + ¼UM + ½PTP), Garmatyuk K.V. noted the positive effect of the $MC4R^{G}$ allele and the $MC4R^{GG}$ genotype on animal productivity. The lowest age to achieve body weight is typical for carriers of the $MC4R^{GG}$ genotype – 164.0 days, which outnumbered carriers of the $MC4R^{AA}$ genotype by 4.5 days or by 2.7% ($p<0.01$) (Harmatiuk, 2019). The results of the study by P. Vashchenko (2019) indicate that the results of DNA typing of the Myrhorod pig breed according to the MC4R gene should

be used as a fixed factor in determining the breeding value by the BLUP method on the grounds of "age of reaching weight 100 kg" and "fat thickness". The author notes that the correlation between the estimates obtained from models with and without the use of data on the genotype of pigs by the MC4R gene, According to the trait, the age of reaching a mass of 100 kg is 0.76±0.109 ($p<0.001$); based on "fat thickness" – 0.71±0.119 ($p<0.001$). When breeding to reduce the thickness of bacon, it is necessary to control the uniformity of its deposition since it has been found that its relative evenness deteriorates with a decrease in the thickness of bacon. Pigs with a lower fat thickness (a large white breed of Hungarian breed) are characterized by a lower coefficient of evenness, inferior to UVB-1 animals by 86.9 % ($p<0.001$).

Further directions of our work include studies of the physicochemical properties and chemical composition of the longest back muscle and subcutaneous fat of young pigs of different breeds and genotypes according to the melanocortin receptor gene MC4R.

Conclusions:

1. It was found that the biochemical parameters of blood serum (activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) of young pigs of large white breed of the controlled population correspond to the physiological norm of clinically healthy animals and according to the leading indicators of fattening and meat qualities (age of reaching body weight 100 kg, days; fat thickness at the level of 6-7 thoracic vertebrae, mm; length of chilled carcass, cm) belongs to class I and class elite.

2. Taking into account the interbreed differentiation of young pigs according to the MC4R gene, it was found that young pigs of the second experimental group ($MC4R^{AG}$) are superior to their peers of I ($MC4R^{AA}$) in terms of average daily weight gain, the age of reaching a body weight of 100 kg, the thickness of fat at the level of 6-7 thoracic vertebrae, and the length of the chilled carcass by an average of 4.58%.

3. The number of reliable associations between interior indicators (activity of AST, ALT), fattening, and meat qualities of young pigs of the controlled population is 28.57 %. This indicates the possibility of using these interior indicators for early prediction of fattening and meat qualities of young pigs.

4. The maximum increase in additional production was obtained from young pigs of the second experimental group ($MC4R^{AG}$**)** – +3.24 %, and its cost, which was obtained from the sale of one head of young pigs of the specified genotype, is + 261.56 UAH or + 6.62 US dollars.

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PROSPECTS OF USING COMPLEX GENOTYPES FOR BETA-CASEIN, PROLACTIN AND LEPTIN GENES IN MARKER-ASSISTED BREEDING IN DAIRY CATTLE

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The analysis of the prospects of using complex genotypes at the loci of beta-casein, prolactin and leptin in marker-assisted breeding programs of Ukrainian Black-and-White and Red-and-White dairy breeds of cattle to create experimental groups of animals producing A2 milk was carried out. Data are given on the peculiarities of the distribution of allele frequencies and genotypes at the loci of beta-casein (c.350C>A, rs43703011), prolactin (c.35333764C>T, rs211032652) and leptin (c.239C>T, rs29004508) in experimental cattle populations. It was established that in both cattle populations, the loci of beta-casein, prolactin and leptin are polymorphic according to experimental mutations. The expediency of using complex genotypes for each of the polymorphic loci for the selection of individuals of both research breeds for the purpose of use in further selection work is substantiated. The number of individuals with the "desired" complex genotypes in each population was determined as $CSN2^{A2A2}PRL^{CC}LEP^{CC}$ *for the Ukrainian Black-and-White dairy breed and* $CSN2^{A2A2}PRL^{TT}LEP^{CC}$ *for the Ukrainian Red-and-White dairy breed. According to the results of the research, it was found out that in the research group of cattle of the Ukrainian Black-and-White dairy breed, the number of individuals with the "desired" complex genotype is 10%, while in the population of the Ukrainian Red-and-White dairy breed it is 17% of the total sample (n =30). It has been proven that the minimum number of individuals required for genetic population studies (n=30, FAO) is insufficient for the effective selection of animals with the desired complex genotypes based on the identified polymorphic loci in both breeds of dairy cattle. Using the obtained data, it is proposed to carry out further marker-assisted selection in order to obtain experimental groups of A2 milk-producing animals based on the selection of individuals with the desired complex genotypes in both experimental cattle breeds using the Gene Pyramiding method. Gene Pyramiding with the markers aims to obtain individuals with the best economic traits according to the optimal breeding scheme, which involves the selection of the desired target alleles and the pyramiding of their most optimal combinations into one complex genotype.*

Key words: polymorphism, population, cattle, allele, genotype, variability.

ПЕРСПЕКТИВИ ВИКОРИСТАННЯ КОМПЛЕКСНИХ ГЕНОТИПІВ ЗА ЛОКУСАМИ БЕТА-КАЗЕЇНУ, ПРОЛАКТИНУ ТА ЛЕПТИНУ В МАРКЕР-АСОЦІЙОВАНІЙ СЕЛЕКЦІЇ У МОЛОЧНОМУ СКОТАРСТВІ

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Проведено аналіз перспективи використання комплексних генотипів за локусами бета-казеїну, пролактину та лептину в програмах маркер-асоційованій селекції української чорно-рябої та червоно-рябої молочних порід корів зі створення експериментальних груп тварин-продуцентів А2 молока. Наведено дані, стосовно особливостей розподілу частот алелей та генотипів за локусами бета-казеїну (c.350C>A, rs43703011), пролактину (c.35333764C>T, rs211032652) та лептину (c.239C>T, rs29004508) у дослідних популяціях корів. Встановлено, що в обох популяціях корів локуси бета-казеїну, пролактину та лептину за дослідними мутаціями є поліморфними. Обґрунтовано доцільність використання комплексних генотипів за кожним з поліморфних локусів для відбору особин обох дослідних порід з метою використання у подальшій селекційній роботі. Встановлено кількість особин з "бажаними" комплексними генотипами в кожній популяції – $CSN2^{A2A2}PRL^{CC}LEP^{CC}$ для української чорно-рябої молочної породи та $CSN2^{A2A2}PRL^{TT}LEP^{CC}$ для української червоно-рябої молочної породи. За результатами досліджень з'ясовано, що у дослідній групі корів української чорно-рябої молочної породи кількість особин з "бажаним" комплексним генотипом становить 10 %, в той час як в популяції української червоно-рябої молочної породи – 17 % від загальної вибірки (n=30). Доведено, що мінімальна кількість особин, необхідна для проведення генетико-популяційних досліджень (n=30, FAO) є недостатньою для ефективного відбору тварин із бажаними комплексними генотипами за виявленими поліморфними локусами в обох породах молочних корів. За використання отриманих даних запропоновано проведення подальшої маркер-асоційованої селекції з метою отримання експериментальних груп тварин-продуцентів А2 молока на основі відбору особин з бажаними комплексними генотипами в обох дослідних породах корів за використання методу Gene Pyramiding. Генний пірамідінг за допомогою маркерів має на меті отримання особин із найкращими економічними ознаками відповідно до оптимальної схеми розведення, яка передбачає вибір бажаних цільових алелів та пірамідування їх найбільш оптимальних комбінацій в один комплексний генотип.

Ключові слова: поліморфізм, популяція, корови, алель, генотип, мінливість.

Despite the clear successes of genomic selection in cattle breeding, the use of specialized types of breeding work based on marker-assisted selection is still relevant worldwide (Gutierrez-Reinoso et al., 2021; Wiggans et al., 2022).

The problems that can be solved due to the use of modern molecular genetic approaches of marker-assisted selection depend on the peculiarities of a specific region/country and, to a large extent, are determined by the specific characteristics of gene pool breeds of local selection (Mrode et al., 2019). In Ukraine, one of the most promising trends in animal husbandry is the production of "organic products", which naturally includes the so-called A2 milk. The uniqueness of this product is due to the genetic characteristics of the producers as different forms of milk, A1 and A2, differ only in the type of beta-casein contained in it (Cieślińska et al., 2019; Sebastiani et al., 2022; Jiménez-Montenegro et al., 2022). At the same time, A1 and A2 are among the most significant, but not the only, allelic forms (Antonopoulos et al., 2021). Alleles A^1 and A^2 differ from each other in the presence of a certain amino acid at position 67 of the beta-casein molecule (Sebastiani et al., 2020). In the case of the A^1 form it is histidine, for A^2 it is proline (Thiruvengadam et al., 2020). Accordingly, different forms of beta-casein are determined by a mutation in the original *CSN2* gene, namely, the presence of adenine in the case of the A^1 allele and cytosine in the case of the A^2 allele (Dai et al., 2016; Kay et al., 2021).

Based on the results of typing, it is possible to select individuals from the population with a specific desired genotype (A^2A^2) for the purpose of obtaining both products (A2 milk) and for use in breeding work (forming populations of A2 milk-producing cattle). From this point of view, Ukrainian breeding cattle populations are quite promising due to the relatively high share of the A^2 allele in the homozygous state (depends on the breed, population, farm, etc.). However, one-time selection of individuals with the A^2A^2 genotype is clearly insufficient for targeted marker-assisted selection. In this case, the genetic environment factor becomes important, which can eliminate all the advantages of obtaining the target product (A2 milk). First of all, this applies to the parameter of the value of the milk yield, which depends on a number of genes. For example, based on the results of molecular genetic examination, it is possible to create experimental populations of cattle of various breeds, which will consist entirely of individuals with the A^2A^2 genotype, which, accordingly, are A2 milk producers. But, despite all the attractiveness of the situation, productive qualities of cattle (milk productivity) can be quite variable and, in general, worse than other groups of animals. Therefore, in order to avoid such a situation, it is necessary to analyze individuals not only for the target gene of beta-casein, but also for a number of loci that belong to the "major" loci, that is, those that are associated with milk yield indicators. In this case, the phenomenon of breed specificity becomes particularly significant as for each individual breed, different genes and allelic variants can contribute to the overall milk productivity. Therefore, determining the possibility of using complex genotypes when creating herds of cattle producing A2 milk is of primary importance for the implementation of marker-assisted breeding programs. From this point of view, prolactin and leptin genes, allelic variants of which are associated with increased indicators of milk productivity of animals, are of considerable interest (along with the beta-casein gene) for Ukrainian Black-and-White and Red-and-White dairy cattle.

The prolactin gene (*PRL*) is one of the most important quantitative trait loci (QTL) in cattle breeding. It encodes protein that belongs to the regulatory hormones of the pituitary gland and takes part in a large number of physiological functions of the body (Dobolyi et al., 2020). It is a key factor in initiating and maintaining the lactation function (Knight, 2001). For the prolactin locus in cattle, a number of promising muta-

tions have been established in various functional elements of the gene (promoter, exon, and others), which, taking into account the physiological value of prolactin as a hormone-regulator of lactation activity, makes it promising for research specifically in dairy cattle breeding (Shah et al., 2021).

The leptin gene (*LEP*) encodes a protein that is directly related to the regulation of fat and energy metabolism. It refers, first of all, to markers of meat productivity of animals (Wang et al., 2020). In addition, allelic variants (for example, A59V mutation) were identified, which are associated with parameters of milk productivity of cattle, as well as with the content of somatic cells in milk (Yazdani et al., 2010; Maletić et al., 2019; Mandefro et al., 2021).

It should be outlined that it is actually impossible to determine all possible variations of complex genotypes based on a large number of loci, due to the unlimited number of variants and the limited number of individuals of this breed in the farm. Therefore, we chose such objects, which have an associative relationship with milk productivity of indicators of Ukrainian Red-and-White and Black-and-White cattle was established by us in previous studies (Kulibaba et al., 2019, 2021).

The purpose of the study. The purpose of the research is to determine the complex genotypes at the loci of beta-casein, prolactin and leptin in the populations of Ukrainian Black-and-White and Red-and-White dairy cattle with further analysis of the possibility of their use in marker-assisted breeding programs to create experimental groups of cattle producing A2 milk .

Research materials and methods. The research was carried out in the laboratory of molecular genetic and physiological and biochemical research in animal husbandry of the Institute of Animal Husbandry of the National Academy of Sciences and in the laboratory of molecular genetic research of the Department of Animal Biology of the National University of Life and Environmental Sciences of Ukraine.

Cattle of the Ukrainian Black-and-White dairy breed (n=30) (SE RH "Hontarivka") and Ukrainian Red-and-White dairy breed (n=30) (SE RH "Hontarivka") were used as the object of research.

Polymorphism of beta-casein, prolactin and leptin loci was determined by mutations of *CSN2* (c.350C>A, rs43703011), *PRL* (c.35333764 C>T, rs211032652) and *LEP* (c.239C>T, rs29004508).

Polymorphism of the beta-casein locus was determined using the allele-specific PCR method according to the algorithm previously presented in the authors' publication (Kulibaba et al., 2023). Polymorphism of the prolactin and leptin loci was determined using the PCR-RFLP method (PCR with restriction analysis).

Appropriate primers were used to amplify experimental genome fragments: for *CSN2* – GCCCAGATGAGAGAAGTGAGG, GATGTTTTGTGGGAGGCTGTTAT and GATGTTTTGTGGGAGGCTGTTAG (Keating et al., 2008); for *PRL* – GTTCTTGCTTTATGTAACACCG and TAGGTCAATCACTCTGAGCA (Kulibaba et al., 2019); for *LEP* – GGGAAGGGCAGAAAGATAG and TGGCAGACTGTTGAGGATC (Haegeman et al., 2000).

In the case of the PCR-RFLP method, appropriate restriction endonucleases were used: RsaI for *PRL* and HphI for *LEP*.

Electrophoretic distribution of amplification/restriction fragments was performed using an agarose gel (1.5%) with ethidium bromide staining. The size of the DNA fragments on the electrophoregram was determined using the GeneRuler 50 bp molecular mass marker (Thermo Fisher Scientific).

Based on the results of genotyping, the main genetic and population indicators were determined: frequencies of alleles and genotypes, the ratio of observed (H_0) and

expected (H_e) heterozygosity values, Wright's fixation index (F_{is}). Research indicators were determined using the GENALEX version 6.5 software package in the Excel 2019 environment (Smouse et al., 2017).

Research results. According to the results of the research, the *CSN2*, *PRL*, and *LEP* loci were found to be polymorphic in each of the certified breeds as individuals with all possible genotypes are present in both populations.

Typing of individuals by experimental loci was carried out using the analysis of the obtained electrophoregrams. Examples of electrophoregrams and features of polymorphism for each of the loci were described in detail by the authors in previous studies (Kulibaba et al., 2019, 2021, 2023)

Beta-casein polymorphism was determined using the allele-specific PCR method. For each allele, there are corresponding amplified fragments, 854 bp in size, on the electrophoregram. The presence of a fragment only for the corresponding allele (A^1 or A^2) indicates the homozygous genotype A^1A^1 or A^2A^2. The presence of both fragments indicates a heterozygous A^1A^2 genotype.

In the case of the prolactin locus, the presence of restriction fragments of size 360 and 56 bp indicates the CC genotype (Rsal-/Rsal-), while the presence of fragments 195, 165, and 56 indicates the TT genotype (Rsal+/Rsal+). The CT heterozygous genotype (Rsal+/Rsal-) is represented by a combination of the above variants – 360, 195, 165 and 56 bp. It should be outlined that each of the PRL alleles additionally contains a monomorphic restriction site for RsaI.

At the leptin locus, the presence of restriction fragments with sizes of 311 and 20 bp indicates the TT genotype (HphI+/HphI+); the presence of one fragment in 331 bp – the CC genotype (HphI-/HphI-). CT heterozygotes (HphI+/HphI-) are presented as fragments 331, 311 and 20 bp.

The frequency values of genotypes and alleles for each of the experimental loci in both breeds of cattle are given in the table.

Table

Values of frequencies of genotypes and alleles at the CSN2, PRL and LEP loci in experimental cattle populations

Locus	Ukrainian Black-and-White dairy breed		Ukrainian Red-and-White dairy breed	
	Genotype frequencies	Allele frequencies	Genotype frequencies	Allele frequencies
CSN2	A^1A^1 – 0.37; A^1A^2 – 0.43; A^2A^2 – 0.20.	A^1 – 0.58; A^2 – 0.42.	A^1A^1 – 0.10; A^1A^2 – 0.57; A^2A^2 – 0.33.	A^1 – 0.38; A^2 – 0.62.
PRL	CC – 0.60; CT – 0.30; TT – 0.10.	C – 0.75; T – 0.25.	CC – 0.46; CT – 0.27; TT – 0.27.	C – 0.60; T – 0.40.
LEP	CC – 0.60; CT – 0.23; TT – 0.17.	C – 0.72; T – 0.28.	CC – 0.47; CT – 0.43; TT – 0.10.	C – 0.68; T – 0.32.

The research results (features of the genetic structure of cattle populations of both breeds) fully correspond to the results obtained earlier (Kulibaba et al., 2019, 2021). No significant changes in genetic and population parameters were found.

Discussion. It should be noted that at the first stage, the goal of our research was to determine the genetic and population parameters in order to analyze the polymorphism of each of the genes in the experimental groups of cattle. That is why, according to FAO recommendations, we used only 30 individuals of each breed for genotyping, as this number is minimal for research. On the basis of the obtained results, an analysis of the possibility of using complex genotypes for the needs of marker-assisted selection was carried out in terms of the perspective of this approach in MAS programs for obtaining herds of cattle producing A2 milk.

According to the results of earlier studies, the formula of promising complex genotypes for the loci of beta-casein, prolactin and leptin for cattle of the Ukrainian Black-and-White dairy breed has the following form $CSN2^{A2A2}PRL^{CC}LEP^{CC}$. In this formula, the $CSN2^{A2A2}$ genotype gives the possibility of obtaining milk that contains only the A2 form of beta-casein (that is, A2 milk directly). In turn, individuals with the PRL^{CC} genotype show higher milk yield values within several lactations compared to individuals with other genotypes (PRL^{CT} and PRL^{TT}). At the same time, the difference in values between individuals with different genotypes can reach 16%. According to the leptin locus, individuals with a genotype homozygous for the C allele are characterized by an increased value of breast milk, up to 19%, which is most denoted during the first lactation.

As we can see, all variants of the loci in the complex genotype formula are in the homozygous state. According to the results of this formula, the animals are milk producers of type A2, while they are characterized by higher milk yield values for different lactations compared to individuals with other genotypes for these loci. The main question, in this context, is whether it is possible to select individuals that meet the formula in the research population of animals? Is the standard sample of 30 heads, which according to FAO recommendations is the minimum for describing genetic-population parameters, sufficient, or should a larger sample be analyzed?

To provide an answer to the above questions, we will carefully consider the results of the analysis of the experimental population of cattle of the Ukrainian Black-and-White dairy breed (n=30), which are listed in the table. As we can see, the limiting factor for selection is the number of individuals with the A^2A^2 genotype at the beta-casein locus (6 heads), provided that a group of A2 milk-producing animals is created. At the same time, the number of individuals with the desired genotype at the prolactin and leptin locus is significantly larger and, in fact, the same (18). Therefore, the minimum value of the number of individuals homozygous for the A^2 allele at the beta-casein locus is a limiting factor for any options for selecting animals from this sample. In addition to everything, the research loci (*CSN2*, *PRL* and *LEP*) are located on different chromosomes (beta-casein gene – on 6; prolactin gene – on 23; leptin gene – on 4, respectively), which makes it impractical to conduct haplotype analysis in contrast to mutations, contained within one locus. This imposes certain restrictions on the possibility of using combinations of genotypes in the form of a single functional unit, which, at the same time, is leveled by the homozygous state of each of the loci in the formula of the desired complex genotypes for the research cattle breeds.

According to the results of typing individuals of the experimental group of animals of the Black-and-White dairy breed with the complex desired genotype $CSN2^{A2A2}PRL^{CC}LEP^{CC}$, only 3 heads were found. Other individuals with the $CSN2^{A2A2}$ homozygous genotype were characterized by the presence of a heterozygous genotype at the prolactin locus and a homozygous/heterozygous TT/CT genotype at the leptin locus ($CSN2^{A2A2}PRL^{CT}LEP^{TT}$ or $CSN2^{A2A2}PRL^{CT}LEP^{CT}$). Thus, under the condition of the established distribution of genotypes by three loci, when analyzing 30 heads of cattle of

the Ukrainian Black-and-White milk breed, the proportion of animals with the desired complex genotype, which can be selected for further breeding work, is only 10%. It should also be noted that it is possible to analyze individuals for further selection for the purpose of creating herds of A2 milk-producing cattle only after bonification (i.e. breeding core), which imposes additional restrictions.

Considering cattle of the Ukrainian Red-and-White dairy breed, the situation is slightly different, which is associated with certain corrections that arise as a result of the peculiarities of the distribution of genotype and allele frequencies by the studied loci (table). It should be noted that in the case of the Ukrainian Red-and-White dairy breed, the formula of the desired complex genotypes has the following form $CSN2^{A2A2}PRL^{TT}LEP^{CC}$. The main difference concerns the TT genotype at the prolactin locus. For the experimental breed of cattle, according to the results of research, the predominance of individuals with the PRL^{TT} genotype compared to PRL^{CT} and PRL^{CC} (which can reach 12%) was established, which led to a change in the formula. In turn, by locus of leptin, as in the case of the Ukrainian Black-and-White dairy breed, CC is the preferred genotype. Individuals with this genotype demonstrate increased values of the standard milk parameter compared to individuals with other genotypes during three lactations (the difference between the indicators of individuals with different genotypes reaches almost 20%). Due to the peculiarities of the distribution of allele frequencies and genotypes, the limiting factor for this breed of cattle is the TT genotype at the prolactin locus. The number of individuals with this genotype is 8 heads. At the same time, the number of individuals with the $CSN2^{A2A2}$ genotype was 10 heads.

According to the results of the analysis, the number of individuals of the Ukrainian Red-and-White dairy breed with the desired complex genotype $CSN2^{A2A2}PRL^{TT}LEP^{CC}$ was 5 heads. Other individuals (with a homozygous genotype for the A^2 allele of the beta-casein locus) were characterized by complex genotypes $CSN2^{A2A2}PRL^{CT}LEP^{CC}$, $CSN2^{A2A2}PRL^{CC}LEP^{CC}$, $CSN2^{A2A2}PRL^{CC}LEP^{CT}$ and $CSN2^{A2A2}PRL^{CT}LEP^{CT}$. Thus, the share of individuals of the Ukrainian Red-and-White dairy breed, which were characterized by the desired complex genotype and are suitable for creating a group of animals producing milk A2, was almost 17% (5 heads).

In a comparative aspect, the selection of individuals with the desired complex genotype by the number of animals is more effective in the population of cattle of the Ukrainian Red-and-White dairy breed (practically twice the number of individuals) in comparison with Black-and-White cattle. However, in any case, for the effective formation of a breeding nucleus, it is necessary to significantly increase the number of individuals for genotyping by experimental loci. The minimum number of individuals (n=30) for genetic population studies is sufficient only to determine the characteristics and ratio of frequencies of alleles and genotypes, but, especially under the condition of increasing the number of research loci, but insufficient for the selection of animals according to complex genotypes.

It should be determined that increasing the number of components of the formula of the desired complex genotype significantly complicates the task, as it leads to an increase in the number of options and, accordingly, to a decrease in the number of individuals with the desired genotype under the condition of a limited sample. We may give an example for a system consisting of two alleles per locus. If individuals are selected based on the results of typing at one locus (A^1 or A^2 allele of beta-casein), there will be 3 different options; for two loci (*CSN2* and *PRL*) – already 9 variants; for three (*CSN2*, *PRL* and *LEP*) – 27 variants of complex genotypes. Accordingly, under the condition of an even distribution of individuals with different complex genotypes in the sample, only 3-4 individuals with the desired complex genotypes can be selected from a population

consisting of 100 individuals. It is quite clear that the peculiarities of the genetic structure of different cattle populations (the ratio of genotype frequencies within groups) lead to certain variations in the number of individuals with complex genotypes, but, in any case, for the selection of individuals, it is necessary to increase the number of analyzed animals.

In addition to all of the above, the need to conduct a primary analysis of the genetic population structure of experimental groups of animals should be noted as an integral component of the general concept of marker-assisted selection, which we have repeatedly emphasized in many publications. Knowledge of the features of the genetic structure (features of the organization of genetic variability in the population) of experimental animal populations makes it possible to predict the prospects of selection at the level of complex genotypes and serves as a foundation for further marker-assisted selection (MAS). This especially applies to the analysis of variations that are not located within the same locus. Taking into account that each of the studied loci (*CSN2*, *PRL*, *LEP*) is in different parts of the animal genome (in different chromosomes), it does not make sense to perform haplotype analysis (as it happens when studying different mutations within the same locus), therefore determination of complex genotypes is, in fact, an alternative option for analysis.

The use of complex genotypes to create herds of A2 milk-producing cattle is also promising for the application of methodological approaches of the Gene Pyramiding system, which, in the context of the conducted research, can become an integral component of the general strategy of MAS – Marker-assisted Gene Pyramiding (Xu et al., 2012). When using such a system, it is necessary to carry out genotyping not only of cattle, but also of breeders according to experimental loci and, in the future, to use only sperm with the specified genotype to obtain offspring of the desired type. Thus, the required number of individuals can be obtained during several generations. It is the combination of different methodological approaches that is the most effective strategy for obtaining high-quality livestock products (A2 milk) against the background of the absence of potential risks of reducing the parameters of total milk productivity due to the influence of "unwanted" alleles of other loci (in the presented work, these are loci of prolactin and leptin).

Conclusions.

1. Based on the results of the research, it was established that the loci of beta-casein (c.350C>A, rs43703011), prolactin (c.35333764 C>T, rs211032652) and leptin (c.239C>T, rs29004508) are polymorphic according to marker mutations in research populations of Ukrainian Black-and-White and Red-and-White dairy cattle.

2. The number of individuals with the "desired" complex genotype $CSN2^{A2A2}PRL^{CC}LEP^{CC}$ in the cattle population of the Ukrainian Black-and-White dairy breed is 10%, while the number of individuals with the "desirable" complex genotype $CSN2^{A2A2}PRL^{TT}LEP^{CC}$ in the cattle population of the Ukrainian Red-and-White dairy breed is 17%.

3. It has been proven that for the effective selection of individuals with the desired complex genotypes based on the identified polymorphic loci in both breeds of dairy cattle, the use of the minimum number of individuals (n=30) is insufficient.

4. For the effective creation of herds of A2 milk-producing cattle, it is recommended to use the proposed complex genotypes for both experimental breeds of cattle using the methodical approaches of the Gene Pyramiding – Marker-assisted Gene Pyramiding system.

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LIPID PEROXIDATION AND THE TOTAL ANTIOXIDANT CAPACITY IN THE PLASMA OF SHETLAND PONY MARES AND STALLIONS INVOLVED IN RECREATIONAL HORSEBACK RIDING: ROLE OF PHOTOPERIOD AND EXERCISE

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This study focuses on the photoperiod-induced variability in the levels of biomarkers of lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS) and the total antioxidant capacity (TAC) in the blood of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC in the blood of Shetland pony mares and stallions involved in recreational horseback riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland). Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years old were used in this study. All horses participated in recreational horseback riding. Training started at 10:00 AM, lasted 1 hour, and consisted of a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min). Blood was drawn from the jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM), and immediately after the exercise test (between 11 AM and 12 AM). Blood samples were taken once per season for one year: summer and winter. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) for determining the malonic dialdehyde (MDA) concentration. The TAC level in samples was estimated by measuring the TBARS level after Tween 80 oxidation. In the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod). The results of the current study demonstrated that changes in the lipid peroxidation and the total antioxidant capacity in the blood of Shetland ponies are photoperiod-, sex-, and exercise-dependent. The maximum statistically significant values for the effect of the photoperiod-exercise combination defined as the F values were observed. The multivariate analysis of the influence of the three parameters: photoperiods, sex, and exercise on the lipid peroxidation and the total antioxidant

capacity in the blood of the ponies indicated the leading role mainly of the photoperiod factor, and next the exercise and sex factors.

Key words: 2-thiobarbituric acid reactive substances, total antioxidant capacity, plasma, exercise, seasonal alterations, photoperiods, Shetland ponies, mares and stallions.

ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ І ЗАГАЛЬНА АНТИОКСИДАНТНА АКТИВНІСТЬ У ПЛАЗМІ КОБИЛ І ЖЕРЕБЦІВ ШЕТЛАНДСЬКИХ ПОНІ, ЯКІ БЕРУТЬ УЧАСТЬ У РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ: РОЛЬ ФОТОПЕРІОДУ ТА ФІЗИЧНОГО ТРЕНІНГУ

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У цьому дослідженні ми зосередилися на вивченні індукованих фотоперіодом змінах рівнів біомаркерів перекисного окиснення ліпідів (реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою, TBARS) і загальної антиоксидантної активності (TAC) у плазмі кобил і жеребців шетландських поні до і після тренування. Ми проаналізували вплив фотоперіоду і фізичних вправ на рівні TBARS і TAC у крові кобил і жеребців шетландських поні, які беруть участь у рекреаційній верховій їзді в центральній частині Поморського регіону (Поморське воєводство, північна частина Польщі). Двадцять один здоровий дорослий шетландський поні (11 кобил і 10 жеребців) віком 6,5 ± 1,4 років були використані в цьому дослідженні. Усі коні брали участь у рекреаційній верховій їзді. Тренування розпочиналося о 10:00, тривало 1 годину і складалося з кросу ходьбою (5 хв), риссю (15 хв), ходьбою (10 хв), риссю (10 хв), ходьбою (5 хв), галопом (5 хв) і ходьбою (10 хв). Кров брали з яремної вени тварин вранці, через 90 хвилин після годування, під час перебування коней у стайні (між 8:30 та 10 ранку) та відразу після тесту з фізичним навантаженням (між 11 ранку та 12 ранку). Забір крові проводили один раз за сезон протягом року: влітку та взимку. Рівень перекисного окиснення ліпідів визначали кількісно за допомогою ТБК-активних речовин (TBARS), зокрема малонового діальдегіду (MDA). Рівень TAC у зразках оцінювали шляхом вимірювання рівня TBARS після окиснення Tween 80. Як показали результати наших досліджень, у жеребців і кобил перед навантаженням (у стані спокою) в літній фотоперіод ми отримали мінімальні значення TBARS, а максимальні – у зимовий фотоперіод. У нашому дослідженні рівень TAC ілюстрував базові рівні ферментативних і неферментативних систем антиоксидантного захисту, які відрізнялися у кобил, ніж у жеребців (статистично значущі в зимовий фотоперіод) як до, так і після фізичного навантаження. Результати поточного дослідження показали, що зміни перекисного окиснення ліпідів і загальної антиоксидантної активності в крові шетландських поні залежать від фотоперіоду, статі та фізичних вправ. Спостерігалися максимальні статистично значущі

значення ефекту комбінації фотоперіод-вправи, визначені як значення F. Багатофакторний аналіз впливу трьох параметрів: фотоперіодів, статі та фізичного навантаження на перекисне окиснення ліпідів і загальну антиоксидантну активність у крові поні вказав на провідну роль переважно фактора фотоперіоду, в порівнянні до таких факторів, як фізичне навантаження та стать.

Ключові слова: реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою (TBARS), загальна антиоксидантна активність (TAC), плазма, фізичні навантаження, сезонні зміни, фотоперіод, Шетландські поні, кобили та лошаки.

The adaptation of a living organism, forming in the process of natural selection, as well as in the background of the natural change of the seasons, with a regularly repeated change in climate, and food conditions, does not manifest itself in the same way as an experimental adaptation to cold (Ebi, K. L., & Rocklöv, J., 2014). Seasonal adaptation occurs in the background of preliminary, advanced preparation of animals (storage of feed, fattening before the crucial season, migration, change of coat, etc.) to difficult conditions (cold, decrease of feed, etc.) with gradual adaptation to them, which cannot be the case with experimental adaptation to cold, since it is almost impossible to create real environmental conditions (Gaughan J. B. et al., 2018; Young B. A., 1981). Thermoregulation in horses primarily depends on heat loss through evaporation, in particular through sweating. Under thermoneutral conditions, these mechanisms are sufficient to enable horses to perform the high-intensity exercise for extended periods of time. Under conditions of thermal stress, especially in high-humidity environments, the efficiency of evaporative heat loss mechanisms is reduced, which can lead to the development of hyperthermia in horses (Guthrie A. J. & Lund R. J. 1998).

The influence of environmental factors is especially susceptible to animals kept in the open when grazing (Schieltz J. M. & Rubenstein D. I. 2016). Meanwhile, knowledge of the biological characteristics of individual breeds of farm animals makes it possible to establish the critical moments of their life, depending on the specific conditions of the external environment. The habitat of farm animals throughout the year is not the same. It is highly susceptible to changes in the seasons of the year (especially when they are kept on pasture all year round) and thus affects the biological needs of animals for feed (energy) at the conditions of keeping. By targeted satisfaction of the physiological needs of the body in certain periods of the season, it is possible to reduce the negative impact of environmental factors and achieve high productive qualities in animals, as well as improve health (Wróbel B. et al.,. 2023)

In our previous study, we also revealed that the seasonal variations in the antioxidant defences, as well as substrates of energy metabolism in the blood of mares and stallions, depending on exercise capacity, could be an important aspect in the ability of endogenous adaptive mechanisms of animals to react in advance to environmental changes associated with seasons (Kurhaluk N. et al., 2022). The influence of seasons and sex on body size and hematological and biochemistry parameters of Noma horses, a native Japanese breed was evaluated by Ono and co-workers (2021). Body size was larger in winter than in summer. Laboratory testing variables, including erythrocytic parameters and urea nitrogen, total cholesterol, and creatinine kinase levels, were higher in winter, while the eosinophil count was higher in summer. These seasonal differences may be related to the increased energy consumption of horses due to heat stress. The higher eosinophil counts may have been related to dermatitis observed in the summer. Stallions tended to have smaller bodies compared with mares (Ono T. et al., 2021).

In the mechanisms of adaptation of the body to free radical lipid peroxidation and the development of oxidative stress at the molecular level, a special place is given. Unlike conventional molecules, molecules and atoms of free radicals (FRs) have one or more electrons with unpaired spin in their outer energy orbital and, therefore, are highly reactive (Jakubczyk K. et al., 2020). FR production in a healthy organism is carried out in small amounts during physiological processes (phagocytosis, oxidation of arachidonic acid, prostaglandin synthesis, mitochondrial respiration, and peroxisomal oxidation) (Di Meo S. et al., 2016). Many enzymatic and non-enzymatic redox processes are known, the products of which are various types of FR, the largest number is formed from oxygen (Pham-Huy, L. A. et al., 2008). Molecular oxygen in the basic state is a biradical that has two unpaired electrons in atomic orbitals. Less than 5% of molecular oxygen undergoes a one-electron reduction in mitochondria and microsomes of cells, especially phagocytes. These reactions result in superoxide anion radical ($\cdot O_2^-$), singlet form of oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and hydroperoxyl radical (HO_2^-) (Ignatowicz, E., & Rybczyńska, M. 1994). The general name of these products is reactive oxygen species (ROS), which have the ability to react with endogenous substrates that form cell structures, and primarily with phospholipid biomembranes (Brieger K. et al., 2012). The consequence of such reactions is the destruction of cellular components, including proteins (with inactivation of enzymes, especially those containing sulfhydryl groups), membrane lipids (with the formation of lipid peroxides from polyunsaturated fatty acids, PUFAs), and nucleic acids (with the formation of breakdowns and mutations) (Ward J. P. T., 2017). Thus, ROS are highly reactive radical oxygen compounds formed as a result of incomplete oxygen reduction.

The entry of aggressive FR into reactions with PUFAs of phospholipids with the formation of hydroperoxides occurs regardless of the cause that caused the increase in ROS levels. The cascade of lipid peroxidation reactions leads to the appearance and accumulation of a wide range of toxic metabolites, the tension of antioxidant defense mechanisms, and the development of oxidative stress (Ayala A. et al., 2014). At a certain intensity, lipid peroxidation (LPO) is a normal physiological process involved in the self-renewal and restructuring of membrane structures, regulation of ion transport, and changes in the activity of membrane-binding enzymes (Spiteller, G., 2003). LPO processes play the role of a necessary link in the biosynthesis of prostaglandins, steroid hormones, and leukotrienes, as well as in the processes of phagocytosis (Ricciotti E., & FitzGerald G. A., 2011). Excessive LPO activation plays an important and sometimes key role in the development of many pathological conditions, which makes it justified to consider this process as a universal typical link in the pathogenesis of various pathological conditions. The action of external prooxidants and the activation of endogenous mechanisms of ROS generation lead to the tension of antioxidant defense mechanisms and the development of oxidative stress, an important pathogenetic factor in many pathological processes and conditions that can manifest itself at the cellular, tissue, and organism levels. LPO and its products, acting as a "primary mediator" of stress represent one of the earliest regulatory mechanisms and are a non-specific pathogenetic link in the development of various diseases (Ayala A. et al., 2014)..

This study focuses on the photoperiod-induced variability in the levels of biomarkers of lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS) and the total antioxidant capacity (TAC) in the blood of Shetland pony mares and stallions before and after exercise. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC in the blood of Shetland pony mares and stallions involved in recreational horseback riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland).

Materials and methods.

Horses. The experiments were conducted in compliance with the Guidelines of the European Union Council and the current laws. Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged 6.5 ± 1.4 years old from the central Pomeranian region in Poland (Strzelinko, N54º30'48.0" E16º57'44.9") were used in this study. All horses participated in recreational horseback riding. The horses were housed in individual boxes, with feeding (hay and oat) provided twice a day at 08.00 and 18.00 h and water available ad libitum. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Exercise. Training started at 10:00 AM, lasted 1 hour, and consisted of a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min).

Blood samples. Blood was drawn from the jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM), and immediately after the exercise test (between 11 AM and 12 AM). Blood samples were taken once per season for one year: summer and winter. Blood was stored in tubes with K_3-EDTA and 3.8% sodium citrate and kept on the ice until centrifugation at 3,000 rpm for 10 minutes. The plasma was removed. The erythrocyte suspensions (one volume) were washed with five volumes of PBS (pH 7.35) three times and centrifuged at 3,000 rpm for 5 minutes.

The 2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration (Kamyshnikov V. S. (2004). This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as the extinction coefficient.

Measurement of total antioxidant capacity (TAC). The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova L. P. et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis. The results were expressed as mean ± S.D. Significant differences between the means were measured using a multiple-range test at min. $p < 0.05$. Data with no normal distribution were log-transformed. Statistical tests with 95% confidence intervals ($\alpha = 0.05$) were applied to determine the significance of differences between the parameters studied (Stanisz A. 2006; Zar J. H., 1999). The data were tested for homogeneity of variance using Levene's test, and normality was checked with the Kolmogorov-Smirnov test. The data of MANOVA analysis were also confirmed by the results of the sum-of-squares test (total SS model) *vs*. residual SS regarding the values of the multiple correlation analysis (R), the coefficient of determination (R^2), and its corrected form reduced by random errors (R^2 adjusted) in the data analysis. The basic statistical analysis (significance of regression slopes, analysis of variance for significance) was done using the STATISTICA 13.3 package (TIBCO Software Inc.). We used the SS test to describe the share of all analyzed biomarkers of oxidative stress (the F test and its significance) (Stanisz A. 2006; Zar J. H., 1999).

Results. We investigated the levels of TBARS and TAC in the blood of the Shetland ponies influenced by the three factors (photoperiods, sex, and exercise). Levels of TBARS and TAC in the blood of Shetland pony mares and stallions before and after exercise in summer and winter are presented in Fig. 1.

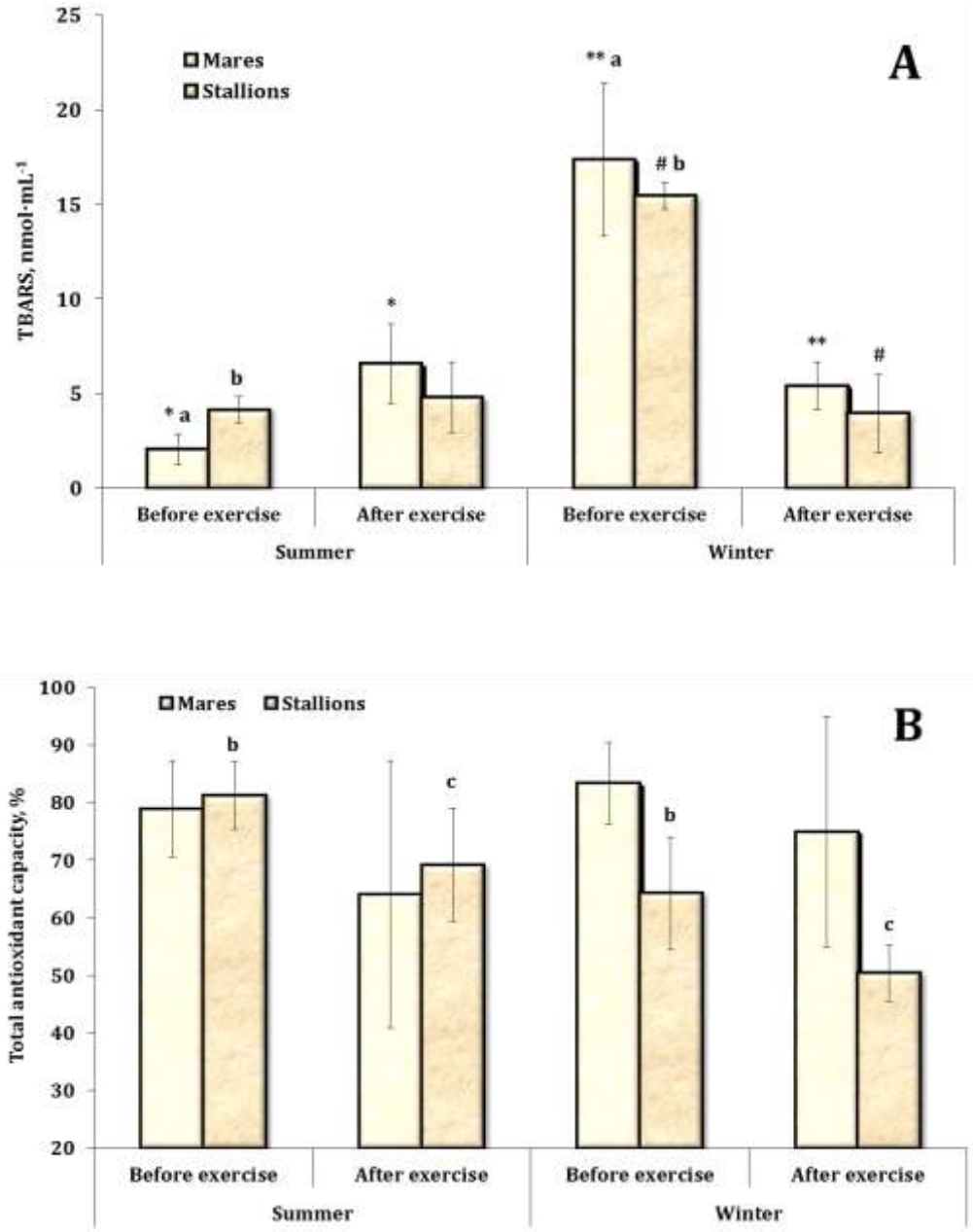

Fig. 1. Levels of 2-thiobarbituric acid reactive substances (TBARS, nmol·mL^{-1}) (A) and total antioxidant capacity (TAC, %) (B) in the blood of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in summer and winter.

Statistically significant differences ($p < 0.05$) in the following dependency groups according to the ANOVA post-hoc Tukey (HSD) test:

** – between values obtained before and after exercise in mares in summer;*

*** – between values obtained before and after exercise in mares in winter;*

 # – between values obtained before and after exercise in stallions in winter;

a – between values obtained in mares before exercise in winter and summer;

b – between values obtained in stallions before exercise in winter and summer;

c – between values obtained in stallions after exercise in winter and summer.

Results of our study revealed that levels of TBARS in the plasma of Shetland pony mares in summer were statistically significantly increased after exercise to (6.58 ± 2.09 nmol·mL^{-1}) compared to the state before exercise (2.05 ± 0.81 nmol·mL^{-1}). On the other hand, in stallions, a statistically non-significant increase in levels of TBARS in the plasma after exercise (4.81 ± 1.85 nmol·mL^{-1}) compared to the state before exercise (4.15 ± 0.70 nmol·mL^{-1}) was observed. The percentage of increase was 221% ($p < 0.05$) for mares and 15.91% ($p > 0.05$) for stallions, respectively. Contrarily, in the winter, levels of TBARS in the plasma of both mares and stallions of Shetland ponies were statistically significantly decreased after exercise to (5.41 ± 1.28 nmol·mL^{-1}) and (3.96 ± 2.04 nmol·mL^{-1}) compared to the state before exercise (17.37 ± 4.05 nmol·mL^{-1}) and (15.46 ± 0.71 nmol·mL^{-1}), respectively. The percentage of decrease was 68.86% ($p < 0.05$) for mares and 74.39% ($p < 0.05$) for stallions (Fig. 1A).

After exercise, levels of TBARS in the plasma of both mares and stallions of Shetland ponies in summer were similar to the values obtained in the winter. Before exercise, levels of TBARS in the plasma of both mares and stallions of Shetland ponies in winter were higher [(17.37 ± 4.05 nmol·mL^{-1}) and (15.46 ± 0.71 nmol·mL^{-1})] compared to the values obtained in the summer, i.e. (2.05 ± 0.81 nmol·mL^{-1}) for mares and (4.15 ± 0.70 nmol·mL^{-1}) for stallions. The percentage of decrease was 88.2% ($p < 0.05$) for mares and 73.16% ($p < 0.05$) for stallions (Fig. 1A).

Levels of the total antioxidant capacity in the plasma of both mares and stallions of Shetland ponies in summer were decreased after exercise to (64.06 ± 23.26 %) and (69.22 ± 9.8 %) compared to the state before exercise (78.89 ± 8.35 %) and (81.26 ± 5.82 %), respectively. The percentage of decrease was 18.8% ($p > 0.05$) for mares and 14.82% ($p > 0.05$) for stallions, respectively. Similarly, in the winter, TAC levels in the plasma of both mares and stallions of Shetland ponies were statistically non-significantly decreased after exercise to (74.94 ± 20.03 %) and (50.40 ± 4.87 %) compared to the state before exercise (83.37 ± 7.06 %) and (64.27 ± 9.82 %), respectively. The percentage of decrease was 4% ($p > 0.05$) for stallions. The percentage of decrease was 10.12% ($p > 0.05$) for mares and 21.58% ($p > 0.05$) for stallions, respectively (Fig. 1B).

Before exercise, TAC levels in the plasma of stallions of Shetland ponies in summer were higher (81.26 ± 5.82 %) compared to the values obtained in the winter (64.27 ± 9.82 %). The percentage of decrease was 20.91% ($p < 0.05$). TAC levels in the plasma of mares of Shetland ponies in summer were at the same levels obtained in the winter. After exercise, TAC levels in the plasma of stallions of Shetland ponies in winter were less (50.40 ± 4.87 %) compared to the values obtained in the summer, i.e. (69.22 ± 9.8 %). The percentage of decrease was 27.18% ($p < 0.05$) (Fig. 1B).

Discussion. The results of our study revealed that in the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod). The next step of our study was to determine statistically significant differences between the values of oxidative stress biomarkers of the maintenance of homeostatic mechanisms of antioxidant defenses in the blood in Shetland ponies depending on the photoperiod, sex, and exercise. TBARS levels in the blood of these horses were shown to differ statistically significantly with a maximum coefficient of variability of $F_{15.152} = 38.47$ ($p = 0.000$). These data were also confirmed by the results of the sum-of-squares test (total SS model) *vs.* residual SS regarding the values of the correlation coefficient

(R = 0.89), the coefficient of determination (R^2 = 0.79), and its adjusted form R^2_{ad} = 0.77 at the value of F = 38.47 (p = 0.000). The plasma of the stallions and mares before exercise (at rest) exhibited minimum TBARS levels in the summer photoperiods and maximum values of the parameter in the winter photoperiods. The ANOVA post-hoc Tukey test (HSD) revealed statistically significant differences in the TBARS levels between the groups in the different photoperiods, depending on the sex and exercise. Before exercise (at rest) for a group of pony stallions, these dependencies were significant for summer-winter photoperiod ($p < 0.05$).

The total antioxidant capacity (TAC) was statistically significantly correlated with the sex, photoperiods, and exercise of the Shetland ponies in our study. The changes in this parameter are probably related to the baseline levels of enzymatic and non-enzymatic antioxidant defense systems in ponies, which are different in mares compared to stallions (statistically significant in the winter period) both before and after exercise (winter period). The TAC data differed statistically significantly ($F_{15.152}$ = 10.71; p = 0.000) in the total SS model vs. residual SS regarding the values of the correlation coefficient (R = 0.71), the coefficient of determination (R^2 = 0.50), and its adjusted form R^2_{ad} = 0.45 at the value of F = 10.06 (p = 0.000).

Various studies on the gradual adaptation of melatonin rhythms to the photoperiod in humans and rodents have been reported in the literature. Murphy and co-workers (2011) revealed that melatonin rhythms in horses may be dependent on light to a greater extent. The authors believe that melatonin is not a suitable marker of the circadian phase in this species, because melatonin biosynthesis in the pineal glands of some ungulates is largely determined by the ambient light cycle, and the circadian oscillator located in the mammalian hypothalamus suprachiasmatic nucleus (SCN) has a minor role in this process (Murphy B. A. et al., 2011). The importance of the hypothalamus SCN, i.e. the site of the main mammalian pacemaker (Murphy, B. A., et al., 2007), has been previously shown in a number of studies. The SCN controls the circadian rhythms of various physiological and behavioral phenomena such as rest and activity cycles, hormone secretion, and body temperature (Nagy, P., et al., 2011). The role of the circadian system is to provide endogenous mechanisms to optimize the survival and adaptation processes. This is important for the formation of anticipatory pathways of adaptive activity of physiological functions. Murphy and co-workers (2007) suggested that the rapid resynchronization of the melatonin rhythm and the central circadian pacing of the horse may exhibit a particularly strong response to restructuring (Murphy, B. A., et al., 2007).

An important role in the physiological effects of melatonin is assigned to its antioxidant properties, as convincingly demonstrated in recent studies (Kurhaluk N., 2021; Kurhaluk N. & Tkachenko H. 2020; Tordjman S. et al., 2017). Possibly, the seasonal dominants of melatonin release help to explain the statistically significant effects of the decrease in TBARS levels in the plasma of the Shetland ponies (Fig. 1). These data are also confirmed by the results of the TBARS levels in the sum-of-squares test (total SS model) vs. residual SS regarding the values of the correlation coefficient (R = 0.89), the coefficient of determination (R^2 = 0.79), and its adjusted form R^2_{ad} = 0.77 at the value of F = 38.47 (p = 0.000). This may suggest higher statistical correlations between these processes in our study. Thus, before exercise (at rest), the plasma of the stallions and mares exhibited minimum TBARS levels in the summer photoperiod and maximum values in the winter photoperiod (Fig. 1).

Schrammel and co-workers (2016) analyzed the effects of artificially long days on rectal temperature, heart rate, heart rate variability, hematology, coat changes, semen parameters, and plasma testosterone concentrations in Shetland stallions stabled overnight and assigned to a control group (CON, n = 9) kept under natural photoperiod, and

a treatment group exposed to a long-day light program from 15 December to 20 March (AL, n = 9). During the 8-month study, rectal temperature, heart rate, and heart rate variability at no time differed between groups. Plasma total protein, hematocrit, leukocyte, and lymphocyte counts first increased and then decreased during the study period but did not differ between groups. Length of the guard hair decreased over time and this decrease occurred earlier in AL than in CON stallions. Hair regrowth was faster in CON than in AL stallions. Total sperm count increased from January to April (AL) and May (CON) but did not differ between groups. Sperm motility and percentage of membrane-intact spermatozoa showed no clear seasonal changes and semen parameters did not differ between groups. Thus, Shetland stallions showed seasonal variations in hair coat and total sperm count but only changes in hair coat but not semen parameters were advanced by a long-day light program (Schrammel N. et al., 2016). In another study, these researchers analyzed the effect of artificially long days on the quality of cooled-stored and cryopreserved semen in Shetland stallions. The motility of cooled-stored semen was reduced in January and increased in stallions kept under a long daylight programme for at least 30 days cold-stored sperm (Deichsel K. et al., 2016). Semen quality was impaired in midsummer when low sperm motility and viability were combined with elevated DNA fragmentation and Ca^{2+} level of sperm (Wach-Gygax L. et al., 2017). The results of Janett and co-workers (2003) demonstrated that in climatic conditions of Switzerland, clear seasonal differences occur in semen quality of fresh and frozen-thawed semen and that cryopreservation of stallion semen should preferably be performed in autumn (Janett F. 2003a). Also, individual and seasonal differences occurred in the semen quality of Franches-Montagnes stallions. Ejaculates collected in autumn (September, October, and November) demonstrated good quality, especially regarding sperm morphology, and were more suitable for cryopreservation because of better motility in frozen-thawed semen collected during autumn than in winter (Janett F. 2003 b).

A growing body of evidence has suggested the beneficial role of regular physical exercise. The results of Mills and co-workers (1994) indicate that lipid peroxidation and the excretion and clearance of hydroxyproline increase when unfit thoroughbreds are strenuously exercised on a treadmill (Mills P. C. et al., 1994). Several studies using exogenous supplementation of vitamin E, vitamin C, and alpha-lipoic acid have shown positive results in decreasing the effects of exercise (endurance and intense exercise)-induced oxidative stress and increasing the antioxidant status based on the markers and antioxidants measured (Kinnunen S. et al., 2009; Marañón G. et al., 2008; McMeniman N. P. et al., 1992; Ono K. et al., 1990; Williams C. A. et al., 2016, 2004), whereas other studies using superoxide dismutase showed little effects on the exercise horses (Lamprecht E. D. & Williams C. A. 2012). In old horses (22 ± 2 years), lipid peroxidation levels and blood antioxidant concentrations were similar to those found in younger but mature (12 ± 2 years) horses both at rest and during exercise (Williams C. A. et al., 2004, 2008). Other studies found that yearlings (18 ± 2.4 months) that are novel to forced exercise had less lipid peroxidation and greater antioxidant status than mature mares (13 ± 2.1 years) prior to exercise training (Smarsh D. & Williams C. 2014). Exercise training reduced oxidative stress markers and improved antioxidant status in mares, whereas few effects were seen in yearlings (White S. H. & Warren L. K. 2017). This indicates that youth provided more defense against oxidative stress due to exercise than the exercise training program (Smarsh D. & Williams C. 2016).

In our previous study, we determined the level of oxidative stress biomarkers in sports horses involved in eventing before and after training (Andriichuk A. et al., 2013). All horses were in regular systematic training and had the same diet. A significant increase in the TBARS level in the blood of horses after exercise was observed. There

were no significant differences in erythrocyte TBARS levels between the resting period and after exercises. Significant decreases by 8% ($p < 0.05$) in the aldehydic derivatives of protein oxidation in the plasma after training were noted. Exercise can induce the activity of the proteasome complex, which is significantly involved in the degradation of oxidatively modified proteins. The preventive effect of regular exercise leads to adaptation to prolonged exercises, which is accompanied by an increase of oxidative stress-induced adaptation and changes in redox homeostasis, increased antioxidant defenses, lower oxidative damage, and increased resistance to oxidative stress. Regularly performed exercise might induce an adaptive enhancement in skeletal muscle and erythrocytes of the defense mechanisms that protect them against oxidative stress (Andriichuk A. et al., 2013).

We also investigated the effect of an exercise test of moderate intensity on oxidative stress biomarkers, antioxidant enzyme activity, and osmotic resistance of erythrocytes in well-trained equine athletes (Andriichuk A. et al., 2013). The exercise test induced a significant increase in erythrocyte values, hemoglobin concentration, and hematocrit in horses of both breeds. Regular training induces activation of the antioxidant enzymes and thereby can reduce oxidative stress in athletic horses. The exercise test in horses of both breeds attenuated oxidative stress and was accompanied by a significant decrease in lipid peroxidation and oxidatively modified proteins in erythrocytes after exercise. Our data suggest that oxidative stress and enzymatic antioxidant defense biomarkers can be used for the monitoring of fitness levels, health benefits, and performance of equine athletes (Andriichuk A. et al., 2016).

The effects of gender differences on the blood oxidative stress biomarkers, antioxidant defenses, and resistance of erythrocytes to hemolytic agents of trained horses before and after exercise were evaluated in other our study (Andriichuk A. et al., 2014). The study was carried out on nine mares and 14 stallions of Ukrainian Warmblood well-trained horses, involved in jumping, eventing, and dressage. Trained stallions showed a decrease in lipid peroxidation and higher glutathione reductase activity, whereas mares presented a higher superoxide dismutase activity after exercise. The resistance of erythrocytes was similar in females and males. No statistically significant differences were observed in the percentage of hemolyzed erythrocytes between after and before exercise. A correlation between the oxidative stress biomarkers and antioxidant defenses in the stallions after exercises was observed, which may indicate a protective response of superoxide dismutase and catalase against exercise-induced oxidative stress (Andriichuk A. et al., 2014). Stallions showed a significant increase in leucocytes and granulocytes amount, as well as erythrocytes, hemoglobin, and hematocrit levels after the exercise test. Pre-exercise level of mean corpuscular hemoglobin concentration was higher in stallions. Trained mares and stallions showed a decrease in lipid peroxidation after exercise. Exercise also caused an increase in the oxidatively modified protein of erythrocytes in stallions indicated by exercise-induced oxidative stress. The resistance of erythrocytes in 0.1N HCl was similar between females and males. No statistically significant differences in the percentage of haemolysed erythrocytes before and after exercise were observed (Andriichuk A. et al., 2017).

The seasonal-induced variations of exercise impact on hematological indices and oxidative stress biomarkers in plasma and erythrocytes in horses involved in recreational horseback riding were also assessed (Kurhaluk N. et al., 2023; Pażontka-Lipiński P. et al., 2017; Tkachenko H. et al., 2016; Witaszek M. et al., 2017). The results of our study showed statistically significant alterations of oxidative stress biomarkers in erythrocytes after the exercise test in autumn. The results observed in our study suggest that ROS production may contribute to exercise-induced damage to the erythrocyte mem-

brane. There were no statistically significant alterations in the derivatives of protein destruction level in the erythrocytes of trained horses involved in recreational horseback riding before and after exercise. Only in the autumn season, aldehydic and ketonic derivatives of oxidatively modified proteins (OMP) increased after exercise by 88% ($p < 0.05$) and 77% ($p < 0.05$), respectively (Tkachenko H. et al., 2016) There were no statistically significant alterations in the aldehydic derivatives of protein destruction level in the plasma of trained horses involved in recreational horseback rides before and after exercise in the spring and summer seasons. In the autumn season, aldehydic and ketonic derivatives of OMP were increased after exercise by 7.2% and 12.8% ($p < 0,05$), respectively. On the contrary, aldehydic and ketonic derivatives of oxidatively modified proteins were decreased after a training session in winter. Increasing the level of oxidative-modified proteins suggests the activation of oxidative stress during a training session in spring and autumn as adaptive changes in the horse's body due to changing ambient temperatures. Significant reductions in aldehydic derivatives of oxidatively modified proteins in the plasma of horses after training in winter are the result of adaptation induced by constant ambient temperature and exercise. This is an important seasonal adaptive reaction to stabilize ambient temperature (Kurhaluk N. et al., 2023, Witaszek M. et al., 2017). The increase in plasma lipid peroxidation level in horses after exercise could be attributed to oxidative damage in various organs mainly in muscle tissue, owing to free radicals being produced, as a consequence of endurance exercise. Based on our results, it is concluded that endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and the athletic performance of horses (Kurhaluk N. et al., 2023, Witaszek M. et al., 2017). A significant increase in erythrocytes amount after exercise tests both in the summer and autumn seasons was observed. Moreover, increased erythrocytes count by 18% ($p < 0.05$) in the autumn compared to the value in the spring season after the exercise test was noted. Hemoglobin level was increased after exercise in the summer season (by 15%, $p < 0.05$). Our results also revealed the increased hematocrit level after exercise tests in the summer, autumn, and winter seasons. We assume that it was due to the release of erythrocytes into circulation as a result of the increased splenic function (Andriichuk A. & Tkachenko H., 2015; Tkachenko H. et al., 2016)

Only with knowledge of the biological needs of the body, it is possible to establish scientifically based feeding norms depending on the season of the year. Knowledge of seasonal adaptive changes in morphological structures, as well as biochemical and physiological reactions of animals makes it possible, using the biological characteristics of individual breeds and offspring, to significantly increase their training capacity and health. Seasonal changes in the thickness of the skin and the structure of the hairline of horses were studied by Shkyratova and co-workers (2019) as signs of adaptation to environmental factors. The minimum skin thickness in winter was found in mares on the back and shoulder blade (4.3 and 4.4 mm), and the maximum – on the side and thigh (4.5 and 4.6 mm). Compared with the summer period, the increase on the side was 0.8 mm, and on the back, shoulder blade, and thigh – 0.4 mm ($p \leq 0.001$). In spring, skin thickening was also noted in the same topographic areas within 0.1-0.3 mm compared to autumn. Quantitative indicators of hair cover varied depending on the season of the year. In winter, the hairline contained more down (23.10%), but fewer guard hairs (68.24%). In summer, there was a lower content of down (4.33%), but more guard hairs – 94.01%. There are sharp seasonal changes in the length of the hair. The longest hairs was found in winter and spring (4.96 and 4.26 cm), and the shortest was in summer and autumn (0.94 and 1.90 cm, respectively) (Shkuratova G. M. et al., 2019).

Schmidt and co-workers (2017) hypothesized that the housing of stallions in a thermoneutral temperature zone during autumn and winter not only influences metabolism and hair shedding but also improves the characteristics of raw and processed semen. These researchers revealed in a study that environmental temperature during autumn and winter had clear results on body temperature as well as hair coat characteristics in Shetland stallions. Simultaneously determined effects on semen characteristics were minimal indicating that reproductive function in the horse is more dependent on day length i.e. the geophysical year than on other environmental factors (Schmidt K. et al., 2017).

Conclusions. We have analyzed the effect of photoperiods and exercise on the levels of TBARS and TAC in the blood of Shetland pony mares and stallions involved in recreational horseback riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland). We obtained the maximum statistically significant values for the effect of the photoperiod-exercise combination defined as the F values. The TBARS levels were indicated as biomarkers of lipid peroxidation in plasma with a maximum value of the adjusted form of the coefficient of determination. In the stallions and mares before exercise (at rest) in the summer photoperiod, we obtained minimum TBARS values, while maximum levels were determined in the winter photoperiod. In our study, the TAC level illustrated the baseline levels of enzymatic and non-enzymatic antioxidant defense systems, which were different in the mares than in the stallions (statistically significant in the winter photoperiod) both before and after exercise (winter photoperiod).

The results of the current study demonstrated that changes in the lipid peroxidation and the total antioxidant capacity in the blood of Shetland ponies are photoperiod-, sex-, and exercise-dependent. The multivariate analysis of the influence of the three parameters: photoperiods, sex, and exercise on the lipid peroxidation and the total antioxidant capacity in the blood of the ponies indicated the leading role mainly of the photoperiod factor, and next the exercise and sex factors.

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BREEDING WORK WITH THE UKRAINIAN BLACK-AND-WHITE DAIRY BREED IN THE SUMY REGION

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Researches were carried out in breeding farms for the breeding of the Sumy inbred type of the Ukrainian black and white dairy breed. The total experimental herd is 1150 cows, 1120 heifers and 62 bulls.

It has been established that the animals mainly come from breeders of four Holstein lines, such as Starbuck 352790 (28%), Bell 1667366 (27%), Chiff 1427381 (26%) and Elevation 1491007 (15%). In recent years, farms have mainly used breeders of three lines, unlike bulls of the Bell 1667366 line. The breeding value of breeders of different lines is slightly different. Bulls of two Holstein lines – Chiff 1427381 and Elevation 1491007 – have the advantage in terms of average breeding value in the amount of milk fat and protein.

The country of origin has a significant influence on the breeding value of breeding bulls. Animals of German selection make up the majority of those used in breeding farms for the breeding of livestock of the Sumy inbred type. A somewhat smaller number of breeders come from the United States of America. The latter are superior to German bulls in terms of average breeding value, both in terms of the amount of milk and the amount of milk fat and protein.

Progeny-descendants, which are more distant from the progenitor of the line, are characterized by a higher average breeding value in comparison with bulls, which are closer to the progenitor according to the pedigree.

In order to create micropopulations with the desired genotypes for beta- and kappa-caseins, homozygous breeders with the A2A2 and BB genotypes are used at the State Enterprise of the Northeast Institute of Agriculture.

The strength of influence of the researched factors on the breeding value is in the range of η^2=11.4-57.9%. The year of the bull's birth and the distance from the progenitor of the line have the greatest influence on this indicator (P<0.05-0.001).

In order to preserve the population of the Sumy inbred type of the Ukrainian black and white dairy breed, it is necessary to apply the proposed measures, which include the use of genetic material of breeders of the Ukrainian black and white dairy breed and a certain set of biotechnological measures.

Key words: bull-breeder, line, genealogy, breed, type

ПЛЕМІННА РОБОТА З УКРАЇНСЬКОЮ ЧОРНО-РЯБОЮ МОЛОЧНОЮ ПОРОДОЮ В СУМСЬКІЙ ОБЛАСТІ

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Дослідження проведені у племінних господарствах з розведення сумського внутрішньопородного типу української чорно-рябої молочної породи. Загальне піддослідне поголів'я складає 1150 корів, 1120 телиць та 62 бугаї.

Встановлено, що в основному тварини походять від плідників чотирьох голштинських ліній, таких як Старбака 352790 (28%), Белла 1667366 (27%), Чіфа 1427381 (26%) та Елевейшна 1491007 (15%). За останні роки в господарствах використовують головним чином плідників трьох ліній, а бугаї лінії Белла 1667366 майже не ні. Племінна цінність плідників різних ліній дещо різниться. Перевагу за середньою племінною цінністю за величиною надою, кількістю молочного жиру та білка мають бугаї двох голштинських ліній – Чіфа 1427381 та Елевейшна 1491007.

Значний вплив на величину племінної цінності бугаїв-плідників має країна походження. Тварини німецької селекції становлять більшість серед тих, що використовувалися у племінних господарствах з розведення худоби сумського внутрішньопородного типу. Дещо менша кількість плідників походять зі Сполучених Штатів Америки. Останні переважають німецьких бугаїв за показниками середньої племінної цінності, як за величиною надою, так і за кількістю молочного жиру та білка.

Плідники-нащадки, що більш віддалені від родоначальника лінії, характеризуються вищою середньою племінної цінністю у порівнянні з бугаями, які за родоводом знаходяться ближче до родоначальника.

З метою створення мікропопуляцій з бажаними генотипами за бета- та капа- казеїнами у ДП ДГ Інституту сільського господарства Північного Сходу використовуються гомозиготні плідники відповідно з генотипами А2А2 та ВВ.

Сила впливу досліджуваних факторів на величину племінної цінності знаходиться в межах $\acute{\eta}^2$=11,4-57,9%. Найбільший вплив на даний показник мають рік народження бугая та віддаленість від родоначальника лінії (P<0,05-0,001).

Для збереження популяції сумського внутрішньопородного типу української чорно-рябої молочної породи необхідно застосовувати запропоновані заходи, які включають використання генетичного матеріалу плідників української чорно-рябої молочної породи та певний набір біотехнологічних заходів.

Ключові слова: бугай-плідник, лінія, генеалогія, порода, тип

Introduction. Dairy cattle breeding is an important component of the agro-industrial complex that ensures food security of Ukraine (Khmelnychyi S. et al, 2020).

In Ukraine, the number of cattle has been decreasing for a long time. At the same time, scientists note the growth of the average milk yield per cow, which currently equals almost 8,000 kg. The reduction of controlled herd leads to the complication of

breeding work with dairy breeds. The Ukrainian black and white dairy breed makes up almost 50% of the breeding livestock of Ukraine, the Holstein makes up a little more than 25%, and the Ukrainian Red-and-White dairy breed makes up 14% (Kruglyak O. V. et al, 2020).

The value of breeders based on certain breeding characteristics significantly affects the effectiveness of the breeding process in dairy cattle breeding. Therefore, scientists recommend the use of bulls-improvers. This is greatly facilitated by the development of biotechnological methods, which allowed intensive exchange of genetic material between different countries (Polupan, Y. P. and Bezrutchenko, I. M., 2022).

Scientists have proven that the effectiveness of selection and breeding work with the dairy breed depends significantly on the successful selection of the breeder. This is due to the fact that the role of heredity in the genetic improvement of the breed reaches more than 90%. This determines the use of breeders-leaders (Polupan Y. P. et al, 2019; Pryima S. V., 2022).

Domestic breeders note that purebred breeding should be the main method of improving local dairy breeds. Nevertheless, the use of the global gene pool of the Holstein breed can be an effective technique for improving breeds. This method is widely used in Ukraine. Scientists claim that animals of the same breed, which are bred in different countries, differ significantly in economic and useful characteristics (Kuziv M. I. et al, 2022; Voitenko S. L. and Sydorenko O., 2019).

The formation of the genealogical structure of domestic breeds was implemented through the theoretical provisions of the linear breeding method. For breeders, initially the development of lines took place through the so-called "long lines". These lines consist of five to six generations. After a certain time, implementation took place through "short lines" consisting of two to three generations. Over the past twenty years, the base of genetic resources is represented by a broad genealogical structure of breeders and their sperm production belonging to 26 lines of the Holstein breed. Moreover, scientists outline the narrowing of the genealogical structure due to the large number of breeders of the Chiff 1427381 and Elevation 1491007 lines (Pochukalin A. Y., et al, 2022).

Domestic scientists state a significant reduction in the genealogical lines of dairy breeds of Ukraine. Thus, only two lines out of twelve, which were approved during the creation of the Ukrainian Red-and-White dairy breed, remained at the beginning of 2023. Most cows of this breed today come from Holstein breeders. The authors note that breeders of the Holstein breed come from different countries. They are representatives of the lines: Astronaut 1458744, Ivanhoe 1189870, Bell 1667366, Bootmake 1450228, Valiant 1650414, Jocko Besn 5694028588, Elevation 1491007, Marshall 2290977, Maple 218036, R. Sovereign 19 8998, Citation 267150, Starbuck 352790 and Chiff 1427381. This indicates that the main method of breeding domestic dairy breeds is absorptive crossing, the consequence of which is an increase in conditional blood for the Holstein breed (Voitenko S. L. et al, 2022; Ilnytska O. Y. et al, 2018).

The effectiveness of the use of Holstein breeders in order to increase the level of milk productivity of local breeds has been proven. However, there is no consensus among scientists and breeders-practitioners regarding the level of desirable conditional blood of the Holstein breed in the genotype of domestic breeds (Pochukalin A. Y. et al, 2022; Sydorenko O. V., 2020; Khmelnychyi L. M., and Vechorka V. V., 2018).

It is known that as a result of purposeful breeding work, breeders in the United States of America, Canada and Israel have achieved the desired indicators of Holstein cattle both in terms of milk productivity and the milk type of animal body structure. However, with a high level of milk yield, low fat and protein content in milk was noted in most herds. Thus, the fat content of milk was in the range of 2.9-3.4%. We should

outline that the recognized record holder Alinde Elen, with an annual milking of 25,242 kg, had a milk fat content of 2.8%. Researchers also claim the influence of the country of origin on the level of milk productivity of cows. The milk productivity of cows in the countries listed above is 9.7-10.4 thousand kg of milk per lactation, which is higher by 1.5 thousand kg compared to cows of European breeding (Netherlands, Belgium, Italy, Germany). However, the content of fat and protein in their milk was lower and amounted to 3.50-3.60% and 3.05-3.10%, respectively. Therefore, scientists claim that milk productivity is genetically determined in each individual population (Krugliak A. P. et al, 2021; Krugliak A. P. and Krugliak T. O., 2021).

Today, the genealogical structure of the Holstein breed in Ukraine includes 16 lines. The most numerous lines are Elevation 1491007, Chiff 142781, Starbuck 352790. Their share is more than 70% (Pochukalin A. Y., and Pryima S. V., 2021).

The important issue is the presence of a breeding base with the necessary level of amplitude of genetic resources. Continuous monitoring makes it possible, if necessary, to analyze and adjust the breeding program of domestic breeds (Pochukalin A. Y. et al, 2022).

Hereditary factors that affect the level of selection traits include paternal origin and lineal affiliation. Conducting constant monitoring of the genealogical structure of the Ukrainian black and white dairy breed proves the influence on the heredity of animals of the world gene pool of the Holstein breed (Shuliar A. L. et al, 2020; Voitenko S. L. and Zheliznyak I. M., 2019). This is what determines the necessity of conducting our research.

The purpose of the work is to study the genealogical structure of the breeders of the Holstein breed, which are used in breeding farms for the breeding of cattle of the Sumy inbred type of the Ukrainian black and white dairy breed, and to establish the influence of lineal affiliation, country of origin and year of birth on the size of their breeding value.

Research materials and methods. The research was carried out at the breeding plant for the breeding of livestock of the Sumy inbred type of the Ukrainian black and white dairy breed of the State Enterprise "Experimental Farm of the North East Institute of Agriculture of of the National Academy of Sciences", the breeders "Pershe Travnya", "Iskra" of the Sumy district, Sumy region. The total experimental herd is 1150 cows, 1120 heifers and 62 breeders.

The influence of such factors as lineal affiliation, country of origin, degree of kinship with the progenitor of the line, and year of birth was determined. Relevant information was obtained when working with pedigree registration documentation (Form 2-Mol cards) and using the SUMS ORSEK database.

The amount of breeding value was determined according to the website https://www.cdn.ca/query.

Statistical processing was performed using the STATISTICA 10 application program package.

Research results. As a result of the conducted research, we found that over the past seven years, Holstein breeders were widely used for the insemination of cows and heifers in Sumy inbred cattle breeding farms of the Ukrainian black and white dairy breed. Accordingly, the genealogical structure of breeding farms of this type is represented by lines of the Holstein breed (Fig. 1).

Almost equal share of cows comes from three Holstein lines: Starbuck 352790, Bell 1667366 and Chiff 1427381 – 26-28%. The share of Elevation 1491007 line cows is somewhat smaller – 15%, less than 5% of the herd structure consists of animals of the Valiant 1650414 line. However, this structure has significant differences in individual

herds. Thus, in the breeding plant of the State Enterprise "Experimental Farm of the North East Institute of Agriculture of of the National Academy of Sciences", there are no animals of the Bell 1667366 and Valiant 1650414 lines at all. In this farm, animals of the Elevation 1491007 (38%) and Chiff 1427381 (34%) lines predominate in number.

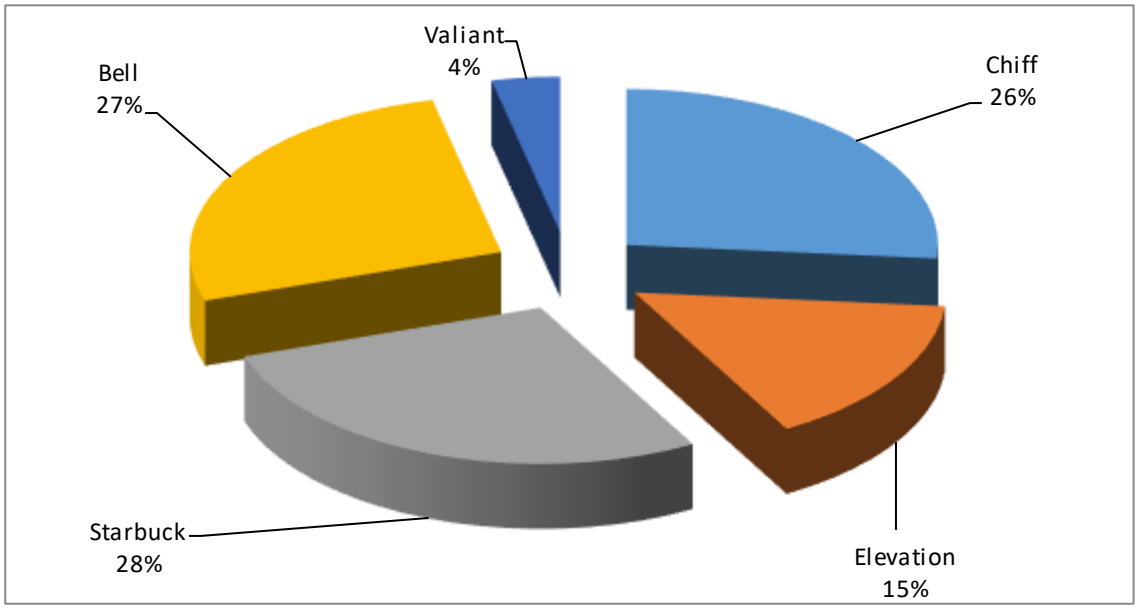


Fig. 1. **Genealogical structure of cows of breeding herds**

According to the level of milk productivity during the first lactation, there was no significant difference between animals of different lines. According to the analysis of the last completed lactation, we note the preference of animals of the Elevation 1491007 line, which have a productivity of more than 8.0 thousand kg of milk per lactation. The animals of the Chiff 1427381 and Starbuck 352790 lines are somewhat inferior to them (Fig. 2).

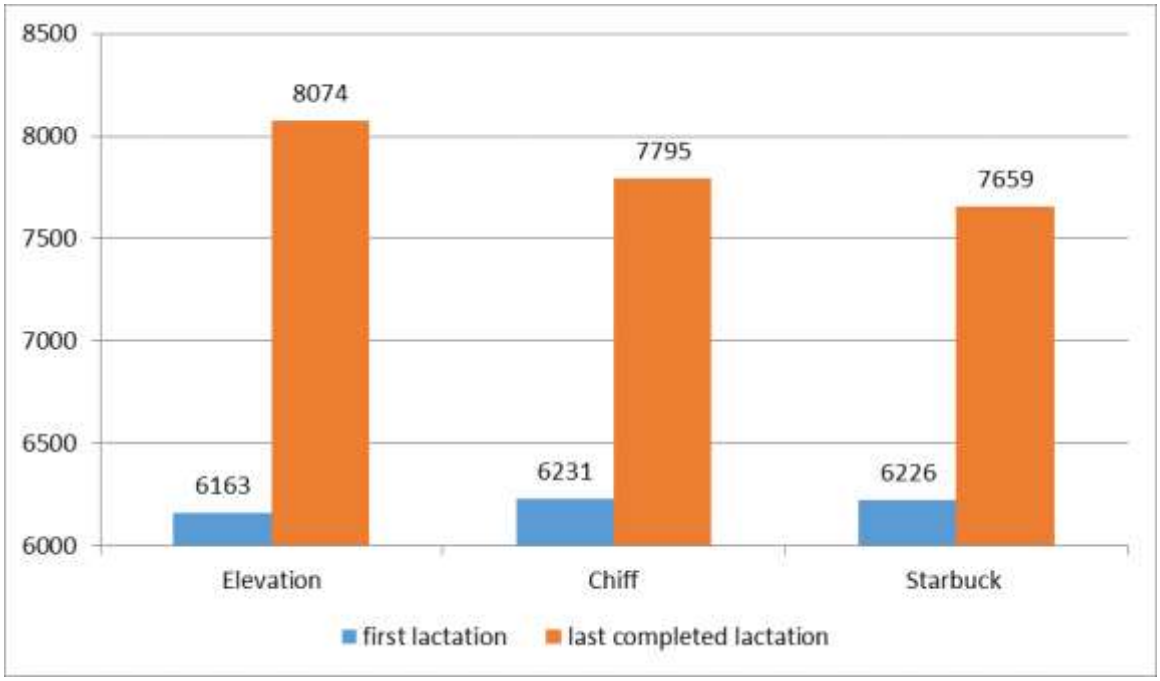


Fig. 2. **The amount of milk yield from cows of different lines**

In the breeding center "Pershe Travnya", the majority of animals come from breeders of the Starbuck 352790 (34%) and Chiff 1427381 (31%) lines, the cattle of the Elevation 1491007 and Bell 1667366 lines make up 17 and 12%, respectively, and the Valiant 1650414 line is only 6%.

Cows of Starbuck 352790 (32%), Chiff 1427381 (28%) and Bell 166366 (27%) lines have a larger share in the genealogical structure of Iskra LLC breeding center. Animals of the Elevation line 1491007 make up 11%, and Valiant 1650414 line – 2%.

Significant changes in the genealogical structure of the studied type were established as a result of the study of the genealogical structure of heifers kept in breeding farms. They do not have heifers that come from breeders of the Valiant 1650414 line, and the share of animals of the Bell 1667366 line has decreased by 25% and is only 2%. The share of animals of the Elevation 1491007 line, which is more than 40%, has significantly increased. The proportions of animals of the Chiff 1427381 and Starbuck 352790 lines did not change significantly (Fig. 3).

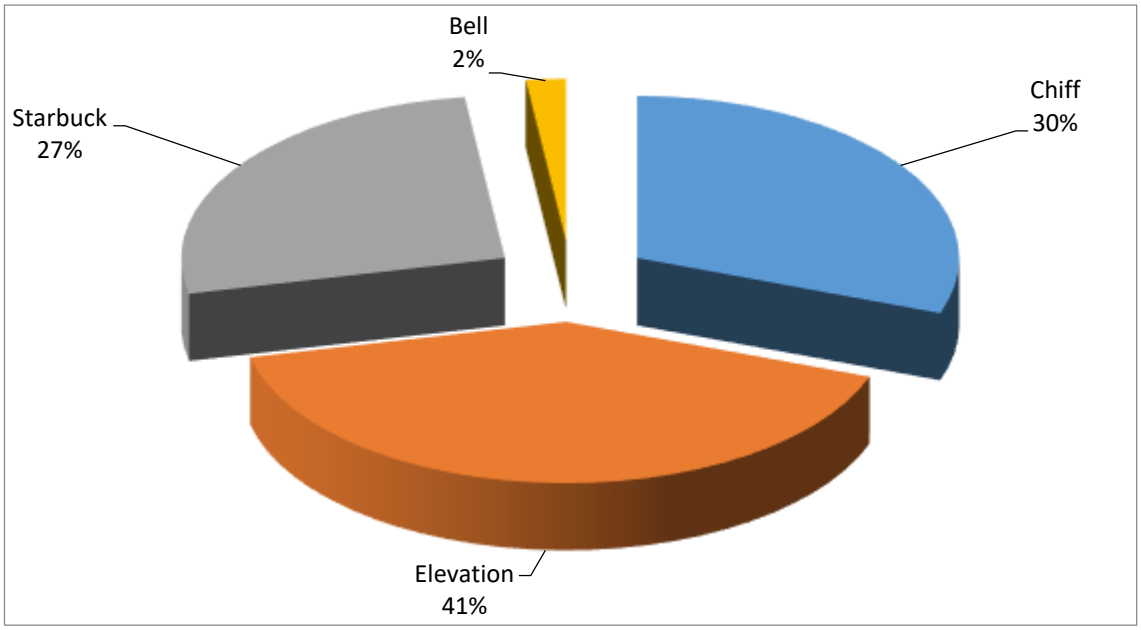


Fig. 3. **Genealogical structure of heifers of breeding herds**

In the breeding plant of the State Enterprise "Experimental Farm of the North East Institute of Agriculture of the National Academy of Sciences", in connection with the termination of the use of breeders of the Starbuck 352790 line, there are no heifers of this genealogical line. Heifers of the Elevation 1491007 and Chiff 1427381 lines have equal shares in the farm (50% each).

Heifers of the Chiff 1427381 line in the holding of "Pershe Travnya" dominated, which made up 48%. Almost the same number of heifers in the farm came from breeders of the Elevation 1491007 (25%) and Starbuck 352790 (22%) lines. Animals of the Bell 1667366 line make up a small share – 5%.

In the pedigree breeder of Iskra LLC, most of the heifers come from Elevation 1491007 line (43%). Animals of Starbuck 352790 and Chiff 1427381 lines accounted for 34% and 22%, respectively. Line Bell 1667366 is not developing in the farm, the number of heifers is only 1%.

According to the results of research, in general, in the studied farms, the most numerous lines of Chiff 1427381, Starbuck 352790, Elevation 1491007 in terms of the number of breeding stock were represented by a larger number of parent bulls, 19, 17,

21 heads, respectively. The number of parent bulls of the Valiant line was 3 heads, of the Bell 1667366 line – 2 heads.

The sires of the Chiff 1427381 line (448±166.6) are distinguished by the highest average breeding value in terms of the greatest milk yield. They prevail over the breeders of the Elevation 1491007 line (380±115.4), although no statistically significant difference was established between them. The average breeding value of bulls of the Starbuck 352790 line (-94±203.0) had a negative value and the difference with the sires of the Chiff 1427381 line was statistically significant ($P<0.05$). The significant difference was also established between the studied parameters of the breeders of the lines Starbuck 352790 and Elevation 1491007 ($P<0.05$). The breeders of the Bell 1667366 line had a negative value of the average breeding value (-79±229.3). The breeders of the Elevation 1491007 line (36±7.6) were distinguished by the higher average breeding value in terms of the amount of milk fat. The difference between them and bulls of Starbuck 352790 (-0.6±4.3) and Bell 1667366 (-16±19.4) lines was statistically significant ($P<0.001$; $P<0.05$, respectively). The average breeding value of the breeders of the Chiff 1427381 line was also higher than that of the breeders of the Starbuck 352790 and Bell 1667366 lines (respectively $P<0.001$; $P<0.05$). According to the amount of milk protein, the average breeding value was higher in breeders of the Elevation 1491007 line (32±5.7). Starbuck 352790 (-4.9±4.7) and Bell 1667366 (-7±8.2) lines were inferior to them with a statistically significant difference ($P<0.001$ and $P<0.01$, respectively). The average breeding value according to the qualitative characteristics of the milk productivity of the daughters of the breeders of the Chiff 1427381 line was 29±8.0 and 21±6.3, respectively, and in terms of the amount of milk protein, they exceeded the bulls of the Starbuck 352790 line with a statistically significant difference ($P<0.01$).

We should outline that the average breeding value of the bulls from which the heifers came was higher than that of the breeders that were the parents of the cows. By the amount of yield, it was 361±165.5 and 141±105.5, respectively; by the amount of milk fat – 13±4.7 and 31±7.9; by the amount of milk protein 26.3±6.2 and 7±3.9 ($P<0.05$).

By country of origin, the majority of breeders are representatives of European breeding, which make up more than 50% of the researched herd (Fig. 4).

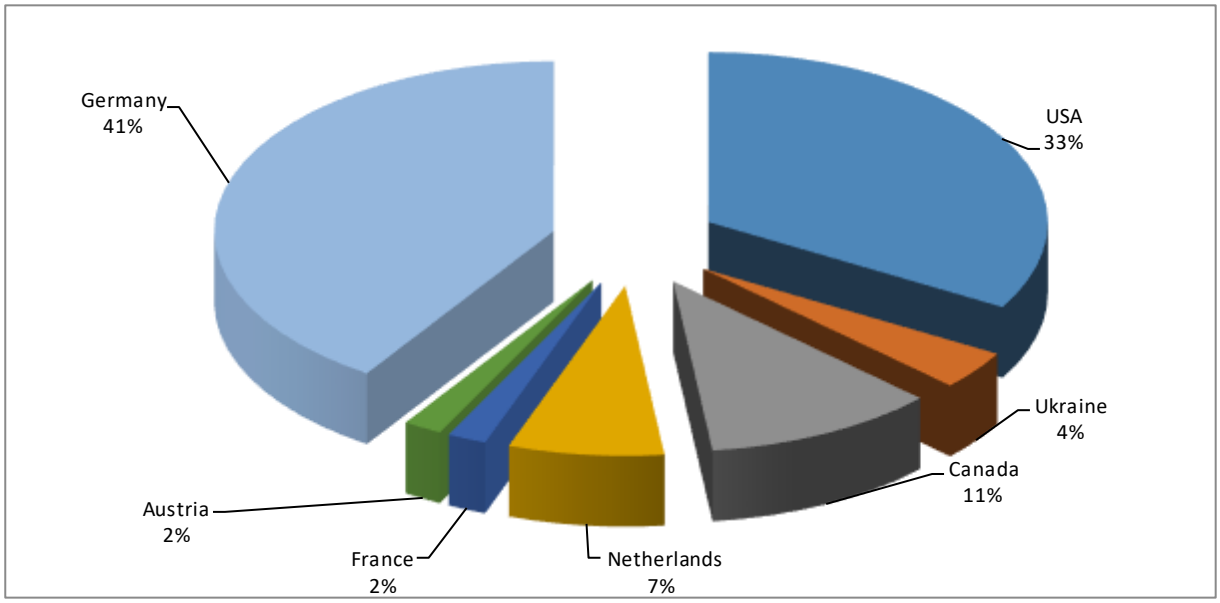


Fig. 4. **Distribution of breeders by country of origin**

In terms of the number of bulls, representatives of the German breed outnumber all others. But at the same time, they have a negative average breeding value by the amount of yield (-26±135.5). Breeders of North American selection make up a little more than 40%, among which the majority are representatives of the selection of the United States of America. They have higher than average breeding value for milk yield (599±137.7) and milk protein (31±5.6) among the studied bulls. They outperform German breeders in these characteristics with a statistically significant difference (P<0.01). By the amount of milk fat of German breeders, bulls of both American (34±7.9) (P<0.01) and Canadian breeding (38±16.9) (P<0.01) prevail.

The most numerous line, Starbuck 352790, originates from the son of the outstanding breeder Elevation 1491009. In the process of breeding the Sumy inbred type, this line develops through the branch of the son of the progenitor of the great Aerostar 383622. There are seven breeders in this branch. Two other branches, which are currently separated into separate lines by individual breeders – Mtoto 6001001962 and Jocko Besn 5694028588 – have a smaller number of used brooders – four and two heads, respectively (Fig. 5).

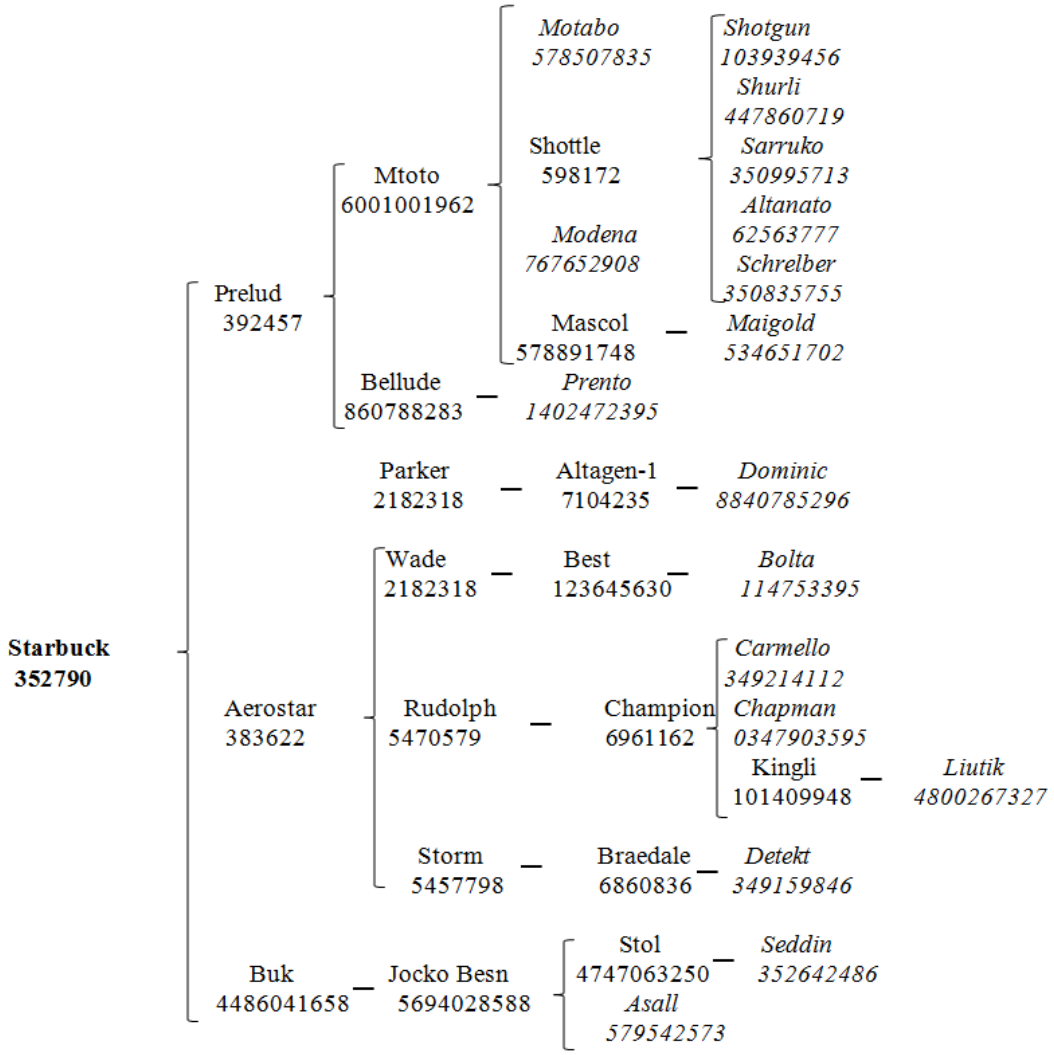


Fig. 5. **Genealogical scheme of the bulls of the Starbuck 352790 line**

Most of the researched breeders of this line are representatives of European selection, namely the countries: Germany, France, the Netherlands, and Ukraine. Only one representative of each represents the Canadian and American selections.

The progenitor of the Elevation 1491007 line is the great-grandson of the famous breeder Vis Ideal 933122. In Ukraine, this is the second line in terms of the number of breeders used in the selection process. Breeders of two lines are used in the breeding work with the brood stock of the Sumy inbred type: with the sons of Bova 1665634 and Tradition 1682485 and grandsons of Cletus 1879085 and Leadman 1983348. With the son of Bova 1665634, the line is represented by nine breeders, and with the line of Tradition 1682485 – by seven ones. Most of the bulls are of American origin – 50%, German – 31%, a small share is presented by Canadian and Dutch breeders (Fig. 6).

Chiff 1427381 line is considered the most numerous line in terms of the number of Holstein breeders in Ukraine (Fig. 7).

The development of this line during the breeding of livestock of the Sumy inbred type occurs similarly to the national Ukrainian through three sons: Mark 1773417, Chiff 1556373, Chiff 1578139.

However, there are differences. If it is considered that in Ukraine, a larger number of breeders come from the Chiff 1556373 line, then this line is the least numerous when breeding the studied inbred type. The share of its bulls from all is only 12%. The most numerous is the group of breeders of the Chiff 1427381 line, which are descendants of the bull Blackstar 1929410. The share of these breeders used in the researched herd is 67%. By origin, the breeders of the Chiff 1427381 line were distributed almost equally among European and North American selections, respectively 47 and 53%. The largest number of bulls came from the United States of America (35%) and Germany (29%).

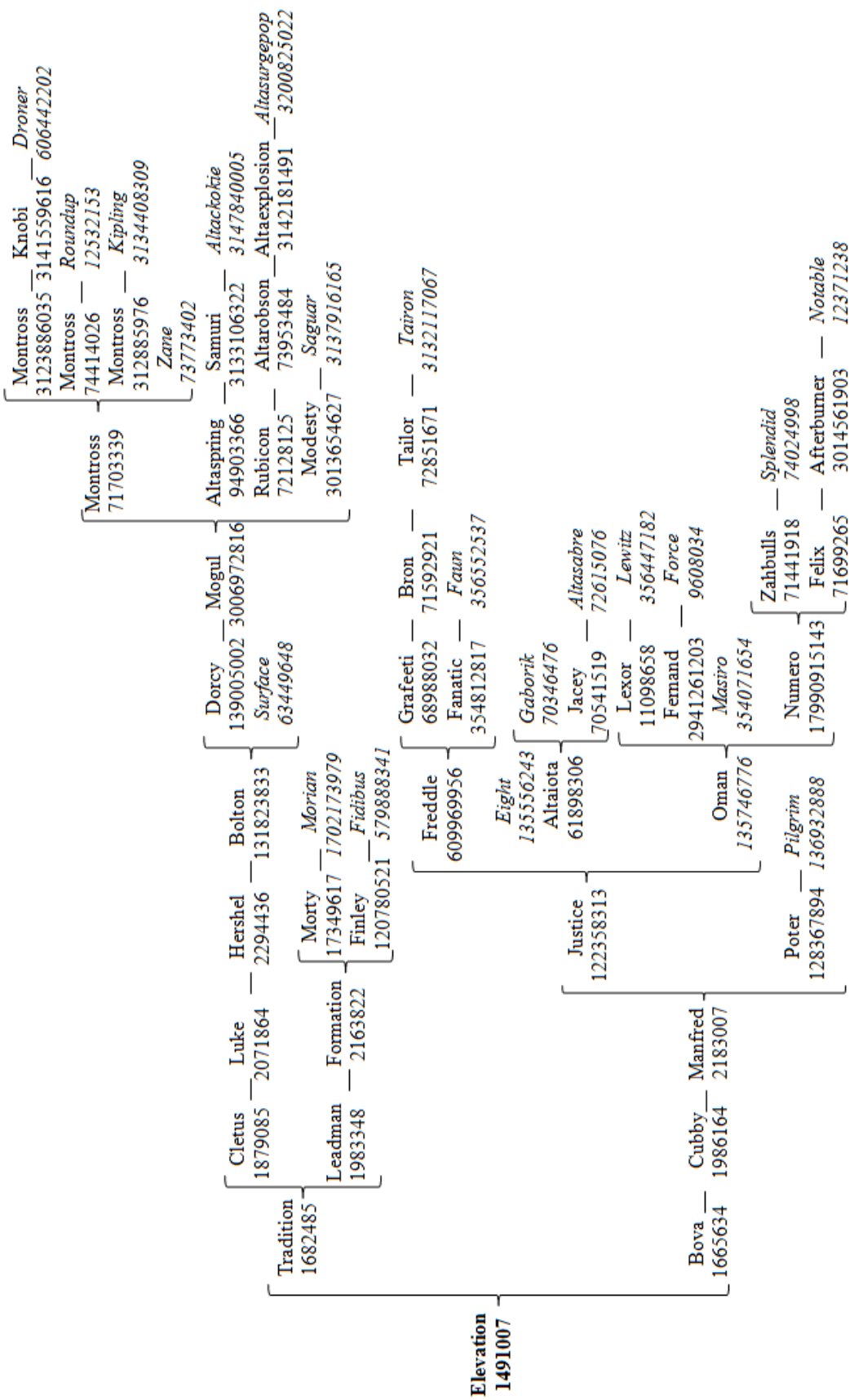


Fig. 6. **Genealogical scheme of the bulls of the Elevation 1491007 line**

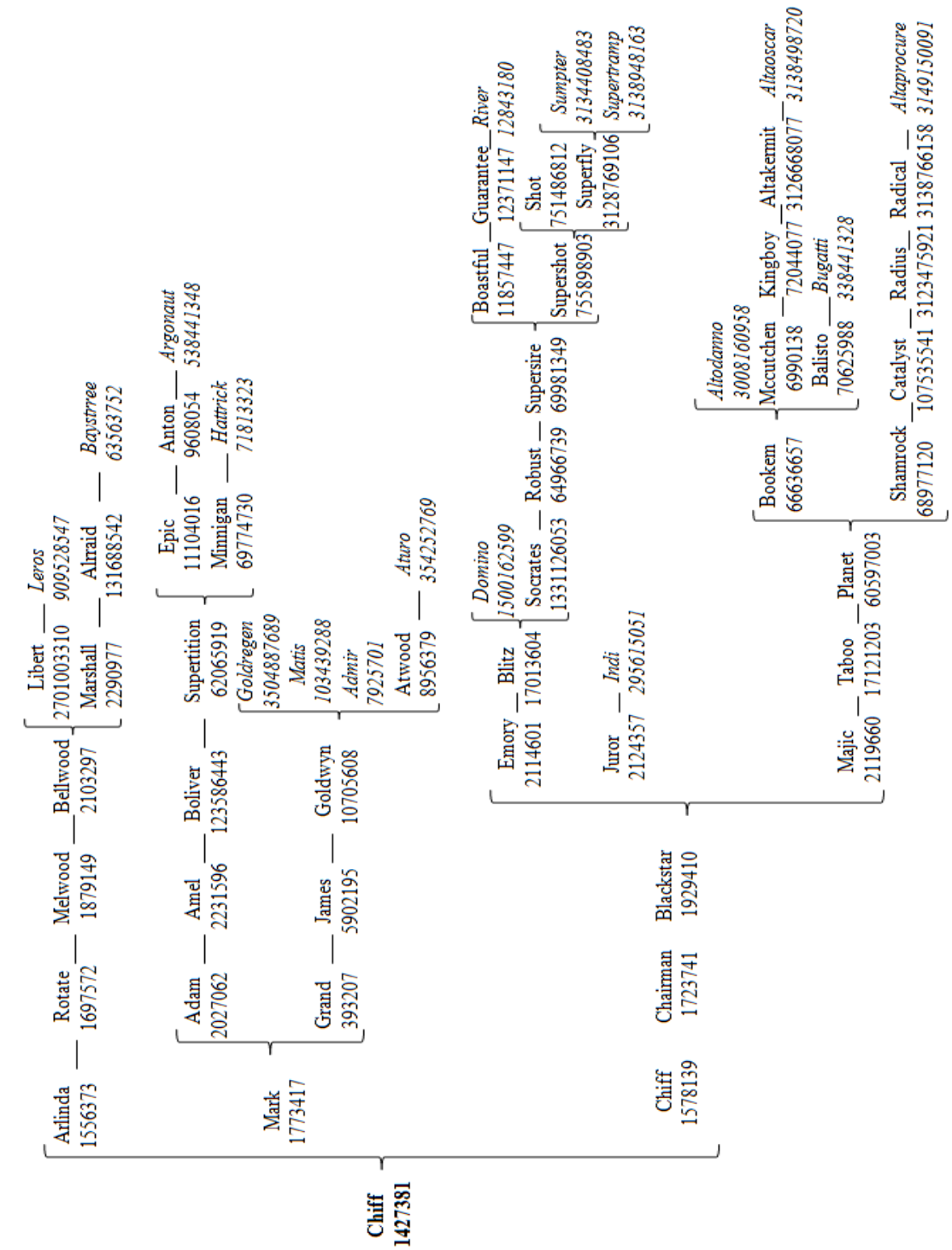


Fig. 7. **Genealogical scheme of bulls of the Chiff 1427381 line**

The Bell 1667366 line is currently represented by three breeders, two of them are of German origin, in the work with the Sumy inbred type. Another bull is a representative of American breeding (Fig. 8).

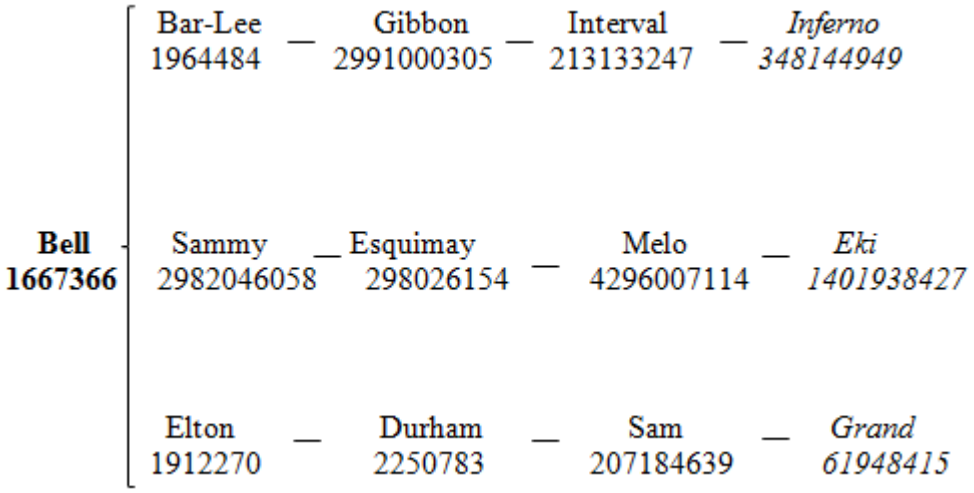


Fig. 8. **Genealogical scheme of bulls of the Bell 1667366 line**

The Valiant 1650414 line is represented by two bulls of the Ukrainian black and white dairy breed and is no longer used in breeding farms today (Fig. 9).

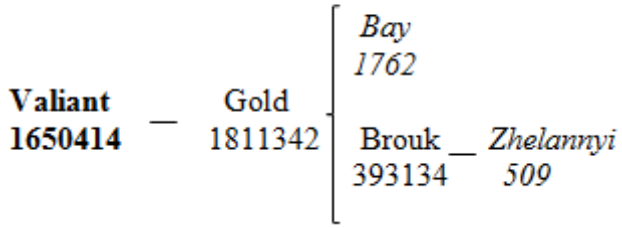


Fig. 9. **Genealogical scheme of bulls of the Valiant 1650414 line**

It was established that the breeders used in dairy herds were located in 2-11 rows from the progenitors of the lines (Fig. 10).

Bulls of the Valiant 1650414 line were in the 2nd and 3rd row from the progenitor, the Bell 1667366 line – in the 4th row. Most of the breeders of the Starbuck 352790 line were in the 4th row, although some moved away from the progenitor to the 5th and 6th rows. The offspring of the Chiff 1578139 and Elevation 1491007 lines were located in more distant rows from the progenitor – 5-11.

Having analyzed the average breeding value of breeders depending on the degree of kinship with the progenitor, we found out that there is a logical increase in this indicator as it was further from the latter. In our opinion, this is due to the fact that foreign breeders pay less attention to the genealogical origin of breeders, and more attention to their breeding characteristics. A statistically significant difference was established in the average breeding value by the amount of yield, considering the breeders located in the third and eleventh rows from the progenitor (1415; $P<0.001$), the fourth and eleventh (778; $P<0.05$), the fifth and eleventh (818; $P<0.01$), sixth and eleventh (858; $P<0.05$). According to the average breeding value for milk yield, a similar tendency of superiority over breeders of the third-sixth row is also characteristic of breeders located in the seventh-tenth rows.

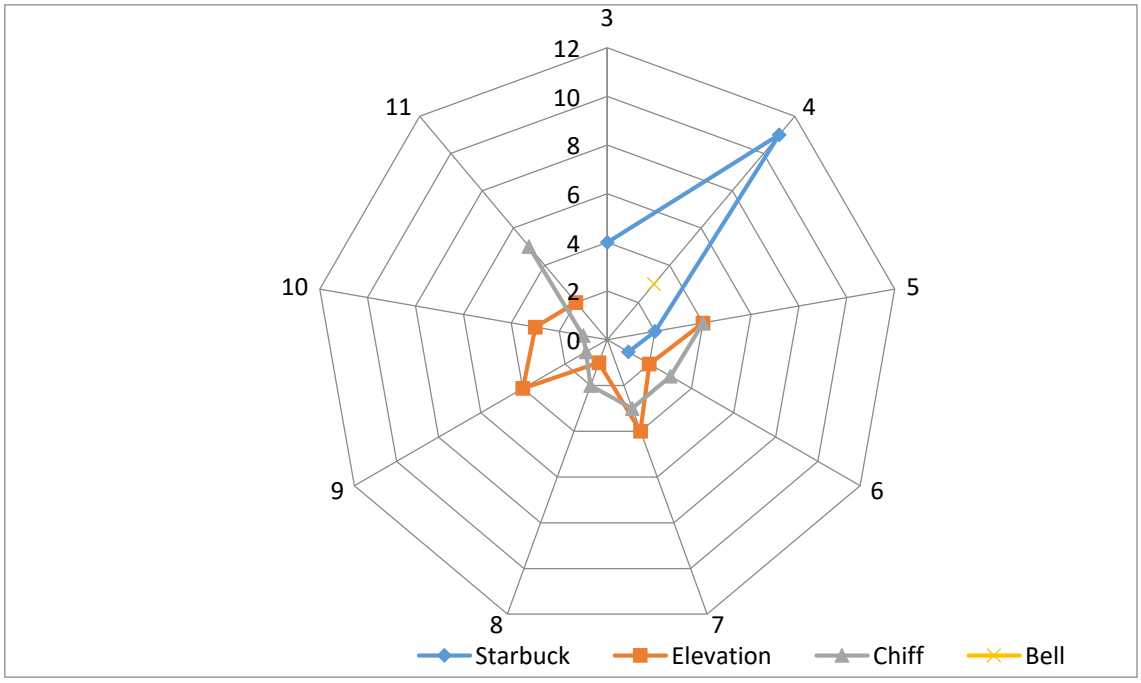
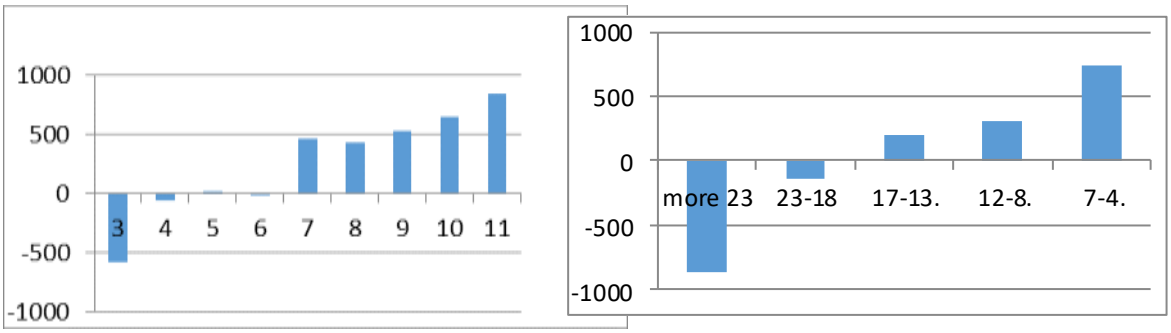


Fig. 10. **Degree of kinship with the progenitor of the line, generations**

This is confirmed by the results of research concerning the influence of the year of birth on the value of the breeding value of the breeder. Thus, we established that with a decrease in the age of the breeder (meaning the year of birth), there is a logical increase in its breeding value. The average breeding value of breeders aged 4-7 years is higher than the breeding value of breeders older than 23 years old by 1611 (P<0.01), aged 23-18 – by 891 (P<0.01), aged 13-17 – by 542 (P<0.05), 8-12 years old – by 433 (P<0.05) (Fig. 11).



The degree of kinship with the progenitor of the line, generations

Age of breeder, years

Fig. 11. **Dependence of the average breeding value on the relationship with the progenitor of the line and the age of the breeder** (age means the number of years from the date of birth of the breeder until 2023)

Similar trends regarding the growth of the value of the average breeding value with a decrease in the degree of consanguinity and the age of bulls are characteristic of the amounts of milk fat and protein.

Recently, domestic breeders began to pay more attention to the determination of the genotype of animals by genes that affect the formation of economically useful traits (Kulibaba R. et al, 2023).

Thus, domestic scientists conduct research on the formation of micropopulations of dairy cattle with the desired genotypes for beta- and kappa-casein. A similar purposeful company is also conducted in the Sumy inbred breeding farm. Such a farm is the State Enterprise "Experimental Farm of the North East Institute of Agriculture of of the National Academy of Sciences".

It was at this farm that a group of cows for the production of A2 milk was formed for the first time in Ukraine. And already in 2020, such milk was put on the shelves of shops in the city of Sumy.

Work on the formation of a herd of animals with the A2A2 genotype for beta-casein continues today. Among the breeders from which the heifers in this farm come, nine out of ten have the A2A2 genotype (one has the heterozygous A1A2 genotype), which is provided for in the selection and breeding plan for working with the dairy herd. In other farms, where similar work is not provided, breeders are used that have not been evaluated for beta-casein genotype. Practitioners may have a question about the breeding value of bulls (n=11) with the A2A2 genotype, from which heifers in farms come. According to the results of our research, these breeders have an average breeding value for milk yield of 854±181.1, the amount of milk fat – 57±10.3, and milk protein – 50±6.0. These values are higher than the average breeding value of breeders (n=26), from which the heifers in the Sumy inbred breeding farms of the Ukrainian black and white dairy breed originate (respectively, 493 ($P<0.05$); 26 ($P<0.05$); 24 ($P<0.05$). That is, the use of bulls evaluated by the genotype of the beta-casein gene will allow not only to obtain milk of the desired quality, but also ensure breeding progress in dairy herds.

Similar work is also being done at the State Enterprise "Experimental Farm of the North East Institute of Agriculture of of the National Academy of Sciences", on the formation of a dairy herd for the production of more pure milk. For this purpose, genotyping of cows for this trait was carried out on the farm, and breeders with the desired genotype of BB according to kappa-casein were assigned to the breeding herd (n=8). Their average breeding value for milk yield is 507±130.2, the amount of milk fat is 56±11.1, and milk protein is 42±7.6, which is higher than the average indicators of breeders with other genotypes for kappa-casein (n=26). A statistically significant difference was established in favor of breeders with the BB genotype in terms of breeding value according to the amount of milk fat in milk ($P<0.05$). It should be noted that in the breeder of Iskra LLC, bulls are used that have not been evaluated for the kappa-casein gene, and in the breeding center "Pershe Travnya" a proportion of the breeders are evaluated, but have AA, AB, AE and VE genotypes. Animals of these genotypes are considered to produce milk with the quality worse for obtaining cheese. Therefore, breeders of these genotypes (n=10) are not recommended to be used in the breeding stock. It should be noted that their average breeding value for milk yield is slightly higher (786±254.4) than experimental animals with the BB genotype, but they are inferior to them in terms of the average breeding value for the amount of milk fat (39±14.6) and protein (37± 10.3). No statistically significant difference was found.

According to the results of univariate variance analysis, we confirmed the presence of statistically significant influence of such factors as lineal affiliation, country of origin, year of birth and degree of kinship with the progenitor of the line on the breeding value of the breeders (Table 1).

Table 1

The influence of individual factors on the breeding value of breeders

Indicator	Factor			
	linear belonging	country of origin	year of birth	degree of kinship
Yield	11.4	16.6*	27.7**	25.8*
Milk fat	26.3**	15.1*	47.6***	48.3***
Milk protein	31.6***	22.1**	57.9***	53.2***

*Note: * - P<0.05; ** - P<0.01; *** - P<0.001;*

It has been established that lineal affiliation does not have a statistically significant effect on the breeding value of bulls by the milk yield. In our opinion, this is due to the fact that selections for increasing the yield have begun to pay less attention to other economically useful traits. It should also be noted that in countries with developed dairy breeding, breeders are usually obtained with the help of interlineal crosses. On the contrary, linear belonging has a statistically significant effect on the indicators of breeding value of the amount of milk fat and protein. This confirms the opinion that breeders began to pay more attention to qualitative indicators of milk productivity. The country of origin of the bull probably influenced all the investigated indicators. The high influence of the year of birth and the degree of kinship with the progenitor on the breeding value of breeders in terms of the amount of milk fat and protein is confirmed by the breeders' interest in improving the quality of milk.

It should be mentioned that the widespread use of breeders of the Holstein breed on the brood stock of the Sumy inbred type of the Ukrainian black and white dairy breed led to a significant increase in conditional blood for the improving breed. The biggest share of first-borns and heifers in breeding farms have conditional blood of the Holstein breed above 95%. This creates a threat of the complete disappearance of the Sumy inbred type of the Ukrainian black and white dairy breed and its transformation into the Holstein breed. This can be prevented by using a family of breeders of the Ukrainian black and white dairy breed, which is stored in the Sumy Breeding Center LLC, for insemination of the brood stock. Another possible direction is the use of biotechnological measures to obtain and preserve biological material (egg cells, epididymal spermatozoa, embryos). However, this work requires financial support from the state.

Discussion. As a result of our research, we can determine that Holstein breeders are widely used in breeding cattle herds of the Sumy inbred type of the Ukrainian black and white dairy breed, which has led to a significant increase in conditional blood for the improving breed. These results fully coincide with the results of other researchers (Kuziv M. I. et al, 2022; Voitenko S. L. and Sydorenko, O., 2019). In recent years, there has been a narrowing of the genealogical structure of breeders of Holstein cattle due to the mass use of breeders of three Holstein lines Starbuck 352790, Elevation 1491007, Chiff 1427381, which is also noted by domestic researchers (Pochukalin A. Y. and Pryima S. V., 2021). The dependence of breeding value of breeders on country of origin and year of birth was also established (Kuziv M. I. et al 2022; Voitenko S. L. and Sydorenko O., 2019). Recently, breeders began to pay attention to the genotypes of breeders for milk proteins (Kulibaba R. et al, 2023). Therefore, we can claim to confirm the general trends regarding the use of breeders of the Holstein breed in Ukraine.

Conclusions 1. It was established that most of the cows originate from breeders of four genealogical lines of the Holstein breed: Starbuck 352790 (28%), Bell 1667366 (27%), Chiff 1427381 (26%) and Elevation 1491007 (15%). At the same time, the genealogical structure of the heifers is represented mainly by three genealogical lines: Elevation 1491007 (41%), Chiff 1427381 (30%) and Starbuck 352790 (27%).

2. The interlineal differentiation of the breeding value of breeders was established by the amount of milk yield, the amount of milk fat and protein. The statistically significant difference was established between individual genealogical lines according to the investigated indicators. The higher average breeding value in terms of milk yield is characteristic of breeders of the Chiff 1427381 line (448±166.6), and in terms of the amount of milk fat and protein – the Elevation 1491007 line (36±7.6) and (32±5.7), respectively.

3. A greater number of breeders are of German and American origin, with the latter prevailing in breeding value for milk yield, amount of milk fat and protein, respectively (599±137.7), (34±7.9), (31±5.6) ($P<0.01$).

4. There is an increase in the average breeding value of bulls with an increase in the indicator of the number of generations between the breeder and the progenitor. As the age of breeders decreases, the average breeding value increases.

5. In the experimental farm, heifers come from breeders with a genotype for beta-casein A2A2 and for kappa-casein BB, which contributes to obtaining animals with the desired genotypes according to these genes.

6. As a result of one-factor variance analysis, a statistically significant influence of linear affiliation, year of birth, distance from the progenitor, country of origin on the breeding value of the breeders was established.

7. In order to preserve the Sumy inbred type, we recommend using a family of breeders of the Ukrainian black and white dairy breed for insemination of the brood stock and applying biotechnological measures to obtain and preserve biological material.

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EFFICIENCY OF THE APPLICATION OF HUMIC PREPARATIONS IN FORMING THE YIELD OF WINTER WHEAT

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The relevance of the use of nitrogen inhibitors is determined by three main aspects, namely, reducing the amount of nitrogen fertilizers and, therefore, the chemical load on the environment, increasing the yield of agricultural crops, and economic feasibility. There is some information about the positive effect of the combined use of a nitrogen inhibitor and fulvic acid preparations, but there is no data on the role of humic substances such as nitrogen inhibitors. The results of the research showed a significant impact of the use of humic preparations on the formation of the yield of winter wheat. The use of UltraBoost for seed for pre-sowing treatment in the amount of 1 kg/t of seeds contributed to an increase in productivity by 0.22–0.29 t/ha, which was 4.4–6.4%. The norm of 1 kg/ha ensured a statistically significant increase in yield by 5–7.9%, which was 0.23–0.4 t/ha. In the variants of the experiment with a rate of 3 l/ha, the increase was 8.8–11% or 0.38–0.51 t/ha. The use of UltraBoost for NH_3 and pre-sowing seed treatment contributed to an increase in yield by 7.1–7.5%. The rate of UltraBoost for NH_3 of 1 l/ha provided an increase in yield by 0.25–0.4 t/ha, which in relative terms amounted to 3.9–7%. Twice as much rate led to an increase in productivity by 0.31–0.69 t/ha or by 10.4%. The use of nitrogen inhibitor UltraBoost for NH_3 leads to an increase in the content of total nitrogen in the soil. In the variants with the application of 1 l/ha, an increase in the content was noted on average by 2.06 g/m^3, which was 14.3%, and in the case of application of 2 kg/ha, the content of total nitrogen in the soil was higher on average by 3.28%, which was 22.7%. The rate of alkaline hydrolyzed nitrogen in these variants was higher by 3.5–8.8%. In variants with a rate of 1 l/ha of UltraBoost for NH_3, the content of total nitrogen increased by 11.5%, and on variants with a rate of 3 l/ha – by 12.7%. The similar trend was also noted for the content of alkaline hydrolyzed nitrogen as the increase was 13.4 and 14.1%, respectively.

Key words: wheat, nitrogen, inhibitors, nutrients, productivity, humic preparations.

ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ ГУМІНОВИХ ПРЕПАРАТІВ У ФОРМУВАННІ УРОЖАЙНОСТІ ПШЕНИЦІ ОЗИМОЇ

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Актуальність застосування інгібіторів азоту обумовлюється трьома головними аспектами – зменшення кількості азотних добрив, а отже, хімічного навантаження на довкілля, підвищення врожайності сільськогосподарських ку-

льтур, економічною доцільністю. Існує інформація про позитивний ефект від сумісного застосування інгібітора азоту та препаратів фульвових кислот, але не зустрічається даних про роль гумінових речовин як інгібіторів азоту. Результати досліджень показали в цілому істотний вплив застосування гумінових препаратів на формування врожайності пшениці озимої. Використання для передпосівної обробки препарату UltraBoost for seed в кількості 1 кг/т насіння сприяло збільшенню урожайності на 0,22–0,29 т/га, що становило 4,4–6,4 %. Норма в 1 кг/га забезпечила статистично достовірну прибавку врожайності на 5–7,9 %, що становило 0,23–0,4 т/га. На варіантах досліду з нормою 3 л/га прибавка становила 8,8–11 % або 0,38–0,51 т/га. Застосування UltraBoost for NH_3 та передпосівної обробки насіння сприяло збільшенню врожайності на 7,1–7,5 %. Норма UltraBoost for NH_3 в 1 л/га забезпечила зростання врожайності на 0,25–0,4 т/га, що у відносному значенні становило 3,9–7 %. Удвічі більша норма призвела до збільшення урожайності на 0,31–0,69 т/га або ж на 10,4 %. застосування інгібітора азоту UltraBoost for NH_3 призводить до збільшення вмісту загального азоту в ґрунті. У варіантах із застосуванням 1 л/га відмічалося збільшення вмісту в середньому на 2,06 г/м^3, що становило 14,3 %, а в разі застосування 2 кг/га вміст загального азоту в ґрунті був більшим у середньому на 3,28 %, що становило 22,7 %. Показник лужногідролізованого азоту в цих варіантах був вищим на 3,5–8,8 %. На варіантах з нормою 1 л/га UltraBoost for NH_3 вміст загального азоту збільшився на 11,5 %, а на варіантах з нормою 3 л/га – на 12,7 %. Подібна тенденція також була відмічена й для вмісту лужногідролізованого азоту – збільшення становило відповідно 13,4 та 14,1 %.

Ключові слова: пшениця, азот, інгібітори, поживні елементи, урожайність, гумінові препарати.

Introduction. The problem of nitrogen losses in agriculture has a multifaceted nature, being felt in both of its main branches – crop and animal husbandry. If in animal husbandry it has, for the most part, an ecological nature, then in the field of plant breeding it also entails a significant economic aspect. First of all, it is related to nitrogen nutrition of plants. The greatest losses of nitrogen are observed, as a rule, in the case of the use of liquid nitrogen fertilizers, anhydrous ammonia, manure, organic fertilizers such as biochar, siderates, etc. Thus, there is a need to develop and implement techniques for the rational use of nitrogen fertilizers. Forms, norms, terms, methods of application of nitrogen fertilizers represent a complex of factors and their interactions, which is complicated by the unpredictability of growing conditions, particularly weather conditions.

The use of biochar in combination with nitrogen inhibitors reduced the emissions of N_2O and NO by 49.1–49.7% and 51.7–55.2%, and NH_3 by 33.4–35.2%, and also increased the yield of wheat by 13-22%, and the biomass of wheat plants – by 38% (Li Q. et.al., 2015, He T. et al., 2018, Dawar K. et al., 2021). In other studies, nitrogen emissions decreased by 23–45% and wheat yield increased by 12% (Li Y. et al., 2015).

The yield increase, depending on the timing of nitrogen application and the use of urease inhibitors, can reach up to 29% if fertilizers are applied in the spring (Mohammed Y. A. et al. 2016, Owens J. L. et al., 2022). However, there are also data on the high efficiency of application at different temperature regimes (13–15 °C) and the frequency of treatments (Wang Y. et al., 2018). The type of soil can also be a significant factor as larger increases at the level of 7.5–12.9% were obtained on soils with a heavy structure that have an alkaline reaction (Abalos D. et al., 2014). On different soils, the agronomic efficiency of inhibitors can be 8.4–31.5%, and grain weight can increase by 9.1% (Kiani S. et al., 2014).

The use of nitrogen inhibitors also affects the assimilation of other nutrients, in particular phosphorus (Bronson K. F. et al., 1991; Ehsanpour F. et al., 2013; Polychronaki E. et al., 2013), which contributes to the increase in yield and the ratio of main and secondary products In addition to increasing yield, the use of nitrogen inhibitors, in particular nitrapyrin, is considered as an alternative to repeated application of nitrogen fertilizers, and can also develop the effect of nitrogen fertilizers for the next crop in the crop rotation (Huber D. M. et al., 1980, Owens J. et al., 2019).

Nitrogen inhibitors can have a different chemical structure and origin, and therefore are not always equally effective. In studies with such inhibitors as trichloroethylphosphodiamidate, diethylphosphoniumtriamide, dimethylphosphoniumtriamide, N-(diaminophosphinyl) cyclohexylamine, N-benzyl-N-methylphosphoniumtriamide and phenylphosphorodiamide, the effectiveness of urea fertilizers was not noted (Schlegel A.J., Nelson D.W., Sommers L.E., 1987), and DMPP inhibitors (3,4-dimethylpyrazole phosphate) and DMPSA (3,4-dimethylpyrazoluccinic acid) reducing N_2O emissions to a minimum do not affect yield reduction (Huerfano X. et al., 2016). In arid conditions, inhibitors also play a role, which consists mainly of reducing emissions into the atmosphere, rather than significantly increasing yield or grain quality (McKenzie R. H. et al., 2010). It is also necessary to take into account the stages of plant development, since the inhibitor may not affect even the content of mineral nitrogen, if it is applied in the initial phases of grain formation (Poškus K. et al., 2021).

Some scientific works emphasize the use of nitrogen inhibitors as a means that reduces environmental risks and does not affect economic efficiency (Kong D. et al., 2021, Mirkhani R. et al., 2021, Hu Y. et al., 2014), and sometimes such an effect may be absent, especially in arid conditions (An H. et al., 2020).

There is some information about the positive effect of the combined use of the nitrogen inhibitor DMPP and fulvic acid preparations (Elsaka M. S., Abo Elsoud H., 2019), but there is no data in the literature on the role of such humic substances as nitrogen inhibitors, so the purpose of this work was to determine the effectiveness of humic preparations, which are food sources for microbes that feed on nitrogen and bacteria, break down stubble residues in the soil, which becomes mineralized and then assimilated by the plant. Instead of reducing bacteria as other inhibitors do, the UltraBoost for NH_3 formula allows nitrogen to bind to soil colloids within the top 8-10 inches of soil.

Relevance and purpose of research. It can be seen from the given material that the relevance of using nitrogen inhibitors is determined by three main aspects such as reducing the amount of nitrogen fertilizers and, therefore, the chemical load on the environment, increasing the yield of agricultural crops, and the economic expediency arising from the previous two factors. At the same time, a scientific and industrial discussion continues on each of the mentioned issues. In Ukraine, the economic aspect is most acutely felt, as the cost of fertilizers has increased sharply, and the prices of agricultural products have reacted negatively as a result of hostilities. The purpose of the research was to determine the effectiveness of using nitrogen inhibitors UltraBoost for NH_3 to increase the yield of winter wheat.

Research materials and methods. UltraBoost for seed is a liquid preparation for pre-sowing seed treatment with a recommended application rate of 0.5–1 kg/t for grain crops, which contains 10% humic, 3% fulvic and 1% ulmic acids. According to the manufacturer, the drug has a local inhibitory effect on the development of *Nitrosomonas* bacteria and stimulates an increase in the content of cytokinins in seedlings. UltraBoost for NH_3 is a liquid preparation intended for simultaneous use with KAS or anhydrous ammonia and is declared a nitrogen inhibitor. It contains 17.8% humic, 4.77% fulvic and 1.19% ulmic acids.

In the first experiment, the influence of varietal properties, drugs and inhibitor application rates on the yield of winter wheat was determined, in the second one, the effectiveness of UltraBoost for NH_3 nitrogen inhibitor application rates in the conditions of farms in the Poltava region was researched. UltraBoost for NH_3 is a CAS inhibitor that contains 16% humic, 3% fulvic and 1% ulmic acids and 7% oligomerized mixture of N-cyanoguanidine.

The experiments were conducted in the production conditions of farms that are in conditions of unstable moisture in the Left Bank Forest Steppe of Ukraine during 2020–2021 (factor A). In one of the experiments, the effectiveness of the nitrogen inhibitor UltraBoost for NH_3 (factor C) was established in the process of growing wheat varieties Boria and Postat (factor B). The cultivation technology used in the farm was taken as control. Methods of application were investigated. In the second experiment with the Smuglyanka wheat variety, the multifactorial complex consisted of year conditions (factor A), farming technology (factor B) and also the rate of application of the humic nitrogen inhibitor UltraBoost for NH_3 (factor C). Factor B combined agrotechnical methods typical for enterprises such as selection of a predecessor, fertilizers, features of crop protection.

The soil of the experimental plots is leached chernozem with a humus content of 3.96%; the depth of the humus horizon is 52–96 cm; pH – 6.0; hydrogen content - 105.6; P_2O_2 – 118.7; K_2O – 119.3 mg/kg. The seed sowing rate was 5 million seeds/ha, fertilizer – 100 kg/ha of KAS in physical volume. Winter wheat varieties studied were Boriya, Postat and Smuglyanka.

The area of the experimental site is 3.08 hectares, the repetition is three times, the placement is randomized. Statistical processing of the results of the experiment was carried out using the methods of dispersion and correlation analysis using the STATISTICA 10.0 program.

The research results showed a significant effect of inhibitor application on the formation of winter wheat yield. The use for pre-sowing treatment of the drug of the same name UltraBoost for seed in the amount of 1 l/t of seeds contributed to an increase in productivity by 0.22–0.29 t/ha, which was 4.4–6.4% (Table 1). The use of the nitrogen inhibitor UltraBoost for NH_3 turned out to be somewhat more effective. The norm of 1 l/ha ensured a statistically significant increase in yield by 5–7.9%, which was 0.23–0.4 t/ha. In the variants of the experiment with an inhibitor rate of 3 l/ha, the increase was 8.8–11% or 0.38–0.51 t/ha.

The obtained results indicate the prospect of using a nitrogen inhibitor in the economic conditions of a significant increase in the price of nitrogen fertilizers. From an economic point of view, it can be assumed that the use of UltraBoost for NH_3 can provide a 10% reduction in fertilizer application rates. However, in further programs, it is necessary to investigate the effect of this inhibitor on different types of soil and moisture conditions, because depending on these factors, the dynamics of changes in the amount and form of nitrogen may be different.

Dispersion analysis made it possible to single out the main factors that had the greatest impact on yield formation (Fig. 1). The largest share was the factor of selection of the variety for cultivation, which was almost 60%, the year conditions played a smaller role during the research period, only 36%.

It is significant that the use of humic preparations had a statistically significant value ($p \leq 0.01$), although it is small, but it is also important for yield management and forecasting – almost 4%. On average, the use of a nitrogen inhibitor and pre-sowing seed treatment contributed to an increase in yield by 7.5%. In the conditions of intensive crop production, such an effect deserves attention, as it has the prospect of not only an

increase in yield, but also a significant economic effect due to the use of lower rates of nitrogen fertilizer application. The total impact of regulated factors in the experiment was 64%. This is quite a high indicator for managing the formation of wheat yield.

Table 1

Yield depending on varietal properties and inhibitor application (2020-2021)

Variety	Way of application	Yield, t/ha	Increase, %
Boria	St (without treatment)	4.54	
	UltraBoost for seed (1 l/t)	4.83	6.4
	t	4.77	5.0
	UltraBoost for NH_3 (3 l/ha)	4.95	9.0
Postat	St(without treatment)	3.86	-
	UltraBoost for seed (1 l/t)	4.19	8.6
	UltraBoost for NH_3 (1 l/ha)	4.12	6.6
	UltraBoost for NH_3 (3 l/ha)	4.28	11.0
Boria	St (without treatment)	5.07	-
	UltraBoost for seed (1 l/t)	5.29	4.4
	UltraBoost for NH_3 (1 l/ha)	5.47	7.9
	UltraBoost for NH_3 (3 l/ha)	5.58	10.1
Postat	St(without treatment)	4.38	-
	UltraBoost for seed (1 l/t)	4.68	6.8
	UltraBoost for NH_3 (1 l/ha)	4.64	5.9
	UltraBoost for NH_3 (3 l/ha)	4.76	8.8
HIP_{01} *Conditions of growing years (factor A)*		*0.05*	*7.5*
HIP_{01} *Variety (factor B)*		*0.04*	
HIP_{01} *Method of application (factor C)*		*0.07*	

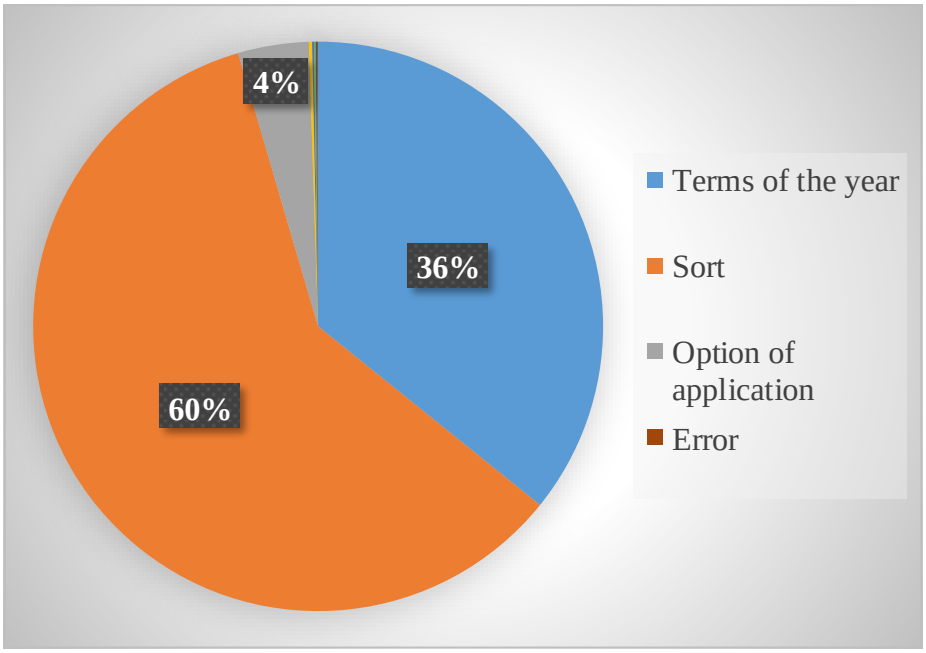

Fig. 1. **The share of the influence of the factors forming the yield of winter wheat varieties depending on the method of application of the nitrogen inhibitor**

In a parallel experiment in different farms of the region with the Smuglyanka wheat variety, where only a nitrogen inhibitor was used, regular increases in yield were also established. And although they were small in relative terms, they were statistically reliable. During the two-year research period, the use of a nitrogen inhibitor made it possible to increase the yield, on average, by 7.1% (Table 2). The rate of UltraBoost for NH_3 of 1 l/ha provided an increase in yield by 0.25–0.4 t/ha, which in relative terms amounted to 3.9–7%. Twice the rate led to an increase in productivity by 0.31–0.69 t/ha or by 10.4%, depending on the cultivation technologies used in the individual farm.

Dispersion analysis of the results of the experiment made it possible to identify the main factors of productivity formation. Cultivation technologies, which differed in the system of fertilization, protection, selection of a predecessor, etc., had the largest share, depending on the cultivation system adopted in each farm separately. Nevertheless, the inhibitor application factor was 8%, which is quite comparable to the previously described experiment. The conditions of the year also had a significant influence, which amounted to 19% (Fig. 2).

Of course, in the case of longer experiments, the conditions of the year can have a much greater influence, especially in years with extreme manifestations of growing factors. Further studies of the effectiveness of nitrogen inhibitors under conditions of temperature, water or salt stress are necessary. It is also advisable to conduct a study on the effectiveness of using this nitrogen inhibitor in combination with anhydrous ammonia.

Table 2

The productivity of the Smuglyanka variety depends on farm conditions and nitrogen inhibitor

Year	Farms	Way of application	Yield, t/ha	Increase, %
2020	FE "Agrosvit SV"	St (without treatment)	6.42	-
		UltraBoost for NH_3 (1 l/ha)	6.67	3.9
		UltraBoost for NH_3 (2 l/ha)	6.73	4.8
	LLC "Karlivske FE "LOS"	St (without treatment)	5.76	-
		UltraBoost for NH_3 (1 l/ha)	6.16	7.0
		UltraBoost for NH_3 (2 l/ha)	6.37	10.6
	FE "Zlatopil"	St (without treatment)	4.95	-
		UltraBoost for NH_3 (1 l/ha)	5.26	6.3
		UltraBoost for NH_3 (2 l/ha)	5.42	9.5
2021	FE "Agrosvit SV"	St (without treatment)	6.90	-
		UltraBoost for NH_3 (1 l/ha)	7.21	4.5
		UltraBoost for NH_3 (2 l/ha)	7.59	10.0
	LLC "Karlivske FE "LOS"	St (without treatment)	6.15	-
		UltraBoost for NH_3 (1 l/ha)	6.47	5.1
		UltraBoost for NH_3 (2 l/ha)	6.79	10.4
	FE "Zlatopil"	St (without treatment)	5.36	-
		UltraBoost for NH_3 (1 l/ha)	5.63	5.0
		UltraBoost for NH_3 (2 l/ha)	5.78	7.8
HIP_{01} *Conditions of growing years*			*0.08*	
HIP_{01} *Variety*			*0.09*	
HIP_{01} *Method of application*			*0.11*	
HIP_{01} *Conditions of years* Conditions of the farm*			*0.14*	

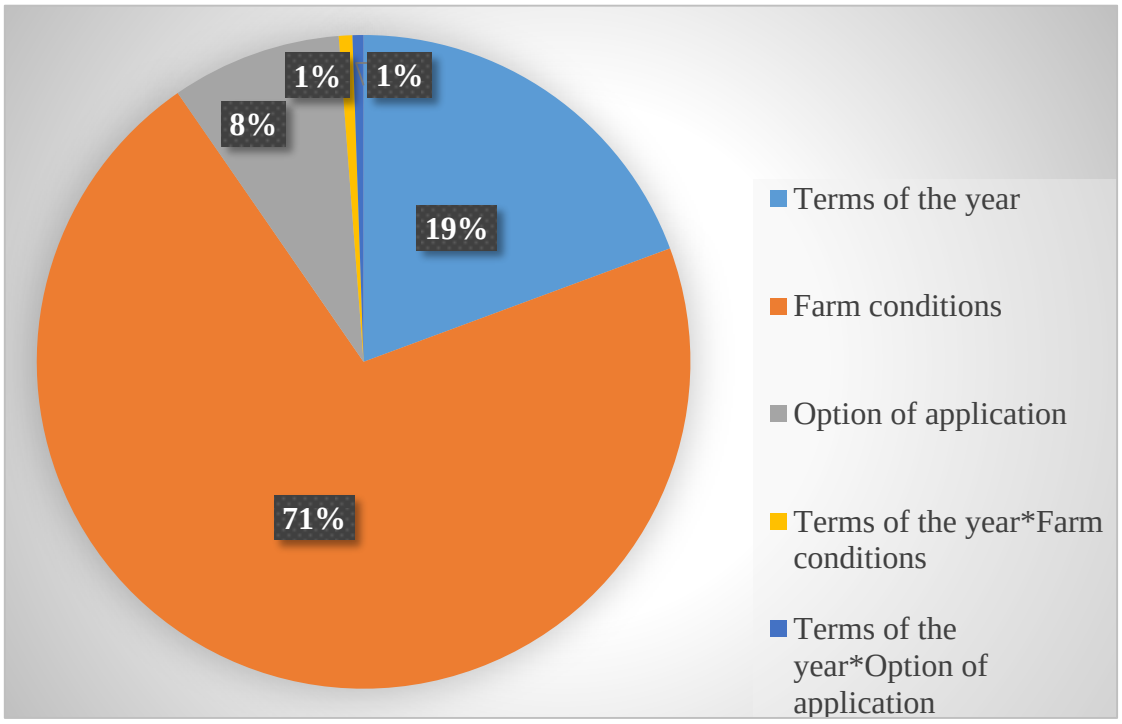

Fig. 2. **The share of the influence of factors forming the yield of winter wheat varieties depending on the use of a nitrogen inhibitor.**

Correlation analysis made it possible to establish a relationship between the nitrogen content in the soil and productivity. which may not always be present. In the first experiment. productivity correlated with the content of total nitrogen r = 0.64 and the content of alkaline hydrolyzed nitrogen r = 0.57 (Fig. 3). In the second experiment, such a dependence was not established although the productivity indicator naturally increased depending on the use of a nitrogen inhibitor.

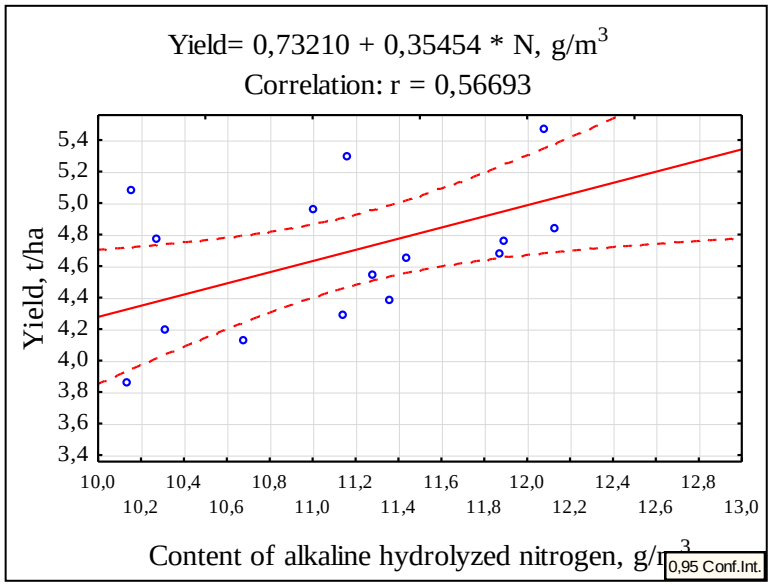

Fig. 3. **Graph of yield dependence on the content of alkaline hydrolyzed nitrogen**

As the results of the experiment showed, the use of the UltraBoost for NH_3 inhibitor leads to an increase in the content of total nitrogen in the soil (Fig. 4). In the first experiment, in the variants with the application of 1 l/ha, an increase in the content of 2.06 g/m^3 was noted on average, which was 14.3%, and in the case of the application of 2 kg/ha, the content of total nitrogen in the soil was higher on average by 3.28%. which was 22.7%.

The rate of alkaline hydrolyzed nitrogen in these variants was higher by 3.5–8.8%. As it can be seen from the research data, a certain increase in nitrogen content was also recorded in the variants where the UltraBoost for seed processor was used. In this variant, the content of total nitrogen increased by almost 5%, and alkaline hydrolyzed nitrogen – by 5.8%.

In the second experiment, a natural increase in nitrogen content due to the use of a nitrogen inhibitor was also observed. On variants with a rate of 1 l/ha of UltraBoost for NH_3, the content of total nitrogen increased by 11.5%, and on variants with a rate of 3 l/ha – by 12.7%. The similar trend was also noted for the content of alkaline hydrolyzed nitrogen as the increase was 13.4 and 14.1%, respectively. Thus, the application of a higher rate did not lead to a proportional increase in nitrogen content and grain yield.

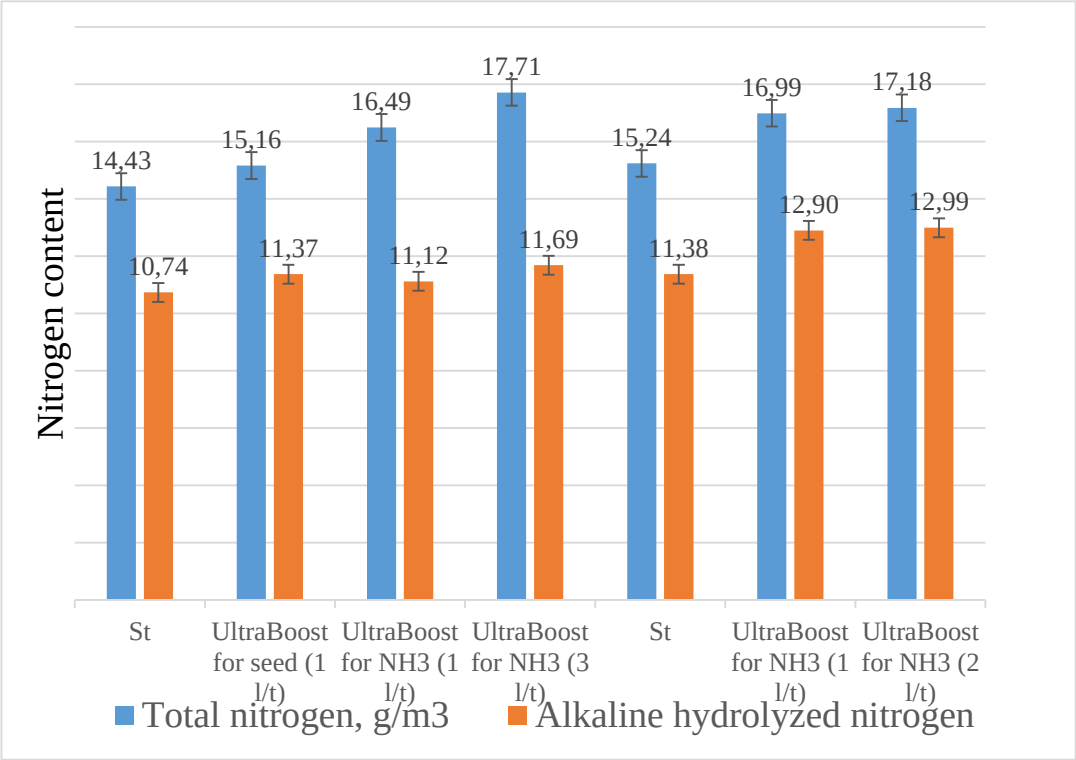

Fig. 4. **The effect of nitrogen inhibitor on the content of total and alkaline hydrolyzed nitrogen in the soil**

Discussion. The obtained research results indicate certain prospects for the use of humic nitrogen inhibitors on the example of UltraBoost for NH_3. The obtained yield increases are correlated with literature data (Mohammed Y. A. et al., 2016; Owens J. L. et al., 022; Kiani, S. et al., 2014), and the increase in the content of total and alkaline hydrolyzed nitrogen – with other sources (Mirkhani R. et al., 2021; Hu Y. et al., 2014; An H. et al., 2020). Both research years were similar in terms of sufficient moisture and

slightly lower temperatures during the spring growing season but later were characterized by insufficient moisture.

Despite the fact that in arid conditions, nitrogen inhibitors may be ineffective (Elsaka M. S., Abo Elsoud H., 2019), in our experiments a statistically significant yield increase was obtained. Moreover, there is still a need for further research, as they also require accuracy and significant multiplicity of observations of different inhibitors under different environmental conditions.

Rather broad scientific discussion in literary sources should also be taken into account, which also notes the lack of direct influence of humic preparations and nitrogen inhibitors on yield formation. After analyzing a sufficient number of literary sources, it is possible to conclude that the research results obtained by scientists are ambiguous depending on the growing conditions and active substances of nitrogen inhibitors.

Conclusions. The main factors that shape the yield of wheat in conditions of unstable moisture are the selection of varieties for cultivation and agro-ecological conditions, the share of which can reach 60 and 71%, respectively. Furthermore, the annual conditions can be 19–36%, and the share of inhibitor use in the formation of yield ranged from 4–8%. The rate of UltraBoost for NH_3 of 1 l/ha provided an increase in yield by 0.25–0.4 t/ha, which in relative terms amounted to 3.9–7%. Twice as much rate led to an increase in productivity by 0.31–0.69 t/ha or by 10.4%. Application of the nitrogen inhibitor UltraBoost for NH_3 resulted in an average yield increase of about 7%, which was statistically significant. Thus, in conditions of unstable moisture, the use of nitrogen inhibitors may have a perspective for use in order to save nitrogen fertilizers and, possibly, increase the yield of winter wheat.

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DISPOSAL OF MANURE AT THE PIGGERY USING BIODESTRUCTORS OF DIFFERENT ORIGIN

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The development of biotechnological methods of disposal of pig waste products is relevant at the current stage. However, the high cost of investment and operation of such technologies limits their application. Therefore, owners of pig farms are constantly looking for compromise solutions that would, on the one hand, ensure compliance with technological requirements for manure removal, and on the other hand, not pollute the environment.

The purpose of the work is to improve existing technologies and develop innovative approaches to biotechnological methods of manure disposal.

Scientific research was carried out at the piggery of Agroprime Holding LLC of the Odesa region with a capacity of 30,000 heads per year. The considered technology of using biological preparations De-Scentase and Complesin for descentization of manure in underground baths, manure storages, manure sites of the complex. The content of gases in the manure subfloor bath of the piggery due to the use of biological preparations was determined. The conducted monitoring of unpleasant scents and harmful gases confirmed the expediency of using new methods of neutralizing unpleasant scents. It was established that the reconstruction of the separator station by installing a metal hangar and a water-dispersion filtration chamber ensured the neutralization and release of scentous gases into the environment and did not affect the ecological state of the environment negatively.

On the basis of the conducted research, the method of wide application of the biological preparation for the disposal of manure has been developed.

The improved technology of manure disposal at the piggery, which involves the addition of the microbial destructor drug Complesin to underground baths, as well as to manure storages and manure sites, helps to reduce the release of harmful gases and accelerates the disposal of manure.

The technology of utilization of liquid and solid fraction of manure by using biological preparation and vermiculture is highlighted. The experience of using equipment for underground application of liquid manure to fields is presented.

Key words: piggery, manure removal, utilization, ecology, harmful gases, biodestructors.

УТИЛІЗАЦІЯ ГНОЮ НА СВИНОКОМПЛЕКСІ ЗА ВИКОРИСТАННЯ БІОДЕСТРУКТОРІВ РІЗНОГО ПОХОДЖЕННЯ

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Актуальним на сучасному етапі є розробка біотехнологічних способів утилізації продуктів життєдіяльності свиней. Однак висока вартість інвестицій та експлуатації таких технологій обмежує їх застосування. Тому власники свинокомплексів постійно знаходяться в пошуках компромісних рішень, які б з одного боку забезпечували дотримання технологічних вимог щодо видалення гною, а з другого – не забруднювали навколишнє середовища.

Метою роботи є удосконалення існуючих технологій та розробка інноваційних підходів до біотехнологічних способів утилізації гною.

Наукові дослідження проведені на свинокомплексі ТОВ «Агропрайм Холдинг» Одеської області потужністю 30 000 голів у рік. Розглянута технологія використання біологічних препаратів Де-Одорази і Комплезіну для дезодорації гноївки у підпідлогових ваннах, гноєсховищах, гнойових майданчиках комплексу. Встановлено вміст газів у гнойовій підпідлоговій ванні свинарника за використання біопрепаратів. Проведений моніторинг неприємного запаху і шкідливих газів підтвердив доцільність застосування нових способів нейтралізації неприємних запахів. Встановлено, що реконструкція сепараторної станції шляхом встановлення металевого ангару і вододисперсійної фільтраційної камери забезпечило нейтралізацію та виділення сморідливих газів у навколишнє середовище і не впливає негативно на екологічний стан довкілля.

На основі проведених досліджень розроблено спосіб широкого застосування біопрепарату для утилізації гною.

Удосконалена технологія утилізації гною на свинокомплексі, яка передбачає додавання мікробного препарату-деструктора Комплезіну у підпідлогові ванни, а також у гноєсховища та гнойові майданчики сприяє зменшенню виділення шкідливих газів і прискорює утилізацію гною.

Висвітлена технологія утилізації рідкої і твердої фракції гною шляхом використання біопрепарату та вермикультури. Наведено досвід застосування обладнання для підземного внесення рідкого гною на поля.

Ключові слова: свинокомплекс, гноєвидалення, утилізація, екологія, шкідливі гази, біодеструктори.

Introduction. Waste from pig farming is considered as one of the most important factors of environmental pollution in most parts of the world. That is why scientists and practitioners of different countries of the world are intensively working on cost-effective methods of manure utilization (Postelga S. et al., 2019; Amon M., et al., 1995; Kyrylenko I. G., et al., 2020; Tokarchuk D. M. et al., 2021; Andretta I. et. al., 2021).

Most of the unpleasant scents coming from pig houses are a complex mixture of volatile gases that are products of animal life. These include ammonia, hydrogen sul-

fide, indoles, skatoles, phenols, mercaptans, and others. Most often, the scent is the result of uncontrolled anaerobic decomposition of manure.

There are many modern technologies for liquid manure removal and disposal and scent minimization, such as ration modification, ventilation, daily manure cleaning, application of biofilters of a vacuum manure removal system and its processing in biogas plants, covering lagoons with plastic film, aromatization of manure with microbial preparations, hydraulic dust capture (Voloshchuk V. M. et al., 2014; Le P. D. et al., 2007; Niraj B. et al., 2021; Norton T. et al., 2013; Zhang, G. et al., 2008).

Changing the diet of pigs to reduce crude protein from 16.5% to 12.5% reduced scent and ammonia emission ($p<0.01$) from pig manure by 12.5% (Yastrebov K. et al., 1997).

Other foreign authors came to the conclusion that feeding pigs with four types of feed with a crude protein content of 130, 160, 190, 220 g/kg increased the rate of scent emission compared to the first by 3.09, 49.22 and 32.60%, respectively, and the level of ammonia emission per animal also increased by 25.08; 89.38; 165.91% (Iregbu G.U. et al., 2014).

American scientists found that well-designed and managed biofilters in livestock complexes can reduce scents and hydrogen sulphide and ammonia by 95 and 80 percent, respectively (Hayes E. T., et al., 2004).

Biogas plants, which not only produce biogas, but also significantly reduce environmental pollution, are an effective means of manure utilization. Anaerobic processing of pig manure in biogas plants helps reduce environmental pollution, protects public health, and also provides valuable organic fertilizer and biogas (Ferrer I., et al., 2009; Gianni K. A., et al., 2012; Zubair M., et al., 2020). However, their wide application in farms is restrained by the economic feasibility of biogas production.

It is known that microorganisms play a leading role in the manure processing. According to Ukrainian scientists, in order to speed up the solution of the problem of manure processing at modern pig farms, a combination of anaerobic fermentation and accelerated biothermal composting technologies should be implemented (Shevchenko I. A., et al., 2012; Shumeiko K., et al., 2020).

It has been established that the microbiological drug TM "Vodogray" contributes to the removal of an unpleasant specific scent from the premises for several days. The author explains their ability to assimilate nitrogen from urea, disinfect the substrate from bacteria that cause purulent anaerobic processes from the release of ammonia and hydrogen sulfide, and insists that future innovations in manure storage, processing and wastewater treatment will be based on biotechnologies (Byedenkov E. L., 2015).

A number of Ukrainian scientists used the microbiological drug "Bioprogress"[1] to dispose manure.

For this, the slatted floor in the compartment for pigs is sprayed with a 50% aqueous solution of the biological drug "Bioprogress" at the rate of 300 g of solution per 1 head of pigs. Spraying is carried out once a week on a clearly defined day 1-2 days after washing away the waste. Next, the waste is sent for separation into fractions, and the liquid fraction is sent to lagoons, where 6 liters of Bioprogress are added to 1 ton of manure.

The disadvantage of this method is that before using the biological drug, firstly, it is necessary to reduce the alkalinity of fresh manure from (pH 8.0) to neutral (pH 6.1-

[1] Pat. 143443. Ukraine. The method of waste disposal of pig farms. Broschak I.S., Malyuta Yu.S., Guivan M.D. and others appl. 10.03.2020 publ. 27.07.2020, Bull. 14

7.0) with appropriate reagents, which increases the cost and complicates the technological operation.

On the basis of the above-mentioned materials, the development of biotechnological methods of disposal of pig waste products is currently relevant.

However, the high cost of investment and operation of such technologies limits their application. Therefore, the owners of pig farms are constantly looking for compromise solutions that would, on the one hand, ensure compliance with the technological requirements for manure removal, and, on the other hand, not pollute the environment.

The purpose of the work is to improve existing technologies and develop innovative approaches to biotechnological methods of manure disposal.

Research materials and methods. The research was conducted on the basis of "Agroprime Holding" LLC of the Odesa region in the sector of fattening young animals. Determination of the effectiveness of the descentization process of liquid manure was carried out in two experimental and one control bath, in the sector for fattening pigs. Complesin biodestructor was added to the first experimental bath (the drug is obtained by deep cultivation of B. Subtilis and B. Licheniformis bacterial associations with the addition of nutrients and minerals) at the rate of 10 liters of working solution per 1 m^3 of manure. In the second experimental bath, 3 g of De-Scentase, which is obtained from yucca extract, suspended in 10 liters of water, were added daily. No drugs were added to the control bath. The experiment lasted for 105 days. Every month, the content of carbon dioxide, ammonia and hydrogen sulfide was determined using a Dozor-S-M-4 gas analyzer.

The scent was assessed using an organoleptic method according to a five-point scale presented in Table 1. If the difference in the scent assessment by individual testers exceeded 1 point, the sample assessment was repeated no earlier than in 30 minutes. The final result of the test was taken as the arithmetic mean of the results of the evaluations demonstrated by the testers. The result was simplified to a whole number.

Table 1

Scale for evaluating the intensity of the scent of manure

Scent	Rating	Score
Scent not perceived	Perfectly	0
Scent perceived as not strong enough	Fine	1
Mild scent	Satisfactorily	2
Strong scent	Unsatisfactorily	3
Scent	unsatisfactorily	4

The research was conducted, and the results were processed according to well-known methods (Ibatullin I.I., et al., 2017).

Research results. The piggery of Agroprime Holding LLC of the Odesa region is an industrial specialized farm that provides continuous, rhythmic production of pork in a closed cycle with a capacity of 30,000 heads per year.

The manure removal system at the complex includes manure baths covered with a grid floor, a vacuum sewer, a separator station, a manure platform, and tanks for the accumulation of manure of various sizes (Fig. 1).

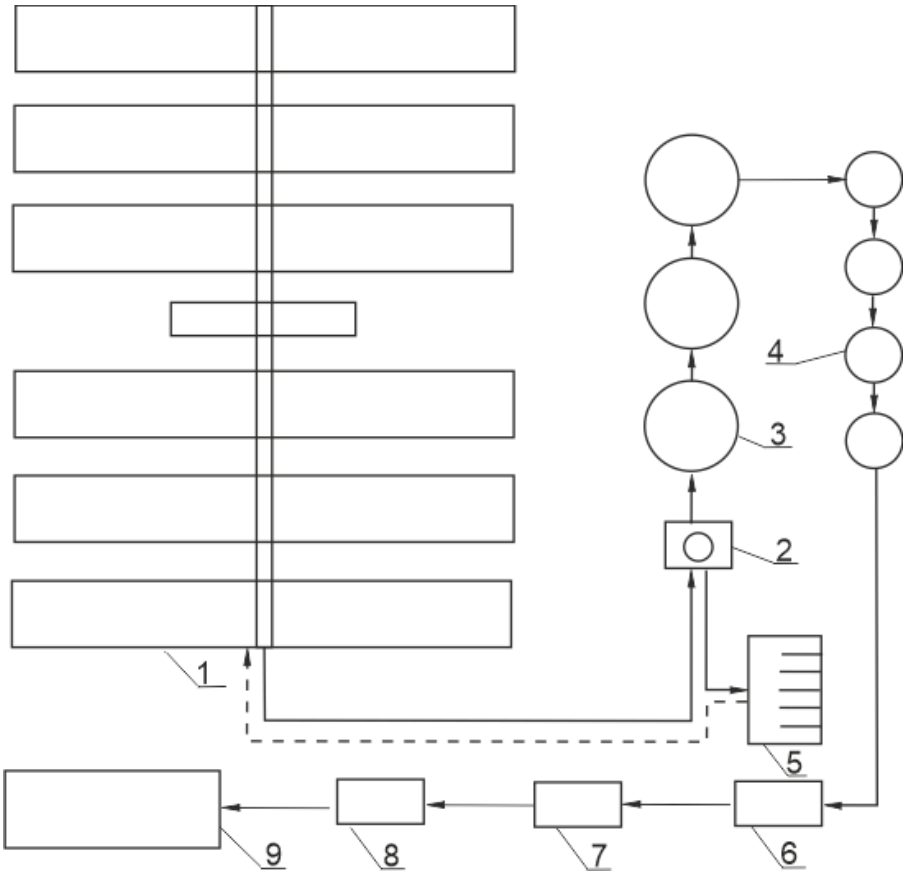

Fig. 1. **The scheme of manure removal and disposal at the piggery:** 1 – manure baths, 2 – separator station, 3 – large metal tanks, 5 – manure platform, 4 – small metal tanks, 6 – diesel pupming station for pumping manure, 7 – trailer conveyor of manure hoses A TOM -2000, 8 – injector cultivator for applying manure A TOM 7 DS, 9 – field.

The results of the use of biological drugs De-Scentase and Complesin for descentization of manure in underground baths are shown in Table 2. We found that during the monitoring process, no stinky scent was felt in the bath, which can be explained by the low level of unpleasant gases.

Table 2

The content of gases in the air of the manure subfloor bath of the piggery

Group	Content in the air		
	CO_2% v	**H_2S mg/m^3**	**NH_3 mg/m^3**
Control	0.14±0.012	6.71 ±0.251	8.13±0.621
1 experimental	0.05±0.002 ***	2.11±0.033 ***	1.21±0.017***
2 experimental	0.07±0.013	3.15±0.054	2.46±0.036

*Note: ***p<0.001.*

These tables indicate the effectiveness of the use of biologics. The level of harmful gases due to the use of biologically active substances was probably lower ($p<0.001$) in the experimental manure baths than in the control ones. The average decrease in the

concentration of hydrogen sulphide and ammonia over a 2-month period decreased by 3.3, respectively; 2.14 and 6.71; 3.30 times. There was also a reduction in the stench in baths treated with Complesin and De-Scentaz.

The use of these biological drugs for manure disinfection improved the microclimate of the room and had a positive effect on the growth energy and body weight of pigs, but the difference was unlikely (Table 3).

Table 3

Feeding qualities of experimental young pigs

Indicator	Underfloor baths of the piggery		
	Control	1 experimental	2 experimental
Livestock, head	30	30	30
Age of animals at the beginning of the experiment, days	70	70	70
Body weight at the beginning of the experiment, kg	27.54±0.81	27.22±0.79	27.48±0.68
Age of animals at the end of the experiment, days	175	175	175
Body weight at the end of the experiment, kg	105.55±1.46	107.95±1.54	108.75±1.86
Average daily increase, g	780.13±15.01	807.34±1726	812.71±16.17

The technological process of manure removal at the pig farm is as follows. Pig excrement falls through the slatted floor into the tubs, which are partially filled with water. Every two weeks, the stop plug is opened and the manure flows through the pipes into the manure storage (manure bath), which is located in the separator station. Next, the manure is fed to an auger, which separates it into liquid and solid fractions.

In order to reduce the scent from the waste products of pigs, we reconstructed the separator station. For this purpose, the manure storage and the platform for the separator were covered with a hangar made of corrugated metal sheets, and a water-dispersion filtration chamber was also installed, which ensured the neutralization and release of scentous gases into the environment (Fig. 2).

The essence of air purification is as follows. In the water-dispersion filtration chamber, fans pump polluted air through the pipes from the manure storage and the platform for the separator. In the upper part of the chamber, nozzles are installed that spray a water-dispersed mixture with which the polluted air mixes and enters the water bath. Next, the purified air is removed from the separator station by an exhaust fan.

The solid fraction of manure in the separator station is additionally treated with Complesin and taken to the manure site, where final disposal takes place in bags and large-sized packages. When adding Complesin to the fresh solid fraction of manure, the disposal process lasted 65-70 days, and without adding – 150-155 days.

The implementation of new innovative solutions in the manure disposal technology at the piggery contributed to the reduction of unpleasant scents from 3 to 1-0 points, which indicates an improvement in the environmental situation.

The liquid manure fraction is poured into tanks, where it is disposed by Complesin microorganisms, which came from underground baths. In order to increase utilization, the working solution of Complesin is additionally introduced into the tanks. Af-

ter the end of the aerobic microbial process, manure is introduced into the soil. For this, we use a pump-diesel station for pumping manure, a trailed manure hose transporter A TOM-2000, an injection cultivator for applying manure A TOM 7 DS, and thus the final disposal of manure occurs and product recycling is achieved (Fig. 3).

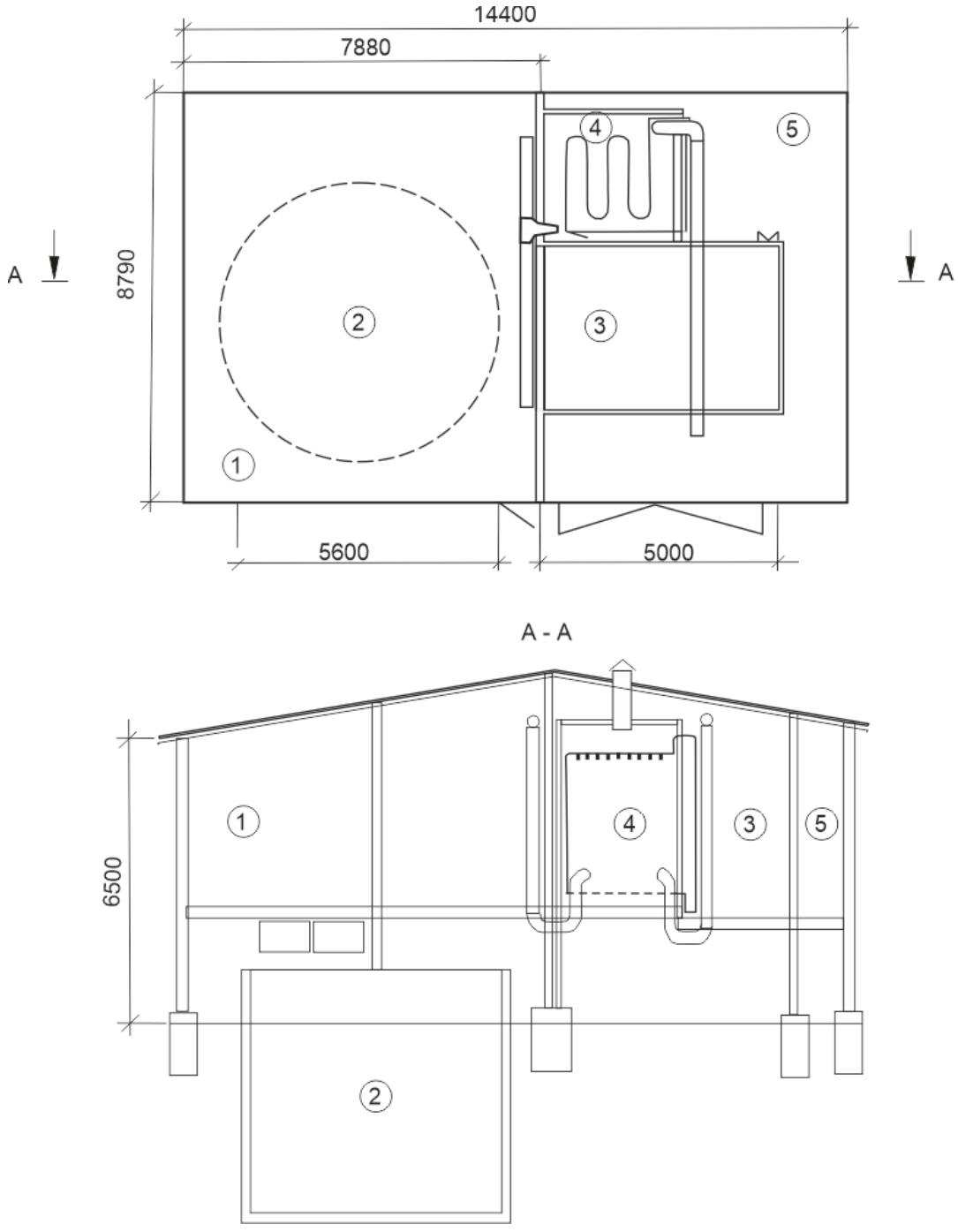

Fig. 2. **Separator station:** 1 – hangar, 2 – manure storage, 3 – site for separator, 4 – water dispersion filtration chamber, 5 – site for technological equipment.

Fig. 3. **Equipment for applying liquid manure to the fields**: 1 – diesel pumping station, 2 – trailed manure hose transporter A TOM-2000, 3 – injector cultivator for applying manure A TOM 7 DS.

The experience of using underground application of liquid manure (to a depth of 20 cm) has shown that in this way nitrogen losses are minimized and unpleasant scents are practically absent.

Discussion. The use of such biodestructors as Complesin and De-Scentase in the technological process of manure disposal at the piggery had a positive effect on the descentization of liquid manure. The obtained data are consistent with a number of foreign authors (Amon M., et al., 1995). It should be noted that the effectiveness of biological drugs is not over with the aromatization of manure. Complesin, in particular, has a prolonged positive effect on other stages of manure disposal.

Conclusions.

1. The technology of manure disposal at the piggery is improved, which involves the addition of the destructor drugs Complesin and De-Scentase to the underground baths, probably helps to reduce the release of harmful gases.

2. It has been proven that the addition of the Complesin biodestroyer to manure, which is in manure storages and manure sites, accelerates manure disposal two times faster than with a traditional manure disposal system.

3. It was established that the improved separator station for separating the solid and liquid fractions of pig manure prevents the spread of foul-scenting gases into the environment and does not negatively affect the ecological state of the environment.

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TECHNOLOGICAL AND CLIMATE FACTORS AFFECTING MILK QUALITY OF DAIRY CATTLE

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One of the most vulnerable spheres of human activity to environmental threats is agricultural production, which, on the one hand, is a source of significant emissions of greenhouse gases which is one of the causes of global warming, and, on the other hand, is the most dependent on its consequences. Ukraine has high positions in the world in many sectors of agricultural production. However, environmental threats are able to cause the transformation of the majority of the climatic and agricultural zones of Ukraine and make adjustments to the development of the agricultural sector of production. The consequences of climate change significantly affect agriculture, which is largely dependent on weather and climate conditions, and, accordingly, the country's food security. The overview of the latest research on the establishment of the main technological and climatic factors affecting the quality of milk was carried out. It has been established that most researchers emphasize such natural factors as breed, seasonal changes of the year, temperature and humidity, age of the cow, stage of lactation and interval between lactations, state of health of the animal and technological factors such as housing conditions, milking technology, fodder and feeding rations, veterinary care. In different countries, cows react differently to heat stress, animals in countries with a hot arid climate with temperatures that exceed the upper critical index for a long time suffer the most. It has been proven that an increase in temperature above the critical temperature leads to oxidative stress of the body, a decrease in the content of dry matter, fat and protein in milk and an increase in somatic cells and microbial contamination of milk. Some researchers associate the predisposition or resistance to heat stress with a genetic component, others with a violation of the conditions of keeping and feeding animals. Thus, the development of further strategic programs to reduce the negative impact of climatic and technological conditions on the production and quality of milk should include breeding programs for creating populations of thermotolerant animals, adaptive technologies that reduce stress, feeding systems with the inclusion of innovative feed products that guarantee a reduction in greenhouse emissions, animal health and food safety.

Key words: dairy cattle, heat stress, technology, milk productivity, milk quality

ТЕХНОЛОГІЧНІ ТА КЛІМАТИЧНІ ЧИННИКИ, ЩО ВПЛИВАЮТЬ НА ЯКІСТЬ МОЛОКА ХУДОБИ МОЛОЧНИХ ПОРІД

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Однією з найбільш вразливих до екологічних загроз сфер людської діяльності є сільськогосподарське виробництво, яке з одного боку є джерелом значних

викидів парникових газів – однієї з причин глобального потепління, а з іншого – найбільш залежне від його наслідків. Україна за багатьма секторами аграрного виробництва сільськогосподарської продукції має високі позиції в світі. Однак екологічні загрози здатні спричинити трансформацію переважної частини кліматичних і сільськогосподарських зон України та внести свої корективи в розвиток аграрного сектору виробництва. Наслідки зміни клімату суттєво впливають на сільське господарство, яке значною мірою залежить від погодних та кліматичних умов, та, відповідно, на продовольчу безпеку країни. Проведено огляд останніх досліджень з встановлення основних технологічних та кліматичних чинників впливу на якість молока. Встановлено, що більшість дослідників наголошують на таких природних чинниках, як порода, сезонні зміни року, температура та вологість повітря, вік корови, стадія лактації та інтервал між лактаціями, стан здоров'я тварини і технологічних чинниках - умови утримання, технологія доїння, корми і раціони годівлі, ветеринарне обслуговування. В різних країнах корови порізному реагують на тепловий стрес, найбільше страждають тварини у країнах із спекотним посушливим кліматом із температурою, що тривалий час перевищує верхній критичний показник. Доведено, що підвищення температури понад критичної, призводить до оксидативного стресу організму, зменшення вмісту сухої речовини, жиру і білка у молоці та збільшення соматичних клітин і мікробного забруднення молока. Схильність або протидію тепловому стресу деякі дослідники пов'язують із генетичною складовою, інші – із порушенням умов утримання і годівлі тварин. Таким чином, розробка подальших стратегічних програм зменшення негативного впливу кліматичних і технологічних умов на виробництво і якість молока мають включати селекційні програми зі створення популяцій термотолерантних тварин, адаптивних технологій, що нівелюють стрес, систем годівлі з включенням інноваційних кормових засобів, що гарантують зменшення парникових викидів, здоров'я тварин і безпеку продуктів харчування.

Ключові слова: молочна худоба, тепловий стрес, технологія, молочна продуктивність, якість молока

Introduction. Global warming is one of the biggest threats caused by human activities as a result of excessive use of fossil fuels as an energy resource (Al-Ghussain, 2019). As global warming increases, ecosystems, plant and animal biodiversity, and food security are threatened (Gauly M. & Ammer S., 2020). It is already widely recognized that the direct and indirect effects of global warming, combined with an increase in the frequency of extreme weather events, are a serious problem for livestock production even in temperate zones such as Central Europe. Global warming causes heat stress, which is an important problem for the dairy industry in many regions of the world. It arises from the inability of the animal's body to distribute the heat load from the metabolism of the body and the surrounding environment. As a result, body temperature rises, breathing and pulse speed up, sweating increases, and shortness of breath appears.

In a thermal environment in which the animal's heat production exceeds heat output, the body stores an increasing amount of heat, which leads to an increase in body temperature. When body temperature rises significantly, a host of homeothermic phenomena are activated, including increased heat loss through respiration and skin evaporation. However, when high temperatures and solar radiation reduce the animal's ability to radiate body heat, feed intake, metabolism, body weight, and milk production decrease, helping to alleviate the thermal imbalance. Although tissue substrates are mobilized, energy metabolism, growth and lactation are reduced (Silpa, 2021). Das R. et al.

(2016) note that dairy cattle are more sensitive to heat stress than meat cattle. This is especially true for highly productive animals, because they generate more metabolic heat.

Modern producers of dairy products divide the influencing factors into natural (breed, period of the year, age of the cow, stage of lactation and the interval between lactations, state of health of the animal) and technological (housing conditions, milking technology, fodder and feeding rations, veterinary care).

The purpose of our work is a review of modern scientific works considering this topic.

Research materials and methods. A systematic review of literature sources was performed by monitoring publications in the databases Scopus, Web of Sciences, Google Scholar, etc., published in Ukrainian and English over the past five years in accordance with the inclusion criteria. The systematization of published data was carried out with the aim of accumulating modern scientific knowledge regarding the factors affecting the quality of dairy products for the further construction of our own methodological basis for research in this direction in domestic conditions, taking into account international experience.

Research results. In 1987, Johnson described a model of the main ecosystem factors affecting a cow's ability to produce milk, grow and reproduce: meteorological (temperature, length of day) and non-meteorological (forage quantity and quality, disease). Environmental temperature and stress signal the hypothalamus and central nervous system to alter feed intake, hormonal functions, and heat production and/or loss, resulting in reduced milk production and fertility.

Quiédeville S. (2022) studied the economic indicators of the dairy sector of the European Union and established the main factors of negative impact: heat stress, which directly affects the reduction of milk productivity, heat and drought, which affect the quantity and quality of fodder for dairy cows.

Scientists are particularly concerned with the impact of climate change on the production of livestock products in a country with an arid climate. Thus, studies of the quality of milk of Holstein cows in the northeastern regions of Iran Toghdory A. et al. (2022) proved that the productivity of livestock is affected by various factors due to the complex interaction between the animal and the environment. These researchers consider the temperature and humidity of the environment to be the main factors influencing the quality of milk. They connect their conclusions with the fact that the temperature increased from 6.2 ºC to 31.3 ºC, which led to a significant decrease in the content of milk protein by 4.09% and fat by 5.75% in milk. Depending on the increase in ambient temperature, the content of nonfat dry matter (SNF) decreased by 1.31% and the content of somatic cells by 16.8%. On the other hand, as the ambient temperature increased, the total content of microorganisms in milk increased by 13.7%.

The increase in air humidity from 54% to 82% caused a significant increase in the content of milk protein (by 4.84%), fat (by 4.84%), defatted dry matter (by 1.06%) and somatic cells (by 10.2 %) in milk, on the other hand, the content of microorganisms decreased by 16.3%. The researchers confirm the results of their studies with correlations between the periods of the year, temperature and air humidity, and the content of milk components and somatic cells in it. The highest percentage of milk fat was recorded by them in January-March, the lowest in May-June. At the same time, milk fat did not change significantly during January-February, sharply decreased in April and remained low until August, increased by 1.24% in September and did not change significantly from then until the end of the year. The mass fraction of protein was the highest in March, the lowest in June. The percentage of mass fraction of dry matter in milk was

the highest in January-March, the lowest in August. The number of somatic cells in milk was the highest in March and the lowest in September-December. The maximum level of total microbial insemination was recorded in May-June, the lowest – in September.

Pacheco-Pappenheim S. et al. (2021) relate the fatty acid and triacylglycerol composition of cow's milk to seasonal changes. In their research, they indicate significant changes in the fatty acid composition of milk with increased intake of C18:3 *cis*-9,12,15 with grass in the spring and summer. The triacylglycerol profile, in turn, depended on the fatty acid composition of milk. Thus, low- and medium-molecular groups of the triacylglycerol profile increased in winter and decreased in summer, while high-molecular groups increased in summer and decreased in winter.

Ayemele A. G. (2021) proved that heat causes oxidative stress in the body, as a result of which the content of reactive oxygen species in cells increases. A significantly higher level of stress indices of catalase, SOD, GSH reductase and malondialdehyde was established in lactating cows in summer. Increased milk production during this period leads to oxidative tissue damage due to the induction of heat stress.

Picinin L. C. A. et al. (2019) established a correlation between climatic factors and physicochemical properties of raw milk, as well as the total volume of milk produced in the conditions of dairy farms in Brazil. The increase in precipitation, average daily temperature and relative humidity had a negative effect on the quality of milk and increased its bacterial contamination. Benavides R. M. et al. (2022) are sure that the most important factors affecting the production and quality of milk, although, in their opinion, solar radiation and wind speed also deserve attention as influencing factors.

In India, dairy cattle experience significant heat stress, because the temperature of the environment in summer exceeds 45 °C, which is 18 °C higher than the upper critical temperature (David B. et al., 2021). At high temperatures, animals spend a lot of feed energy to speed up breathing and sweating, and therefore, in the summer, milk production is reduced by half.

In the conditions of Korea, Eunjeong J. et al. (2023) made a large-scale analysis of the impact of climate change and heat stress on the milk production of Holstein cows and recognized critical environmental parameters on cow productivity and milk quality. Scientists emphasize the importance of implementing appropriate feeding management strategies that take into account climatic factors to ensure optimal performance and stability of Holstein cows in changing environmental conditions.

In Pakistan, Rahman M.A. et al. (2019) identified critical risk factors threatening the dairy production system due to climate change. The most influential of them are the lack of genetic studies and research on the assessment of feed safety, laboratories for disease detection, water shortages, and innovations in the processing of milk and dairy products.

Magan J. B. et al. (2021) in the conditions of Scotland proved that even at too low environmental temperature there was a negative impact on milk production. At the same time, cows that calved for the first time suffered the most. The researchers suggest taking into account the factor of low environmental temperature in the conditions of climate change when implementing the technological strategy of dairy farming.

Experimenting on Holstein cows, Tančin V. et al. (2020) proved the effect on milk quality indicators of the season of the year, hotel activity, number and stage of lactation, and the content of somatic cells.

Ataallahi M. et al. (2023) correlated cortisol concentration in pasteurized and sterilized dairy products with temperature and humidity index during milk production in northeastern South Korea. In their experiments, cortisol levels fluctuated in samples

produced during the summer and autumn months of the year, and were the highest in July (211.9±95.1 pg/ml) and August (173.5±63.8 pg/ml). Researchers suggest that monitoring cortisol residues in dairy products can serve as an alternative indicator of heat stress in dairy cattle. Other South Korean scientists Lee D. et al. (2023), after examining 1.5 million experimental data, established a negative effect of heat stress on productivity and milk quality of Holstein cows.

In the work, Feliciano R. J. et al. (2020) indicated that climate change affects the microbial safety of dairy products. In conditions of heat stress, susceptibility of lactating cows to microbial contamination increases, accordingly, microbial contamination of milk increases. The researchers suggest the development of models to quantify microbial contamination to assess risk mitigation strategies, a key step in prioritizing a climate-resilient dairy industry. The same researchers (Feliciano R. J. et al., 2023) studied milk quality indicators (content of mass fractions of fat and protein) in different weather conditions throughout the year and established seasonal patterns. However, other authors Magan J. B. et al. (2021), Hayes E. et al. (2023) associate seasonal changes in milk quality primarily with the cow's diet. Correa-Calderón A. et al. (2022) add that no less than 40% of the decrease in milk productivity is associated with a decrease in the consumption of dry matter of feed, the rest is associated with a direct effect on the general metabolism and cellular heat resistance. The decrease in milk synthesis is associated with insufficient consumption of dry matter (Gao S. T. et al., 2019). They associate this process with biological changes in the mammary gland. Based on their experiments, it can be assumed that heat stress reduces the transcription of genes related to metabolism and increases the concentration of genes related to inflammation in mammary gland tissues, due to which milk protein synthesis decreases.

Marai I. F. & Habeen A. A. M. (2010) note that industrial and domestic animals are more prone to heat stress losses than indigenous ones. In this direction, studies on determining the endurance of cows of different genotypes to heat stress were conducted by Voytenko S. L. et al. (2020), who proved that under the natural and ecological conditions of the Poltava region, cows with different Holstein bloodlines, as well as female offsprings of different breeders, reacted differently to stress factors.

Researchers from the USA Sigdel A. et al. (2019) prove a negative genetic relationship between cow productivity and resistance to heat stress, and believe that selection for productive traits without taking into account thermotolerance will lead to greater dependence on heat stress.

Piddubna L. et al. (2021) found a significant level of influence of the genetic component on the quality of milk of cows of the Ukrainian black and white dairy breed. Therefore, the content of mass fractions of fat and protein depended on the origin of the cow's sire by 11.4; 31.0 and 31.5%, respectively, while linear belonging affected the indicated indicators with half the level.

Silpa M. V. et al. (2021) also see overcoming the impact of heat stress on the animal body in the activation of genomic selection, which is based on already identified biomarkers that regulate heat stress and milk productivity, and also contribute to the discovery of additional characteristics related to adaptation, production and reduced emission of methane That is, it is the task of the genetics is to obtain a well-adapted thermotolerant animal capable of producing under different climatic conditions and releasing less methane per unit of used feed. In this context, Cassandro M. (2020) believes that understanding the adaptive properties of livestock is a key factor in counteracting the effects of global climate change. The tool for this understanding is the identification of genes that form adaptive phenotypes. Therefore, the importance of using technolo-

gies that increase the efficiency of land and fodder use and mitigate the negative impact of animal husbandry on biodiversity, ecosystems and global warming is understood.

To prevent the negative effects of heat stress on dairy cows, researchers suggest more detailed management of feeding and installation of cooling systems on farms. Thus, Avendaño-Reyes L. et al. (2021) proved that in conditions of high heat stress, additional cooling at night made it possible to lower the body surface temperature of Holstein and Jersey cows, slightly reduce the content of triiodothyronine and increase the content of thyroxine, and increase the amount and quality of milk. Tao S. et al. (2020) also emphasize that the most effective way to reduce the negative impact of heat stress on the animal body is to reduce the temperature through shading and evaporative cooling.

Ukrainian scientists (Sediuk, I. et al., 2023; Zolotarov A.P. et al., 2023) studied the possibilities of reducing the influence of high ambient temperature on milk productivity of cows using different approaches to the organization of feeding technology. It has been proven that the introduction of 1.5 kg of a protein feed additive with protected protein (TEP-mix) into the diet of high-yielding cows helps to increase the productivity of experimental cows (in terms of base milk) by 15.7% (4.3 kg). Modernization of the feeding ration of high-yielding cows by increasing the content of undegradable protein in the rumen to the norm during temperature stress contributed to better adaptation of animals in terms of productivity and quality indicators of milk: an increase in the mass fraction of fat by 0.67% and the mass fraction of protein by 0.26%, with content of somatic cells 285.06±81.0...409.3±134.3 thousand/cm3.

Min L. et al. (2021) claim that feeding strategies with the inclusion and dosage variations of dietary fats, dietary fibers, dietary microbial additives, minerals, vitamins, buffer solutions of metal ions, plant extracts, etc., are capable of mitigating the negative effects of heat stress in dairy cows, increasing production of milk and content of protein and fat in milk.

Razzaghi A. et al. (2023) are confident that in dairy cows exposed to stressors: social (housing conditions, regrouping, feed delivery), physiological (beginning of lactation and calving), or physical (heat or cold stress), body reactions include changes in energy balance and distribution of nutrients. The ability of an animal to synthesize milk fat depends largely on the availability of substrates for lipid synthesis from the diet, ruminal fermentation, or adipose tissue reserves, all of which can be altered under conditions of stress. Indeed, milk fat concentration is particularly sensitive to changes in diet and environment, where a wide range of nutritional and technological factors influence milk fat yield.

Gerun I. et al. (2021) established that the quality and safety of milk directly depends on the technology of its production. With the use of the latest technology of milk production, with 2,000 cows kept indoors without tethering, the number of somatic cells and total insemination of milk did not exceed 221.1 thousand/cm^3 and 70.8 thousand CFU/cm^3, which corresponds to the "extra" grade. Moreover, the incidence of mastitis in cows did not exceed 6.4%. Milking of cows was carried out on the "Parallel" milking plant of the DeLaval company. The effectiveness of the use of "Parallel" MM in relation to the quality and technological properties of milk is proven by Lutsenko M. et al. (2021). In their opinion, the better quality of milk is due to the fact that the "Parallel" milking machine provides a higher intensity of milk yield in the first minute of milking (compared to the "Carousel" milking machine).

Researchers published interesting facts about dairy cows that were kept on pastures in the conditions of the Amazon region of Brazil (Vieira R. K. R., 2022). According to their data, an increased content of somatic cells was observed in the milk of cows

that grazed on irrigated pastures. These animals were 5.03 times more likely to develop mastitis than those grazed on non-irrigated pastures.

Gunn K. M. et al. (2019) studied the cost-effectiveness of four heat stress reduction strategies at minimal cost (tent, shading), moderate (forced ventilation), high (ventilation and condensation), and intensive (indoor air conditioning) and found that intensive technologies allowed for an increase in annual milk production. The studies were carried out under conditions of increasing heat stress, the annual milk losses were the highest in the western, southwestern, southern and southeastern regions of the USA. Therefore, in these regions, increasingly intensive technologies for reducing temperature stress using air cooling are needed.

Borshch O.O. (2021) studied the dynamics of the productivity and quality of milk of cows in the central part of the Forest Steppe of Ukraine under different technological options for keeping and proved that under the conditions of an increase in the average annual temperature, the use of continental-scaled spatial composite structures for housing dairy cattle reduces the effect of temperature stress on the animal body.

In our research, we have become convinced that it is probably desirable to add to further scientific research a Swiss mathematical model that helps to identify the general thermal effects in the production of dairy products and, at the same time, on empirically visible veterinary costs and feed purchases at the farm level (Bucheli J., 2022). However, the temperature regimes for different regions should be taken into account, because animals from the northern countries have time to recover from the heat stress they receive in the summer.

Discussion. Stating the review of modern research and forecasts of well-known scientists regarding the influence of technological and climatic factors that affect the quality of cow milk, it should be noted that climate change will continue to affect domestic animal husbandry, and therefore the development of potential strategies for mitigating this negative impact is of particular importance. It is necessary to pay attention, first of all, to the adaptation of safety management programs for the production of animal husbandry products to climate change forecasts.

EU regulations currently set high requirements for the quality of milk and its processing products. It is known that the quality of milk depends on genetic and technological factors, but in the conditions of environmental changes, it is important to take into account the factors of the influence of the surrounding environment, to follow the forecasts of changes in order to improve technologies for the comfort of dairy cattle. In this regard, it is necessary to adapt all current programs for managing the safety of production of animal husbandry products. Local and regional heat stress reduction strategies for dairy cattle should be adjusted according to climate projections for different temperature zones to mitigate potential losses of dairy production due to heat stress. The decrease in the body temperature of animals during the heat promotes their consumption of more feed, and the introduction of special feed elements into the diet reduces the release of electrolytes from the body through the skin. Thus, the development of mineral supplements for regulating the acid-alkaline balance of the animal body is needed. No less important is the search for genes associated with thermotolerance, namely, markers for breeding programs to increase the adaptive properties of animals.

According to the climate forecasts of different regions of Ukraine, it is necessary to adjust local and regional strategies for reducing the impact of negative factors on dairy cattle in order to mitigate potential losses of dairy production. The climatic zones of Ukraine are quite diverse, due to their geographical location, they often belong to the zones of risky agriculture: arid steppes, mountain and forest areas with increased precipitation, the Carpathians and Crimean Mountains, the Black Sea and Azov coasts. That is

why, for different climatic conditions, well-adapted breeds of animals were once created. Therefore, in different climatic regions of Ukraine, research is needed on the adaptation of animal husbandry under the influence of climate changes, as well as in the search for ways to prevent them: breeding, technological, physiological, veterinary. Particular attention is paid to the development of stress-free and adaptive animal husbandry technologies taking into account actual and possible environmental changes, the spread of ecological and organic production technologies for animal husbandry, as well as the creation of anti-stress systems and feeding rations using technologies of targeted nitrogen release, regulation of stability and the content of rumen microflora in ruminants, the development and use of preparations and probiotics that are anti-stress products of synergistic action, vitagens, immunomodulators, organic acids, mineral compounds. In the conditions of climate change, the least vulnerable to environmental risks will be livestock enterprises that use modern technologies for keeping and feeding animals.

Conclusions. The lives of people all over the world depend on the quality of food, among the most important of which are milk and dairy products. Obtaining high-quality milk depends on successful dairy farming, which, in turn, depends on many natural and unnatural factors. Environmental factors, such as temperature, humidity, wind speed, and radiation directly affect the production of dairy products, and scientists consider thermal stress to be the most influencing factor. Researchers from different countries have proven that climate change causes not only a decrease in the quantity of dairy products, but also its quality. It is possible to reduce the impact of thermal stress by selecting animals with a thermotolerant genotype, adjusting the feeding system with the inclusion of innovative substances that reduce oxidative stress, and technological means of protecting animals from critical temperatures.

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APPROACHES TO ESTIMATING GREENHOUSE GAS EMISSIONS FROM THE BY-PRODUCTS OF LIVESTOCK FARMING IN UKRAINE

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Climate change due to human activity in developed countries leads to numerous cases of deterioration of living conditions in all regions of the planet. However, it is possible to change this situation. To do this, it is necessary to maintain the global temperature at today's level by reducing emissions of greenhouse gases into the atmosphere. Agriculture makes a significant contribution to anthropogenic global warming, particularly livestock. Animal manure and the soils cultivated with it are the most important sources of emissions from livestock after intestinal methane. Experts estimate that nitrous oxide and methane produced in pastures and manure processing systems can account for up to a quarter of on-farm emissions, so it is important to identify strategies to reduce the flow of these gases. To solve the problem of global warming, it is necessary to control a number of positions, one of which is the assessment of actual emissions of greenhouse gases and, in particular, in the production of livestock products.

The article presents a new approach to increasing the accuracy of greenhouse gas emissions calculations. To determine the gross energy in the methodology of effective practice, the values of the indicators of clean energy are used to maintain the needs of animals for the continuation of vital activities and taking into account their productivity. When expanding the approaches for obtaining raw data for determining greenhouse gas emissions from by-products of livestock farming to determine gross energy, use the indicators of the content of crude protein, crude fat, crude fiber, and non-nitrogenous extractives in the diet.

The yield of animal excrement is calculated based on the weight and composition of the feed, taking into account the digestibility of the organic matter of the feed and the relative content of organic matter.

When separating livestock by-products (organic waste) into solid and liquid fractions, the actual data characterizing the quality of separation on individual elements of the technological line are the mass and moisture content of effluents entering processing and obtained at the exit, the mass and moisture content of the liquid fraction, and the mass and moisture content of the solid fraction.

Key words: livestock by-products, nutrient digestibility, greenhouse gases, separation efficiency, liquid, solid fractions.

ПІДХОДИ ДО ОЦІНКИ ВИКИДІВ ПАРНИКОВИХ ГАЗІВ З ПОБІЧНОЇ ПРОДУКЦІЇ ТВАРИННИЦТВА В ГОСПОДАРСТВАХ УКРАЇНИ

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Зміна клімату через діяльність людини у розвинених країнах призводить до численних випадків погіршення умов мешкання у всіх регіонах планети. Проте є спроможність змінити цю ситуацію. Для цього необхідно утримати глобальну температуру на сьогоднішньому рівні за рахунок зниження викидів до атмосфери парникових газів. Сільське господарство робить значний внесок в антропогенне глобальне потепління, зокрема тваринництво. Гній тварин і оброблювані ним ґрунти є найважливішими джерелами викидів від худоби після кишкового метану. За експертними оцінками, закис азоту та метан, що утворюються на пасовищах і системах переробки гною, можуть складати до чверті викидів, що утворюються на фермі, і тому важливо визначити стратегії скорочення потоків цих газів. Для вирішення проблеми глобального потепління необхідно контролювати ряд позицій, однією із яких є оцінка фактичних викидів парникових газів і, зокрема, при виробництві тваринницької продукції.

У статті пропонується новий підхід до підвищення точності розрахунків викидів парникових газів. Для визначення валової енергії в методології ефективної практики використовуються значення показників чистої енергії на підтримання потреб тварин для продовження життєдіяльності та з урахуванням їх продуктивності. При розширенні підходів для отримання вихідних даних з визначення викидів парникових газів з побічної продукції тваринництва для визначення валової енергії використовувати основі показників вмісту сирого протеїну, сирого жиру, сирої клітковини, безазотистих екстрактивних речовин в раціоні.

Вихід екскрементів тварин розраховується за масою та складом кормів з урахуванням коефіцієнт перетравлення органічної речовини корму та відносний вміст органічної речовини.

При розділенні побічної продукції тваринництва (органічні відходи) на тверду і рідку фракції фактичні дані, що характеризують якість розділення на окремих елементах технологічної лінії – це маса і вологість стоків, що надходять на оброблення та отримані на виході, маса і вологість рідкої фракції та маса і вологість твердої фракції.

Ключові слова: побічна продукція тваринництва, перетравність поживних речовин, парникові гази, ефективність розділення, рідка, тверда фракції.

Introduction. Human activity has led to climate change, and, as a result, to numerous cases of worsening weather conditions in all regions of the planet, moreover, to the change of the entire climate system of the Earth, and these changes are reflected in the state of the atmosphere, ice caps, oceans and land. According to a report by the Intergovernmental Panel on Climate Change (IPCC), anthropogenic greenhouse gas

(GHG) emissions were the main cause of global climate warming of about 1.1 °C between 1850 and 1900. According to scientists' forecasts, this indicator may increase by another 1.5^0 C in the next 20 years. This leads to the melting of glaciers and, as a result, to a rise in the level of the world ocean.

Similar data regarding Great Britain are given by Kendon M. et al. (2023). They note that 2022 was the warmest not only in terms of air temperature, but also of water near the coast in almost 150 years of research.

Tomczyk A.M. et al. (2021) outline the impact of climate change on the decrease in snowfall in Poland over the past 54 years.

In the article, Paul M.J. et al. (2019) provide multi-year observational data on the impact of air temperature change on precipitation, water temperature, and water quality in the United States.

The main reason for this is the greenhouse effect, which is caused by greenhouse gases such as water vapor, carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), etc. Increasing greenhouse gas emissions provoke extreme climate changes, such as floods, droughts, and heat, which induce reactive oxygen species and oxidative stress in plants (Cassia R. et al., 2018).

In order to meet the global demand for food, humanity is forced to increase the number of animals, but this does not necessarily lead to a proportional increase in GHG production.

Livestock farming, especially large ruminants, and its related products make a significant contribution to anthropogenic emissions of the highly active GHGs methane and nitrous oxide (MacLeod M.J. et al., 2018). According to Gerber P.J. et al. (2013), global livestock production contributes to approximately 7.1 Gigatons of CO_2-equivalent emissions. The amount of GHG from domestic animals is equal to 14.5% of anthropogenic emissions of greenhouse gases every year, of which CH_4 from livestock is about 44% of this total amount. The production of beef and milk from cattle accounts for the majority of emissions – 41% and 20% of the sector's emissions, respectively. These indicators from the pig and poultry industries (meat and eggs) are 9% and 8%, respectively. Feed production and processing account for 45% of emissions, ruminant intestinal fermentation for 39%, manure storage and processing for 10%, and the rest for processing and transporting animal products.

Achieving net "zero emissions" of long-lived climate pollutants (LLCPs), primarily carbon dioxide (CO_2) and nitrous oxide (N_2O), is considered vital to halting the warming trend.

The food system is the main cause of increased CO_2 emissions due to land-use change, mainly as a result of clearing land for crops or pasture. Net CO_2 emissions associated with land use are estimated to be about 14% of annual anthropogenic CO_2 emissions (Le Quéré S. et al., 2018).

Shorter-lived greenhouse gases than carbon dioxide are automatically removed from the atmosphere over a shorter period of time, so emissions will not continue to accumulate for a very long time like CO_2. These are methane and nitrous oxide.

Methane in livestock farming is mainly produced as a result of intestinal fermentation and manure handling. Methane as a result of enteral fermentation is a by-product of the digestion of feed materials, mainly roughage. Most CH_4 from ruminants is produced in the rumen and is exhaled or regurgitated by the animal. During enteral fermentation in the rumen, methanogenic microorganisms generate CH4 from hydrogen (H_2) and CO_2 produced by protozoa, bacteria, and anaerobic fungi (Martin S. et al., 2010; Morgavi D. et al., 2010; Tapio I. et al.., 2017).

As reported by Shibata M. and Terada F. (2010), the amount of CH_4 emissions depends on animals (i.e. type of digestive tract, stage of production, age and weight), feed (i.e. quality, quantity and composition) and environmental temperature. The amount and quality of forage affects the amount of energy, nitrogen and minerals available to microorganisms in the rumen. The protein content of the feed has a negative effect on CH_4 production, while the fiber content has a positive effect.

According to the US Environmental Protection Agency (EPA, 1995), only a small part of CH_4 is produced in the large intestine of ruminants and is excreted through flatulence.

According to Aguirre-Villegas H.A. and Larson R.A. (2017), manure (faeces and urine) is the second largest source of GHG emissions after enteric methane, accounting for about 7% of agricultural CH_4 and N_2O emissions.

Ruminants do not convert nitrogen well – only 5–30% of consumed is assimilated, and the remaining 70–95% is excreted in faeces and urine (Luo J. et al., 2010).

According to Dangal S.R.S. et al. (2019), about 2.2 million tons of N_2O–N are emitted annually from pastures in the world, including 74% from anthropogenic sources. Animal feces and urine are the largest source of N_2O emissions in pastures (54%), followed by manure (13%) and nitrogen fertilizers (7%).

In order to reduce GHG emissions throughout the world, and in Ukraine in particular, numerous studies are being conducted in this direction. Thus, Pinchuk V.O. and Borodai V.P. (2019) calculated the emission of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) from by-products of animal origin (manure) in different regions of Ukraine, categories of farms, types of farm livestock and systems of cleaning, storage and use of manure in Ukraine according to the IPCC methodology.

Emission reduction methods (low-emission housing systems) for various types of domestic animals have been developed and proposed (Furdychko O.I. et al., 2016).

Some scientists suggest making adjustments to the composition of diets (van Cleef F.O.S. et al., 2022; Bica R. et al., 2022), or using feed additives to reduce greenhouse gas emissions (Owens J.L. et al., 2020).

Others propose to process animal manure. For example, adding the natural mineral glauconite to the dung substrate of cows can reduce the level of CO_2 and CH_4 by 5%, respectively (Vorobel M. et al., 2018). And the use of superphosphate and slaked lime in different ratios to pig manure contributes to the reduction of carbon dioxide emissions by 6.1-25.5%, methane by 3.8-11.5%, and nitrogen oxide by 5.4-17.4%, respectively % (Vorobel M., 2022).

In 1988, the Intergovernmental Panel on Climate Change (IPCC) was established. The purpose of its creation is the coordination of scientific activities to determine the amount of GHG emissions, the development of calculation methods, their refinement and adaptation to national conditions, and the assessment of scientific knowledge related to climate change. The Intergovernmental Panel's mission is to provide policymakers with regular assessments of the scientific basis of climate change, its impacts and future risks, as well as options for adaptation and mitigation.

In the methodology of the IPCC (2006), it is noted that effective practice should meet the need to use assessment methods for each country, in particular gross energy consumption, based on the use of methods specifically defined for that country. To determine the gross energy in the proposed methodology of effective practice, the values of the indicators of net energy for maintaining the vital activity of the animal, physical activity, lactation, work, physicality, and digestive energy are used.

For more efficient processing of livestock by-products (organic waste) on farms, it is recommended to separate them into solid and liquid fractions. This makes it

possible to reduce direct emissions of GHG to the atmosphere, improve processing and disinfection of manure, as well as its processing in biogas plants. However, this practice is not widespread in Ukraine. Thus, according to the data of the Association of Pig Breeders of Ukraine, among farms with a herd of 1,000 to 50,000 pigs, when manure is prepared for use, separation into solid and liquid fractions occurs only in 43%.

In the methodology of effective practice, there are no approaches for determining greenhouse gas emissions during the preparation of livestock by-products using fractionation technologies, as well as approaches for determining the output of organic waste (IPCC, 2006).

The purpose of the work is to expand the approaches to obtaining raw data for determining greenhouse gas emissions from livestock by-products within the framework of the effective practice of the methodology of the Intergovernmental Group of Experts on Climate Change.

Research materials and methods. When expanding the approaches for obtaining initial data for determining greenhouse gas emissions from by-products of livestock farming to determine gross energy, indicators of the content of crude protein (CP), crude fat (CF), crude fiber (CFb), and non-nitrogen extractive matters (NES) in the diet were used according to DSTU 8066:2015[1].

When separating livestock by-products (organic waste) into solid and liquid, the actual data characterizing the quality of separation on individual elements of the technological line (units, devices and installations) are the mass and moisture content of effluents entering processing and obtained at the exit, mass and the moisture content of the liquid fraction and the mass and moisture content of the solid fraction (Piskun V., 2007).

On the basis of the balance equations of the mass consumption of the jet through the separation element, the following data can be obtained: efficiency of separation into liquid and solid fractions; concentration of dry matter in liquid and solid fractions of effluents.

Research results. Based on the characteristics of animal rations, gross energy is determined taking into account the structure of the herd and feed according to the formula (DSTU 8066:2015[1]):

$$GE = 0.240*CP + 0.398*CF + 0.201*CFb + 0.175*NES \quad (1)$$

where GE is gross energy, in MJ/kg of dry matter;
CP is crude protein content in the dry matter of the forage, %;
CF is crude fat content in dry matter, %;
CFb is crude fiber content in the dry matter of the forage, %;
NES is the content of nitrogen-free extractive matters in dry matter, %;

As an example, Tables 1 and 2 show the results of the evaluation of the zoochemical composition of feed and calculations of gross energy consumption by cows of the "Hontarivka" branch of the "Hontarivka" State Enterprise of the Kharkiv region. The zoochemical composition of feed was evaluated at the testing center of the Institute of Animal Husbandry of the National Academy of Agrarian Sciences of Ukraine. According to the calculations, it was established that the gross energy of the ration of a dairy cow is 363.5 MJ, and that of a dry cow is 191.1 MJ.

[1] DSTU 8066:2015. (2015). Feed fr farm animals. Methods for determining of energy content and nutrient value. *Derzhspozhivstandard*. Kyiv. 15 p.

Table 1

Assessment of the composition of fodder at SE SF "Hontarivka", the Kharkiv oblast

The name of the type of test, measurement unit	Test results					
	Silage		Hay		Combined feed	
	on natural wet matter	on completely dry matter	on natural wet matter	on completely dry matter	on natural wet matter	on completely dry matter
Wet matter, %	73.71	0	10.35	0	10.41	0
Dry matter, %	26.29	100	89.65	100	89.59	100
Ash, %	1.60	6.09	5.91	6.59	5.01	5.59
Crude fat, %	2.46	9.36	1.03	1.15	2.64	2.95
Total nitrogen, %	0.352	1.339	0.953	1.063	2.317	2.586
Crude protein, % (N × 6.25)	2.20	8.37	5.96	6.65	14.48	16.16
Crude fiber, %	7.82	29.75	36.90	41.16	4.78	5.34
NES, %	12.21	46.44	39.85	44.45	62.68	69.96
Calcium, %	0.209	0.795	0.981	1.094	0.483	0.539
Phosphorus, %	0.049	0.186	0.228	0.254	0.428	0.478
Exchange energy, MJ	2.60	9.89	6.70	7.48	10.94	12.21

Further, within the framework of the effective practice of the IPCC methodology, we determine the rates of release of volatile solids taking into account the obtained values of gross energy by sex and age groups and the coefficient of methane emissions.

Table 2

Consumption of gross energy by cows at SE SF "Hontarivka", the Kharkiv oblast

Fodder	Quantity of fodder, kg	Content, %					Gross energy per head, MJ	Gross energy for all livestock, MJ
		Dry matter	Protein	Fat	Fiber	NES		
Dairy cows n=284								
Combined feed	8	89.59	16.16	2.95	5.34	69.96	131.7	37389.5
Silage	32	26.29	8.37	9.36	29.75	46.44	166.9	47404.4
Hay	4	89.65	6.65	1.16	41.16	44.45	64.9	18443.2
Total							363.5	103237.1
Dry cows n=86								
Combined feed	2	89.59	16.16	2.95	5.34	69.96	32.9	2830.26
Silage	21	26.29	8.37	9.36	29.75	46.44	109.5	9420.44
Hay	3	89.65	6.65	1.16	41.16	44.45	48.7	4187.34
Total							191.1	16438.04

Fattening cows n=13								
Combined feed	6	89.59	16.16	2.95	5.34	69.96	98.7	1283.62
Silage	32	26.29	8.37	9.36	29.75	46.44	166.9	2305.55
Hay	3	89.65	6.65	1.16	41.16	44.45	48.7	632.97
Total							324.8	4222.14
Non-calved cows n=65								
Combined feed	2	89.59	16.16	2.95	5.34	69.96	32.9	2139.15
Silage	21	26.29	8.37	9.36	29.75	46.44	109.5	7120.1
Hay	3	89.65	6.65	1.16	41.16	44.45	48.7	3164.85
Total							191.1	12424.1

The value of the rate of release of volatile solids will be determined by equation (2):

$$VS = \left[GE \cdot \left(1 - \frac{DE\%}{100} \right) + (UE \cdot GE) \right] \cdot \left[\left(\frac{1 - ASN}{18.45} \right) \right]; \quad (2)$$

where *VS* is the release of volatile solids per day based on the mass of dry organic matter, kg *VS*/day;

GE is gross consumed energy, MJ/day;

DE is feed digestibility, in percent (for example, 60%);

(*UE GE*) is energy lost in urine (urine energy), expressed as a fraction of GE. For most ruminants, the energy lost in urine can generally be assumed to be 0.04 *GE* (reducing to 0.02 for ruminants and in pig diets containing 85% or more grain).

ASN is the ash content of the manure, calculated as a fraction of the dry matter intake of the feed (for example, 0.08 for cattle).

18.45 is the conversion factor for *GE* ration based on 1 kg of dry matter (MJ/kg).

To determine the emission of greenhouse gases in the form of direct and side emissions of N_2O, it is necessary to know the daily output of livestock by-products. However, in the effective practice of the IPCC methodology, there are no approaches to determining the daily yield of livestock by-products. The daily yield of animal by-products can be calculated by formula (3) (Piskun, V., 2007):

$$Q_m = Q_{ex} + L + I + D + W_p + W_r, \quad (3)$$

where Q_{ex} is the mass of excrement, kg per day;

L is litter mass, kg per day;

I is mass of extraneous inclusions, kg per day;

D is mass of feed residues, kg per day;

W_p is mass of process water, kg per day;

W_r is is the mass water used to remove manure from the premises, kg per day.

The output of animal excrement is calculated based on the weight and composition of feed:

$$Q_{ex} = k_u * \left(1 - \frac{k_d}{100}\right) * R_t * \frac{O_r}{O_e} \quad (4)$$

where k_u is is the coefficient of use of fodder;

k_d is weighted average coefficient of digestion of organic matter of the diet by animals, %;

R_t is total weight of feed ration, kg;

O_r is relative content of organic matter in the feed ration, %;

O_e is the relative content of organic matter in excrement, %.

The average weighted coefficient of digestion of organic matter is calculated according to formula (5):

$$k_d = \frac{\sum_{j=1}^{m} R_j * O_{rj} * k_{nj}}{\sum_{j=1}^{m} R_j * O_{rj}} \quad (5)$$

where m is the number of feed types included in the ration;

R_j is the mass of the type of fodder included in the ration, kg;

k_{nj} is coefficient of digestion of organic matter of the jth feed;

O_{rj} is the relative content of organic matter in the jth feed, %.

The relative content of organic matter in the ration is determined by formula (6):

$$O_r = \frac{\sum_{j=1}^{m} R_j * O_{rj}}{\sum_{j=1}^{m} R_j}, \quad (6)$$

According to the presented formulas, the yield of manure was determined due to the amount of organic matter in the ration for a group of 7 cows. It was established that the output was 5.194±0.177 kg of organic matter on average for the group. And the actual output of manure, according to the data of the balance experiment, conducted in the conditions of the physiological yard of IAH of the National Academy of Sciences, amounted to 5.018±0.437 kg.

Separation of livestock by-products into fractions. Liquid waste is a big problem for any livestock enterprise. In places of their accumulation, a number of problems arise, related to environmental pollution, ecological deterioration, the possibility of poisons entering water bodies (including those included in the water intake system).

In the presence of own fields, the problem seems to be less, but the effluents can be applied as fertilizers, no earlier than after 12 months, after the effluents have undergone a natural composting process. Thus, even long-term composting retains the potential danger of infection. In the absence of fields, the problem of disposing of sewage becomes unsolvable.

The removal of solid particles from sewage, that is, the separation of livestock by-products into fractions, is a key point in solving this problem, the purpose of which is to reduce the content of polluting components of manure, which will allow to reduce the volume of settling tanks, simplify the application technology, increase the efficiency of biological treatment and minimize the harmful impact on the environment, reduce unpleasant scent a minimum.

Storing livestock by-products in open storage facilities does not contribute to a significant reduction in the amount of GHG emissions into the atmosphere, so in developed countries, closed storage facilities have begun to be built. Therefore, the question arises – why not use them as tanks for settling and separating fractions into liquid and solid with subsequent use in a biogas plant to obtain biofuel.

Livestock by-products are often used as a substrate for anaerobic digestion, as they have a neutral pH and the mix of microbes needed for this process naturally. In addition, animal by-products are often used as a basis for anaerobic digestion because many wastes are acidic, contain little natural microbes and nutrients necessary for anaerobic digestion, such as trace elements, vitamins, nitrogen, etc. (Mahmudul H.M. et al., 2021). Also, to ensure optimal technological parameters of methane fermentation, effluents are prepared with their separation into sediment and liquid fraction. Reducing the amount of lignocellulosic material due to fractionation will allow to accelerate the process of anaerobic fermentation and, accordingly, the processing of GHG into biofuel (Li Y. et al., 2020).

The actual data characterizing the quality of waste separation on individual elements of the technological line (units, devices and installations) are the mass M and moisture W of the wastes entering the treatment and received at the exit, the mass M_l and moisture W_l of the liquid fraction and the mass M_s and moisture W_s solid fraction (Piskun, V., 2007).

The following data are obtained on the basis of the balance equations of the mass consumption of the jet through the separation element:

h_l, h_s – efficiency of separation into liquid and solid fractions;

C_l, C_s – concentration of dry matter in liquid and solid fractions of effluents.

Three main options for obtaining separation parameters are considered:

1. If M, W, W_l, W_s are given, then the last characteristics of the separation quality are determined by formulas (7):

$$M_s = M*(W_l - W) / (W_l - W_s); \quad (7)$$

Giving an example, wastewater output during pork production is 250 tons of manure with a moisture content of 95.4%. When divided into fractions, a liquid fraction with a moisture content of 98.5% and a solid fraction with a moisture content of 75.3% are obtained. According to formula 6, 38.87 tons of solid fraction and 211.13 tons of liquid fraction will be obtained. To determine greenhouse gas emissions from solid and liquid fractions, we determine the gross energy in the fractions based on the indicators of the content of CP, CF, CFb, NES according to DSTU 8066: 2015.

The elements are determined according to the equations below.

$$M_l = M * (W - W_s) / (W_l - W_s) \text{ або } M_l = M - M_s;$$
$$C_l = 1 - 0{,}01*W_l; \quad C_s = 1 - 0{,}01*W_s; \quad C = 1 - 0{,}01*W;$$
$$h_l = 1 - (M_l *\cdot C_l) / (M * C) = 1 - [M_l \cdot (100 - W_l)] / [M \cdot (100 - W)];$$
$$h_s = 1 - (M_s *\cdot C_T) / (M * C) = 1 - [M_s * (100 - W_s)] / [M * (100 - W)] \text{ or}$$
$$h_s = 1 - h_l \quad (8)$$

2. If M, W, h_p, W_s are specified, then M_l, M_s, W_l, h_s are determined by formulas (9):

$$M_s = M * h_l \cdot (100 - W) / (100 - W_s); \qquad M_l = M - M_s;$$
$$h_s = 1 - h_l; \qquad W_l = 100 - M * h_s / (100 - W) * M_l. \quad (9)$$

3. If M, W, h_l, W_l are given, then M_l, M_s, W_s, h_s are determined by formulas (10):

$$h_s = 1 - h_l; \quad M_l = M * h_s \cdot (100 - W)/(100 - W_l); \quad M_s = M - M_l;$$
$$W_s = 100 - M * h_l \cdot (100 - W)/M_s. \qquad (10)$$

The proposed approaches to the methodology for estimating greenhouse gas emissions from livestock by-products from the determination of gross energy through the value of crude protein, crude fat, crude fiber and nitrogen-free extractive matters in the dry matter of feed and the quality of separation on individual elements of the technological line in the technologies of preparation for use will allow to increase the accuracy determination of greenhouse gas emissions.

Discussions. Efficient practice in the IPCC methodology uses the determination of gross energy (GE) for cattle and sheep based on the total net energy required and the energy availability characteristics of the forage(s) (Equation 11)

$$GE = \left[\frac{\left(\frac{NE_m + NE_a + NE_l + NE_{work} + NE_p}{REM} \right) + \left(\frac{NE_g + NE_{wool}}{REG} \right)}{\frac{DE\%}{100}} \right] \qquad (11)$$

where: *GE* = gross energy, MJ/day;

NEm = net energy needed to support the animal, MJ/day;

NEa = net energy for physical activity of the animal, MJ/day;

NEl = net energy for lactation, MJ/day;

NEwork = net energy for work, MJ/day;

NEp = net energy required for pregnancy, MJ/day;

REM = ratio of net dietary energy available for maintenance to digestible energy intake;

NEg = net energy required for growth, MJ/day;

NEwool = net energy required for wool production during the year, MJ/day;

REG = ratio of net energy in the diet available for growth to digestible energy consumed;

DE% = digestible energy expressed as a percentage of gross energy.

It should be outlined that the values of net energy required to maintain the vital activity of the animal, net energy for physical activity of the animal, net energy for lactation, net energy for work, net energy required for pregnancy are determined by other formulas. To use these formulas, you need to know net energy for animal growth, average body weight per herd, body weight of an adult female, body weight of an adult female of average fatness, average daily weight gain of an animal per herd, amount of milk produced per day, fat content in milk, number of working hours per day, etc. It is also necessary to know the ratio of net energy in the ration, which is available for life support, to the easily digestible energy consumed, as well as the ratio of the net energy in the ration, which is available for growth, to the easily digestible energy consumed, easily digestible energy, which is expressed as a percentage of gross energy. This is in case we define gross energy.

The analysis of literary sources showed that in practice determination of methane emissions due to gross energy, which is determined according to the above formula 9, is practically not carried out. For this, the simplest method of determining GHG

emissions is used by the emission coefficient for the corresponding livestock according to formula 11 (Pinchuk, V.O., Borodai, V.P., 2019; Das N.G. et al., 2020):

$$CH_{4manure} = \sum_{(A)} \frac{EF_{(A)} * N_{(A)}}{10^6}, \quad (12)$$

where $CH_{4manure}$ is CH_4 emissions as a result of cleaning, storage and use of manure for the established animal population, thousand t/year;

$EF_{(A)}$ is the emission coefficient for the established animal population, CH_4 kg/head/year (IPCC, 2006);

$N_{(A)}$ is average annual population of the A species/categories of animals; A – species/category of animals.

The emission coefficient for the established animal population depends only on the average annual temperature and climatic conditions and does not include data on the influence of the amount of gross energy in the animal ration on its (coefficient) value.

According to the State Statistics Service of Ukraine, milk yield per cow in 2006 was 3,896 kg, and in 2022 – 5,119 kg. Nevertheless, the same coefficient is used to determine methane, without taking into account livestock productivity.

Therefore, the determination of methane emissions through the indicator of gross energy of feed, taking into account crude protein, crude fat, crude fiber and non-nitrogenous extractive matters, is simpler and more accurate.

References include a CH_4 prediction equation formulated by Shibata, M. & Terada, F. (2010) using only dry matter intake (DMI) and adopted in Japan to estimate emissions from ruminants for the National Greenhouse Gas Inventory Report :

$$Y = -17{,}766 + 42{,}793X – 0{,}849X^2,$$

where Y is CH_4 production (l/day),

X is DMI (kg/day).

It should be noted that using the equation for forecasting CH_4 emissions only using the dry matter consumption indicator leads to some inaccuracy. After all, in different rations with the same amount of dry matter, but different amounts of crude protein, crude fat, crude fiber and non-nitrogen extractive matters, when calculating emissions, a distinctive result with a significant error will be obtained.

Therefore, the authors believe that the proposed calculation approach is more accurate and easier to use in practice.

Conclusions. As part of the effective practice of the methodology of the Intergovernmental Panel on Climate Change (IPCC), the authors propose to expand the approaches to obtaining raw data for determining greenhouse gas emissions from livestock by-products:

- to determine gross energy, use the value of crude protein, crude fat, crude fiber and nitrogen-free extractives;
- the output of animal excrement should be calculated based on the weight and composition of feed, taking into account the digestibility coefficient of organic matter of the *j*th feed and the relative content of organic matter in the *j*th feed;
- on the basis of the balance equations of the mass consumption of the jet through the separation element, obtain the following data: efficiency of separation into liquid and solid fractions; concentration of dry matter in liquid and solid fractions of effluents.

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THE INFLUENCE OF REGENERATED LITTER ON THE PRODUCTIVE PERFORMANCE AND WELL-BEING OF REARING YOUNG CHICKENS

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Due to the shortage and high cost of bedding materials, the regeneration of used reinforcement and its repeated use is an urgent problem in modern poultry farming. However, at the same time, there is a threat of the negative impact of such litter on poultry. Several studies have proved the possibility of raising broiler chickens on regenerated bedding. Less studied is the option of using regenerated waste in the cultivation and maintenance of poultry with a long production cycle rearing young stock or adult birds. In the State Experimental Poultry Station National Academy of Agrarian Sciences of Ukraine (SEPS NAASU), the regeneration technology of used litter has been improved. In this study, the impact of the use of litter regenerated according to the developed technology in the rearing of young chickens on the microclimate of the poultry house, well-being, and zootechnical indicators of poultry was studied. The research was carried out on the experimental farm of the SEPS NAASU. According to the results of the investigation, although the initial moisture content of the regenerated litter was higher than the new one ($p<0.05$), after ten weeks of rearing young animals, the litter moisture content in both variants no longer had significant differences. During the growing period, the content of ammonia and carbon dioxide in the air of the experimental premises did not exceed the maximum permissible concentrations. Still, in the practical room, it was higher than in the control room, especially in the initial cultivation period. The leading difference was 4.4 mg/m3 for ammonia and 0.05% for carbon dioxide. In terms of ammonia content, the difference between the experimental and control variants was statistically significant up to 8 weeks of age ($p<0.05$) and carbon dioxide content up to 6 weeks of age ($p<0.05$). There was no negative impact of regenerated litter on the well-being indicators (condition of feather cover, knee joints, and paw feet) and zootechnical indicators of rearing young stock (safety, body weight, and feed conversion). This indicates the possibility of using regenerated litter in the rearing of young chickens without a negative impact on poultry.

Keywords. Litter, regeneration, multiple use, rearing chickens, microclimate, poultry welfare, zootechnical indicators.

ВПЛИВ РЕГЕНЕРОВАНОЇ ПІДСТИЛКИ НА ПРОДУКТИВНІ ПОКАЗНИКИ І БЛАГОПОЛУЧЧЯ РЕМОНТНОГО МОЛОДНЯКА КУРЕЙ

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У зв'язку з дефіцитом та високою вартістю підстилкових матеріалів регенерація використаної підсилки та багатократне її використання є актуаль-

ною проблемою в сучасному птахівництві. Однак при цьому існує загроза негативного впливу такої підстилки на птицю. Можливість вирощування курчат-бройлерів на регенерованій підстилці була доведена низкою досліджень. Менш вивчена можливість застосування регенерованої підстилки при вирощуванні та утриманні птиці з тривалим виробничим циклом-зокрема ремонтного молодняку або дорослої птиці. В ДДСП НААН удосконалено технологію регенерації використаної підстилки. У цьому дослідженні вивчали вплив використання регенерованої за розробленою технологією підстилки при вирощуванні ремонтного молодняку курей на мікроклімат пташника, благополуччя та зоотехнічні показники птиці. Дослідження були проведені на експериментальній фермі ДДСП НААН. Як засвідчили результати досліджень, хоча початкова вологість регенерованої підстилки була більшою, ніж нової ($p<0,05$), після 10 тижнів вирощування молодняку вологість підстилки в обох варіантах вже не мала істотних відмінностей. Впродовж періоду вирощування вміст аміаку і вуглекислого газу в повітрі дослідних приміщень не перевищував гранично допустимих концентрацій, однак в дослідному приміщенні він був більший, ніж контрольному, особливо в початковий період вирощування. Максимальна різниця досягала: за вмістом аміаку 4,4 мг/м^3, вуглекислого газу 0,05%. За вмістом аміаку різниця між дослідним і контрольним варіантом була статистично вірогідною до 8-тижневого віку ($p<0,05$), вмістом вуглекислого газу до 6-тижневого віку ($p<0,05$). Не встановлено негативного впливу застосування регенерованої підстилки на показники благополуччя (стан пір'яного покриву, колінних суглобів та стоп лап) та зоотехнічні показники ремонтного молодняку (збереженість, живу масу та конверсію корму). Зазначене свідчить про можливість використання регенерованої підстилки при вирощуванні ремонтного молодняку курей без негативного впливу на птицю.

Ключові слова: підстилка, регенерація, багатократне використання, ремонтний молодняк курей, мікроклімат, благополуччя птиці, зоотехнічні показники.

Entry. Due to the shortage of bedding materials and the increased cost, poultry farmers are increasingly interested in their repeated use after appropriately carrying out regeneration. The main renewal task is the destruction or inactivation of pathogenic microflora (Merino, 2018; Roll et al., 2011; Oliveira et al., 2015).

The most common way to regenerate used litter is to disinfect it by biothermal composting in piles (Wang, 2019; Schmidt et al., 2013). This regeneration technology involves raking litter in poultry houses during the period of their sanitation into piles with a height of 0.3-1.7 m and keeping them in piles for 3-8 days. In the process of composting, the used litter is heated to a temperature of 55-70 °C, which ensures its disinfection. During the composting period, it is possible to turn the piles over to move the outer layers of the piles into their middle for better disinfection.

Methods of disinfection of used litter, such as adding quicklime to the litter, shallow fermentation, or processing in special bioreactors, are also known (Lopes et al., 2013; Vaz et.al., 2017; Benedet et.al., 2021).

Poultry farmers are also concerned about the possible negative impact of the use of regenerated litter on the microclimate of poultry houses, production indicators, and well-being of poultry since its physical, mechanical, and thermal properties, moisture absorption capacity are significantly different from those of fresh litter (Cressman, 2014; Dornelas et al., 2020; Dornelas et al., 2023).

The effect of using regenerated litter compared to fresh litter on the leading indicators of broiler chicken rearing has been studied in several studies.

Abougabal and Taboosha (2017) and Abougabal (2019) raised broilers on fresh litter, a mixture of fresh and regenerated litter, regenerated litter, and regenerated alum-treated litter. The results of the experiment showed that the differences between the variants in most indicators (live weight of poultry, feed intake, feed conversion rate, safety, European Production Efficiency Index, slaughter yield and carcass characteristics, behavior patterns, overall economic efficiency of production) were insignificant and statistically unreliable. However, numerically, some advantages favored the variant using fresh litter. More significant differences ($P<0.05$) were observed with different types of litter in terms of dermatitis of the soles of the feet and the condition of the feather cover, which were worse with the use of regenerated litter. Adding alum to compostable reused litter helped improve them.

Kyoung et al. (2017) studied ammonia emissions when poultry were kept on single and reusable litter. It was found that, as a rule, in the latter case, an increase in the emission of this gas by 1.3 – 2.0 times was recorded; however, in most cases, the total content of ammonia in the air of the poultry house did not exceed the maximum permissible concentration.

In an experiment by Garces-Gudino et al. (2018), broiler chickens reared on litter regenerated after cycles 2 and 3 had higher live weight, daily weight gain, feed intake, preservation, and European production efficiency factor ($P<0.05$) than chickens raised on new litter. The authors concluded that regenerating used litter is an excellent option to reduce bird droppings production and improve the productivity of broiler chickens.

Kalita et al. (2012) conducted three experiments in which broiler chickens were raised on new, regenerated after two and three use cycles, and mixed (50%:50%) litter. In broiler chickens raised on mixed litter and used for 2 and 3 cycles, there was a tendency to increase live weight. At the same time, in the 3rd experiment, the live weight of chickens raised on mixed litter was statistically significantly ($P<0.05$) higher than the live weight of chickens raised on fresh and regenerated used litter. In all three experiments, the authors did not note a statistically significant difference between the variants regarding feed conversion, preservation, and yield of gutted carcass. During the research, an organoleptic assessment of broiler meat was also carried out. Meat from chickens raised on mixed and used litter had an advantage in tenderness and juiciness ($P<0.05$) compared to broilers raised on new bedding. However, no significant difference was found between the samples regarding "flavor" and "overall acceptability" of meat.

Bucher et al. (2020) and Pepper et al. (2021) note that with repeated use of litter, a microbe is formed in it, which negatively affects harmful microflora and is characterized by a probiotic effect in the intestines of poultry, which ultimately has a positive effect on the safety and productivity of poultry.

Grimes et al. (2003) regenerated used turkey litter by heat treatment in a screw system at 95 °C or 220 °C. No significant differences were found in live weight, feed conversion, and safety of turkey poults reared on new heat-treated bedding.

About 13% of farmers in Canada raise turkeys on reclaimed litter (Staaveren et al., 2020). However, the effect on poultry performance of the use of regenerated litter compared to fresh litter is not reported by the authors.

Despite the generally positive results of using regenerated litter when growing poultry abroad, poultry farmers in Ukraine still have concerns about its disinfection in the process of biothermal treatment in the traditional way and the possible negative impact of repeated use of litter on the production performance of poultry. Both abroad and in Ukraine, the possibility of using regenerated litter in the cultivation and maintenance

of poultry with a long technological cycle – rearing stock or parent flock of chickens and turkeys – has not been sufficiently studied.

The State Experimental Station of Poultry Farming of the National Academy of Agrarian Sciences of Ukraine (DSSP NAAS) has improved the regeneration technology of used litter. The improved technology involves adding a special microbiological preparation to the litter and, in addition, irradiating the surface of compost piles with ultraviolet radiation of the bactericidal range or disinfecting it with ozone. The developed experimental methods ensured the intensification of the process of its biothermal treatment by 1 – 9.4°C, a decrease in ammonia emission by 1 – 17 mg/m2 per 1 hour, seeding with enterobacteria by 1.1 log10, fungal microorganisms by 2-5 times (Ryabinina & Melnik, 2022).

Purpose of research. To study the effect of litter regenerated according to improved technology on the productive performance and well-being of rearing young chickens.

Materials and methods.

The study was carried out on the experimental farm "Conservation of the State Poultry Gene Pool" of the Children's Agricultural Enterprise of the National Academy of Agrarian Sciences of Ukraine in the period from July 10 to November 16 in a poultry house for raising young chickens in two isolated sections measuring 4x5 m with an area of 20 m3 each with separate independent ventilation systems. In one section, new bedding (a mixture of pine shavings and sawdust) was used. In another section, regenerated litter made of the same materials was used after one rearing cycle of Chickens. The initial moisture content of the new reinforcement was 15.6%, and the regenerated 25.7%. In quantitative terms, at the induced humidity, the mass of the regenerated litter exceeded the mass of the new litter by 4.7 times, and in terms of dry matter content by 4.1 times, since during the first cycle of use, poultry excrement was added to the bedding material.

The regeneration of the used litter was carried out according to the previously developed experimental method, which involved the addition of the microbiological preparation Bioseven (Vetolac) manufactured by PE "BTU-Center" to the litter, its biothermal treatment for ten days in a pile 70 cm high and irradiation of the pile surface with bactericidal ultraviolet radiation (UVB wavelength 253.7 nm) according to the regime: daily once a day for 20 minutes. Microbiological preparation Bioseven (Vetolak) contained microorganisms of probiotic action not less than 1x10 10 CFU/l: Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus fermentum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, whey. The estimated dose of UVB was 50 J/m2.

Each section housed 150 heads. Day-old young chickens of the G-2 line of the Plymouth Rock white breed with a stocking density of 7.5 heads/m2. The standard temperature and air exchange was maintained in the sections (according to VNTP-APK-04.05). Also, the norms of feeding poultry of both groups were the same, following the Recommendations on rationing of feeding of poultry (IP et al., 2014). During the research period, the following indicators were studied and determined in both sections:

– litter moisture – according to GOST 26713−85 (once every two weeks, five samples from each section)

- the content of harmful gases (ammonia and carbon dioxide) in the air: using a portable 3-component gas analyzer Dozor S-M - once every two weeks at the same time - 5 measurements at the level of poultry in each section;

- mortality of poultry and causes of death – according to the data of daily registration and autopsy of carcasses;

- the live weight of birds: by weighing 50 of the same birds once a month on electronic scales with an accuracy of + 1 g;

- feed consumption by groups – by daily accounting;

- feed conversion – by calculation;

- plumage condition was assessed in the entire population of the experimental bird at the end of the experimental period visually on a point scale from 0 to 3, where 0 meant very clean and 3 - very dirty (De Jong et al., 2011).

- frequency and severity of foot lesions by pododermatitis: visually in the entire population of experimental birds at the end of the experimental period on a point scale from 0 to 4, where 0 points meant no lesions, and 4 points meant half of the foot area was affected (Hocking et al., 2011);

- the condition of the knee (hock) joints was assessed visually in the entire population of the experimental bird at the end of the experimental period in 3 categories, where category 01 meant no lesions, 02 – more than 25% of the hock area was affected, 03 – more than 50% of the hock area was affected (Bignon et al., 2015);

- the quality (structure) of the litter was assessed visually once a month on a 4-point scale (from 0 to 3): 0 – dry and loose; 1 - slightly damp, with a small degree of caking; 2 – moister and caked; 3- wet, with a high degree of caking (Kheravii et al., 2017);

– statistical processing of research results was carried out using application software for OS Windows - Microsoft Excel;

- The European Factor Efficiency Index (EPEF) was determined according to the generally accepted formula:

$$EPEF = \frac{live\ weight, kg \times conservation, \%}{growing\ period, days \times forage\ conversion, kg/kg} \times 100$$

Research Results

Dynamics of humidity and quality (structure) of litter. As mentioned above, the initial moisture content of the new reinforcement was 15.6%, and the regenerated 25.7%. That is, the difference was 9.1% ($p<0.05$). Subsequently, the moisture content of both the new and the regenerated litter increased. At the same time, the difference in humidity between them decreased (Fig. 1) since the moisture content of the new litter increased faster than that of the regenerated litter, primarily due to its much lower total amount. After ten weeks of rearing young animals, the moisture content of the litter in both variants no longer had significant differences. At the end of the growing period, the litter moisture content in the control section was 32.3%, and in the experimental section, 31.1%.

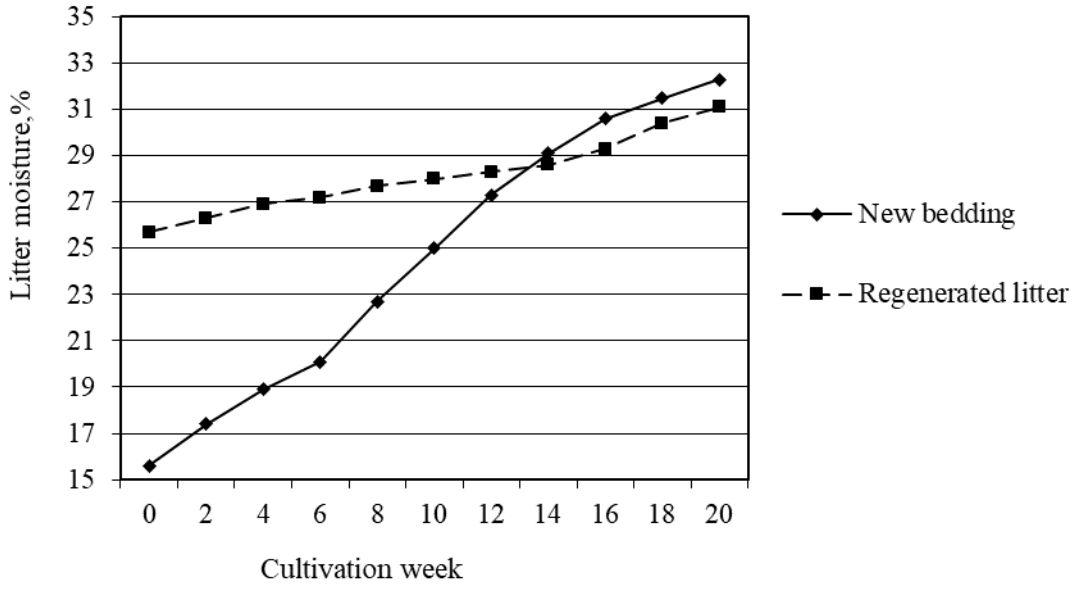

Fig. 1. **Moisture dynamics of new and regenerated litter**

The dynamics of litter quality (structure) in both sections is shown in Table 1.

Table 1

Dynamics of litter quality (structure) in the experimental and control sections

Month (week) of cultivation	Quality (structure) of litter	
	Control (new litter)	Experiment (regenerated litter)
0(0)-before planting chicks	0	1
1(4)	0	1
2(8)	0	1
3(12)	1	1
4(16)	1	1
5(20)	2	2

The structure of the litter during the rearing period of the rearing stock varied from dry and loose (new litter in the control section) or slightly moist with a small degree of caking (regenerated litter in the experimental section) before planting the chicks to more moist and caked in both sections at the end of the rearing period.

Dynamics of the content of harmful gases in the air of the experimental and control sections. The content of harmful gases (ammonia and carbon dioxide) in the air of the experimental and control sections during the entire period of cultivation did not exceed the maximum permissible concentrations (MPC) [15]. However, in the experimental section, it was higher than in the control section (Figs. 2 and 3). A more significant difference was observed in the initial cultivation period, for ammonia by 4.4 mg/m3 and carbon dioxide by 0.05% on the first day of cultivation. In terms of ammonia content, the difference between the experimental and control variants was statistically probable up to 8 weeks of age ($p<0.05$) and carbon dioxide content up to 6 weeks

of age ($p<0.05$). At the end of the growing period, the content of harmful gases in the air of the experimental and control sections was almost equal.

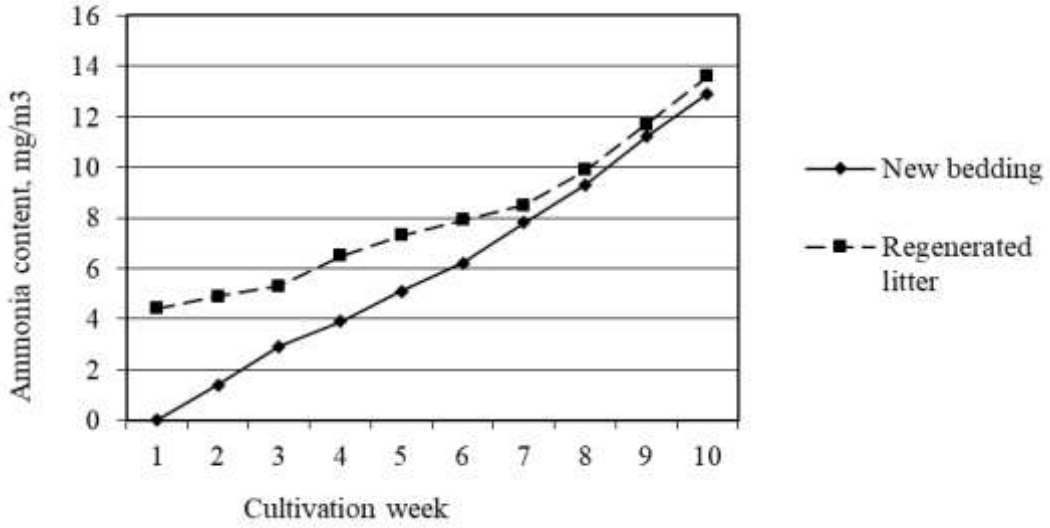

Fig. 2. **Dynamics of ammonia content in the air of premises for rearing young chickens using new and regenerated litter**

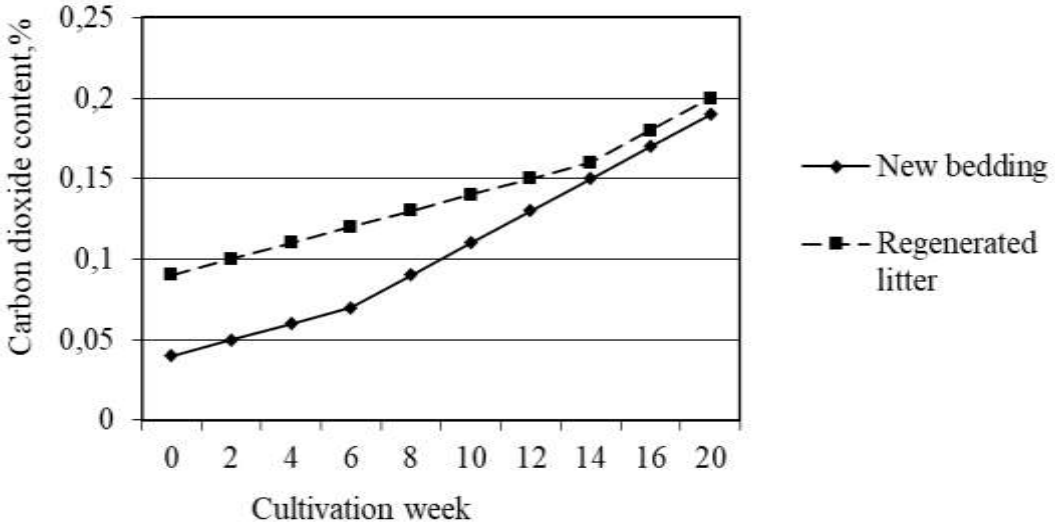

Fig. 3. **Dynamics of carbon dioxide content in the air of premises for rearing young chickens using new and regenerated litter**

Indicators of poultry welfare. Such indicators characterizing the bird's well-being as the state of plumage, the frequency and severity of damage to the feet by pododermatitis, and the condition of the knee (hock) joints were studied. The state of the plumage of young chickens at the end of the experimental period did not have significant differences in the control and experimental groups. Plumage contamination in both groups was minor, and it was also estimated at an average of 1 point (low degree of contamination). The number of birds with lesions of the knee (hocks) and with pododermatitis was also small. The severity of the lesion was also minor (see Table 2).

Table 2

Plumage condition, frequency and degree of damage to hocks and soles of the feet by pododermatitis in rearing young chickens when rearing on fresh and regenerated reinforcement

Indicators	Control (fresh litter)	Experiment (regenerated litter)
Number of birds evaluated	141	142
Assessment of the degree of contamination of the feather cover, points	1 (slight degree of contamination)	1 (low degree of contamination)
Number of birds with lesions of the knee joints, head.	7	6
Incidence of knee joint involvement, %	5,0	4,2
Degree of damage to the knee joints in poultry, points	01	01
Number of birds with lesions of the feet by pododermatitis	12	14
Frequency of manifestations of pododermatitis, %	8,5	9,9
Assessment of the degree of damage to the feet by pododermatitis, points	1	1

Zootechnical indicators. There were no significant differences between the groups of rearing chickens reared on fresh and regenerated litter and in terms of the leading zootechnical indicators (see Table 3). The live weight of the bird corresponded to the characteristics of the G-2 line for chickens of the appropriate age. The safety of young animals was within the permissible standards (VNTP-APK-04.05). The causes of death of the bird were not related to the quality of the litter.

Table 3

The main zootechnical indicators of rearing chickens when grown on new and regenerated litter

Indicators	Control (new litter)	Experiment (regenerated litter)
Duration of the experiment, weeks	20	20
Initial number of chickens, head.	150	150
Preserved to 20 weeks of age, goal.	144	143
Safety, %	96,0	95,3
Average live weight of chickens at the age of one day, g	42,8±1,12	41,7±0,87
Average live weight of birds (g) at age, weeks:		
4	356,5±5,3	345,4±4,5
8	851,8±14,2	839,1±15,9
12	1190,7±17,3	1178,4±19,6

16	1465,2±22,9	1457,2±23,7
20	1843,3±24,1	1835,4±25,9
Average weight gain during the growing period, kg	1,801	1,794
Homogeneity of livestock by live weight, %	72,0	70,8
Received conditioned youngsters, head.	131	130
Yield of conditioned young animals, %: of the number grown of the number planted for cultivation	 91,0 87,3	 90,9 86,7
Feed consumption during the growing period, kg/head	8,8	8,7
Feed conversion rate, kg/kg	4,996	4,849
Feed consumption per 1 grown conditioned pullet, kg	10,1	10,1

European Factor Efficiency Index. The EPEF in the control and experimental groups was 25.30 and 25.77, respectively; that is, there were also no significant differences.

Discussion. The moisture content of the litter at the end of the period of rearing and keeping poultry can be up to 45%. According to Karthiga (2018), the optimal moisture content of the litter used for regeneration is 28-32%. Suppose the actual moisture content of the used litter is less than this value. In that case, biothermal processes during its regeneration will not ensure the litter is heated to the temperatures required for pasteurization – 55-70°C (Lopes et al., 2015). This necessitates the addition of water and, as in our case, a higher initial moisture content of the regenerated litter, respectively 25.7%, compared to 15.6% for the new litter ($p<0.05$). Subsequently, the moisture content of both the new and the regenerated litter increased due to the constant supply of excrement. However, due to the 4.7 times greater total initial weight of the regenerated litter or 4.1 times in terms of dry matter, its moisture content increased more slowly than the new one. However, the difference in moisture between the litter variants remained statistically significant until the 10th week of cultivation. After ten weeks of rearing young animals, the litter moisture content in both variants no longer had significant differences and was, in the control section, 32.3%, and the experimental section, 31.1%. A similar trend was observed by Abougabal and Taboosha (2017). The difference in the structure of the new and regenerated litter, which was assessed according to the point system, was also observed only in the initial period of cultivation. At the same time, it must be borne in mind that before regeneration, "cakes" were removed from the litter. Further, this difference decreased, and in the last weeks of cultivation, the condition of the litter was estimated at 2 points - relatively wet and caked.

When rearing young chickens on regenerated litter in the air of the poultry house, an increase in the content of ammonia and carbon dioxide was observed, respectively, by 3.5-1.1 and 2.1-1.1 times compared to their rearing on new litter. In terms of ammonia content in the air, the difference between the cultivation options was statistically significant from the first to the eighth week of cultivation ($p<0.05$), in terms of carbon dioxide in the air from the first to the sixth week of cultivation ($p<0.05$). At the same time, during the entire cultivation period, their concentration in the air did not exceed the maximum permissible. An increase in ammonia emissions from the regenerated litter compared to fresh litter was observed, and Kyong from singing. (2017) and Coufal with Sang. (2006). In all cases, this increase can be explained by a higher

amount of organic matter in the regenerated litter due to an overall higher amount of organic matter (in our case, 4.1 times in terms of dry matter) and a greater degree of decomposition. At the same time, the content of these harmful gases in the air of the experimental room did not exceed the maximum permissible concentration at similar (according to the norms of VNTP-APK-04.05) in the experimental and control versions of the levels of air exchange in the premises.

However, the most important criteria determining the possibility of repeated use of litter after properly carried out regeneration, of course, are harmlessness to poultry welfare and productive indicators of poultry compared to the use of new litter. In our study, no significant difference was found between these options when rearing young chickens for indicators that characterize the well-being of poultry as the state of plumage, the condition of the knee joints, and the condition of the soles of the paws. In both variants, these indicators were within acceptable limits. Similar data in the rearing of broiler chickens were obtained by Cressman, M. D. (2014) and Oliveira, M. from Singing. (2015), while Abougabal (2019) observed a negative effect of regenerated litter on the indicated indicators.

In our case, there were no significant differences between the experimental and control groups of rearing young animals, which were reared, respectively, on regenerated and new litter, and in the leading zootechnical indicators: safety, live weight by age and feed conversion, as well as such a generalizing indicator as the European Production Factor Efficiency Index (EPEF). The live weight of the bird corresponded to the characteristics of the G-2 line for chickens of the appropriate age. The safety of young animals was within the permissible standards (VNTP-APK-04.05). The causes of death of the bird were not related to the quality of the litter. When growing broiler chickens, the absence of significant differences in the leading productive indicators of chickens raised on new and regenerated litter was noted by Abougabal (2019) and Kalita with singing. (2012), although Cressman (2014) and Garces-Gudino sang. (2018) The best performance was obtained when broilers were raised on regenerated bedding and Nagaraj sang. (2007) when grown on new litter. This indicates the need to continue research in this direction, in particular, taking into account economic factors.

Conclusions

1. The used litter, regenerated according to the technology improved in the DSP NAAS, can be used in rearing young chickens without adversely affecting the welfare and productive performance of poultry.

2. It was found that the moisture content of the regenerated litter was 1.6-1.2 times higher than the new one ($p<0.05$) from the first to the eighth week of rearing young animals. With further cultivation, the difference in moisture between the types of litter decreased and became insignificant.

3. When rearing young chickens on regenerated litter, there was an increase in the content of ammonia in the air of the poultry house by 3.5-1.1 times and carbon dioxide by 2.1-1.1 times compared to their rearing on new litter. In terms of ammonia content in the air, the difference between the cultivation options was statistically significant from the first to the eighth week of cultivation ($p<0.05$), in terms of carbon dioxide content in the air from the first to the sixth week of cultivation ($p<0.05$). At the same time, during the entire cultivation period, their concentration in the air did not exceed the maximum permissible.

4. Rearing of rearing chickens on regenerated litter did not adversely affect the studied indicators of poultry welfare and zootechnical indicators of rearing.

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ACCUMULATION OF ^{137}Cs, Pb, Cd, Cu IN THE MUSCLE TISSUE AND LIVER OF PIGS GETTING DIFFERENT RATIONS

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Experimental studies on young pigs of the large white breed were conducted on the territory of the physiological yard of the Polissia Institute of Agriculture of the National Academy of Sciences (III zone of radioactive contamination due to the accident at the Chernobyl Nuclear Power Plant). To conduct the experiment, 2 groups of experimental animals were formed: Group I (control) was fed with grain mixture № 1 with narrow-leaved lupine (alkaloid-free) and sunflower cake; II group (experimental) received grain mixture № 2 with genetically modified soy. Experimental pigs were kept in one room according to the accepted technology in group cages equipped with a wooden floor. The mode of feeding and drinking, parameters of the microclimate in both groups were the same.

According to the results of tests conducted at the Ukrainian Laboratory of Quality and Safety of Agricultural Products, it was established that soybeans contain genetically modified deoxyribonucleic acid (DNA), have the target sequence of the 35S+FMV promoter of the cauliflower mosaic virus and the NOS terminator (nopaline synthase) of Agrobacterium tumefaciens.

The specific activity of ^{137}Cs in the longest back muscle of pigs varied by group within 3.7-10.3 Bq/kg and did not exceed permissible levels (DR-2006 = 200 Bq/kg). When used as part of the GM soybean grain mixture, the concentration of radiocesium in the muscle tissue of animals of the II (experimental) group relative to the control decreased by 6.6 Bq/kg, or by 64.1% with a statistically significant difference (P> 0.95). Moreover, the accumulation rate of ^{137}Cs in the longest back muscle of piglets was 0.46-0.95 and was 2.1 times higher in young pigs that received grain mixture No. 1 without GM soybeans, compared to the use of grain mixture No. 2.

The concentration of Pb in the slaughter products of experimental animals was significantly lower than the MPC, while the level of contamination of the longest back muscle of pigs Cd exceeded the regulatory requirements by 1.40-1.44 times. The introduction of 60% (by mass) of corn gain and 10% of GM soybeans into the grain mixture instead of the same amount of wheat grain, lupine and sunflower cake for fattening young pigs in the III zone of radioactive pollution contributes to a smaller accumulation of heavy metals in meat: Pb – by 17.5%, Cd – by 2.8%, Cu – by 4.2%. Therefore, the transition coefficients of Pb and Cd into the muscle tissue of animals of the II (experimental) group relative to the analogues of the I (control) group increased by 1.24% and 3.02% respectively, and Cu decreased by 0.23%.

Key words: pigs, ^{137}Cs, Pb, Cd, Cu. GM soy, the longest back muscle, liver.

НАКОПИЧЕННЯ ^{137}Cs, Pb, Cd, Cu У М'ЯЗОВІЙ ТКАНИНІ І ПЕЧІНЦІ СВИНЕЙ ЗА ВИКОРИСТАННЯ РІЗНОТИПОВИХ РАЦІОНІВ

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Експериментальні дослідження на молодняку свиней великої білої породи проводили на території фізіологічного двору Інституту сільського господарства Полісся НААН (ІІІ зона радіоактивного забруднення внаслідок аварії на ЧАЕС). Для проведення досліду сформовано 2 групи піддослідних тварин: І група (контрольна) – згодовували зерносуміш №1 з люпином вузьколистим (безалкалоїдним) і макухою соняшниковою; ІІ група (дослідна) – отримувала зерносуміш №2 з генетично модифікованою соєю. Піддослідних свиней утримували в одному приміщенні згідно з прийнятою технологією – у групових клітках, обладнаних дерев'яною підлогою. Режим годівлі та напування, параметри мікроклімату в обох групах були однаковими.

За результатами випробувань, проведених в Українській лабораторії якості і безпеки продукції АПК, встановлено, що соя містить генетично модифіковану дезоксирибонуклеїнову кислоту (ДНК), має цільову послідовність промотора 35S+FMV вірусу мозаїки цвітної капусти та NOS-термінатора (нопалінсинтази) Agrobacterium tumefaciens.

Питома активність ^{137}Cs в найдовшому м'язі спини свиней коливалася по групах у межах 3,7-10,3 Бк/кг і не перевищувала допустимих рівнів (ДР-2006 = 200 Бк/кг). За використання у складі зерносуміші ГМ-сої, концентрація радіоцезію в м'язовій тканині тварин ІІ (дослідної) групи відносно контролю знижувалася на 6,6 Бк/кг, або на 64,1 % за статистично значущої різниці (P>0,95). Водночас кратність накопичення ^{137}Cs в найдовшому м'язі спини підсвинків складала 0,46-0,95 і була більшою у 2,1 рази в молодняку свиней, які отримували зерносуміш №1 без ГМ-сої, порівняно з використанням зерносуміші №2.

Концентрація Pb в продуктах забою піддослідних тварин була значно нижчою за ГДК, тоді як рівень забруднення найдовшого м'язу спини свиней Cd перевищував нормативні вимоги в 1,40-1,44 рази. Уведення до складу зерносуміші 60 % (за масою) дерті кукурудзи і 10 % ГМ-сої замість аналогічної кількості дерті пшениці, люпину і макухи соняшникової за відгодівлі молодняку свиней у ІІІ зоні радіоактивного забруднення сприяє меншому нагромадженню важких металів у м'ясі: Pb – на 17,5 %, Cd – 2,8, Cu – на 4,2 %. При цьому коефіцієнти переходу Pb і Cd у м'язову тканину тварин ІІ (дослідної) групи відносно аналогів І (контрольної) групи підвищувалися на 1,24 % і 3,02 % абс. відповідно, а Cu - знижувалися на 0,23 % абс..

Ключові слова: свині, ^{137}Cs , Pb, Cd, Cu. ГМ-соя, найдовший м'яз спини, печінка.

Introduction. As a result of the accident at the Chornobyl NPP, large areas of Ukrainian Polissia were contaminated with radioactive substances ^{137}Cs, ^{90}Sr, etc. Their entry into the body of animals with fodder rations requires the use of technologies for the production of livestock products that make it possible to produce ecologically safe

and high-quality food products for the population living in these regions (Zubets M.V. et al., 2011; Krasnov V.P. et al., 2015). Regardless of the general trend of stabilization of the radiation state, it should be noted that the levels of radioactive contamination of agricultural products in some regions of Polissia of Ukraine are ten times higher than the pre-accident level and in some cases, especially in peasant farms, remain significantly higher compared to the existing regulations (Romanchuk L. D. et al., 2019).

Pollution of the Polissia zone by heavy metals such as Pb, Cd, Cu remains an equally important problem (Savchuk I. et al., 2022). These chemical elements and their compounds are toxic in high concentrations, which negatively affect the health, productivity of animals, and the quality of livestock products (Peng L. et al., 2015; Martyshuk T.V. et al., 2019).

Heavy metals are metallic elements with a high density that gradually accumulate in the food chain with negative effects on human health. Some elements are necessary (Fe, I, Co, Zn, Cu, Mn, Mo, Se) to support various physiological functions and are usually added as feed additives to the ration of animals. Other metals (Cd, Pb, Hg) have no established biological functions and are considered pollutants (Hejna M. et al., 2018). Heavy metals are potentially dangerous due to their toxicity, bioaccumulation and biomagnification when they are in living tissues and accumulate more than they are excreted (Jarup L., 2003).

According to the authors (Mamenko O.M., Portiannik S.V., 2021), in areas with high anthropogenic pressure, in order to reduce the accumulation of harmful substances in the body of animals and obtain ecologically safe livestock products, it is very important to balance the rations of animals in terms of protein, trace elements and vitamins. It was established that with a shortage of sugar, protein, and mineral substances in the rations, and, as a result, low productivity of animals, the accumulation of ^{137}Cs and heavy metals in milk and meat increases significantly compared to complete feeding. Complete protein nutrition weakens the toxic effect of harmful substances, reduces the absorption of ^{137}Cs and heavy metals from the gastrointestinal tract and increases their removal from the body (Razanov S. et al., 2022).

Taking into account the wide range of biological and toxic effects of radionuclides and heavy metals, which causes a negative impact on the internal organs and systems of animals (Roggeman S. et al., 2014; Hashemi S., 2018), it is worth noting that the necessity of improving the system of management of the livestock industry and animal feeding in areas of increased man-made load of agricultural production. Therefore, research on the search for types of feed and rations of animals with the aim of reducing the accumulation of ^{137}Cs, Pb, Cd, Cu in livestock products during their production in the III zone of radioactive contamination due to the accident at the Chernobyl nuclear power plant is quite relevant.

The purpose of the research is to determine the accumulation and transfer of ^{137}Cs, Pb, Cd, Cu in the muscle tissue and liver of pigs when using different high-protein feeds in the composition of fodder grain such as narrow-leaved lupine and sunflower meal and GM soybeans.

Research materials and methods. Experimental studies on young pigs of the large white breed were conducted on the territory of the physiological yard of the Polissia Institute of Agriculture of the National Academy of Sciences. To conduct the experiment, 2 groups of young pigs were formed using the method of balanced groups according to the methodological provisions of Ibatullin I.I. and Zhukorskyi O.M. (2017). The average indicators by which the animals were characterized were almost equal in both groups. Experimental pigs were kept in one room according to the accept-

ed technology in group cages equipped with a wooden floor. The mode of feeding and drinking, parameters of the microclimate in both groups were the same.

According to the scheme of the experiment, during the comparative period, the animals of both experimental groups received grain mixture No. 1, which consisted of concentrated fodder of local production, grown in the III zone of radioactive contamination, with the addition of PMVA (Table 1).

Table 1

The composition of grain mixtures during research, % by mass

Concentrated fodder	Grain mixture № 1	Grain mixture № 2
Wheat grain	60	-
Corn grain	-	60
Triticale grain	25	25
Sunflower cake	5	-
Narrow-leaved lupine (alkaloid-free)	5	-
GM soybeans extruded	-	10
PMVA	5	5
In total	100	100

The difference in the feeding of the experimental pigs in the main period of the research was that the animals of the I (control) group received the same feed as in the comparative period of the experiment. Simultaneously, in the II (experimental) group of piglets, 60% of wheat grain (by weight) in the ration was replaced by 60% of corn grain, and 10% of cake and lupine – with a similar amount of genetically modified extruded soybeans.

On average, the animals consumed slightly different amounts of mixed grain per day: I group – 2.52 kg, II group – 2.29 kg. The nutritional value of 1 kg of fodder for piglets was also different: I group – 1.16 ECO, 11.6 MJ of exchangeable energy and 869 g of dry matter; II group – 1.36 ECO, 13.6 MJ of exchangeable energy and 866 g of dry matter. During the period of raising young pigs, the concentration of energy in 1 kg of dry matter of the ration was: I group – 1.33 ECO and 13.3 MJ of exchangeable energy, II group – 1.57 ECO and 15.7 MJ of exchangeable energy. Based on the calculation of 1 kg of dry matter of the ration, there were 155-121 g of digestible protein (norm 109-118 g) and 42.5-64.5 g of fiber, which is slightly lower than the existing norms for fattening pigs (norm 66-76 g) (I.I. Ibatullin, O.M. Zhukorskyi, 2016).

Determination of the specific activity of ^{137}Cs in feed and livestock products was carried out on a SEG-0.5 spectrometer. The preparation of samples of plant and animal origin for the determination of heavy metals in their composition was carried out by the method of dry mineralization, the analysis was carried out on the atomic absorption spectrometer "Kvant-2A" according to DSTU 7670:2014.

Transition coefficients (CP) of heavy metals (Pb, Cd, Cu) in the "ration – production (muscle tissue and liver)" chain were determined by the formula: TC = Chmp./Chmr. x 100, where TC is the transition coefficient; Chmp is the content of heavy metals in animal products, mg/kg; Chmr is the content of heavy metals in the daily ration, mg (Mamenko O.M., Portyanyk S.V., 2019). This coefficient is a relative integrated indicator, which in % reflects the migration of heavy metals from the ration to products, which allows for a comparative assessment of the transfer of pollutants when using different fodder mixtures for feeding pigs.

Research results. In the conditions of modern man-made pollution of the environment, ecological problems, improvement of the quality of livestock products and their food safety become extremely relevant.

The specific activity of ^{137}Cs in the fodder of the rations fluctuated within insignificant limits 8.0-10.8 Bq/day and was greater by 2.8 Bq/day or by 35.0% in the I (control) group than in the II (experimental)) group. This is due to the consumption of experimental pig herds of grain mixes of different composition. Probably, the accumulation of radionuclide in winter wheat and lupine compared to corn and GM soybeans was significantly greater.

According to the research results, a significant intergroup difference in the concentration of ^{137}Cs in the longest back muscle and an improbable difference in the liver of the experimental young pigs were established (Table 2).

Table 2

Concentration of ^{137}Cs in ration feed and products (n=3; M ± m)

Groups of animals	^{137}Cs concentration			
	average daily ration, Bq	products, Bq/kg	± to control group	
			Bq/kg	%
The longest back muscle				
I - control	10.8	10.3 ± 1.8	-	-
II - experimental	8.0	3.7 ± 0.3*	-6.6	-64.1
Liver				
I - control	10.8	6,0 ± 1,7	-	-
II - experimental	8.0	4.0 ± 1.0	-2.0	-33.3

*Note. * - P>0.95*

The specific activity of ^{137}Cs in the longest back muscle of the animals ranged from 3.7 to 10.3 Bq/kg and did not exceed permissible levels (DR-2006 = 200 Bq/kg). Therefore, when grain mixture No. 2 is used as part of the ration, the concentration of radiocesium in the muscle tissue of young pigs of the II (experimental) group, relative to the control, decreases by 6.6 Bq/kg, or by 64.1% (P>0.95).

A similar pattern was observed in the accumulation of ^{137}Cs in the liver of experimental young pigs as this indicator was the lowest in animals of II group (4.0 Bq/kg), and the highest in the analogues of I group (6.0 Bq/kg). Compared to the longest back muscle, the concentration of ^{137}Cs in the liver of animals of the control group was lower by 41.7%, while this indicator was higher by 8.1% when pigs were fed with grain-mix № 2.

The parameter that characterizes contamination of animal husbandry products with radionuclides depending on their entry into the body of animals with feed is the multiplicity of accumulation. This is the ratio of the nuclide content in an organ, tissue or organism as a whole to the content in the daily ration (B.S. Priester, 2007).

In our studies, the accumulation rate of ^{137}Cs in the longest back muscle was 0.46-0.95 and was 2.1 times higher in young pigs that received grain mixture № 1 with wheat, lupine and cake, compared to grain mixture No. 2 with corn and genetically modified soy (Fig. 1).

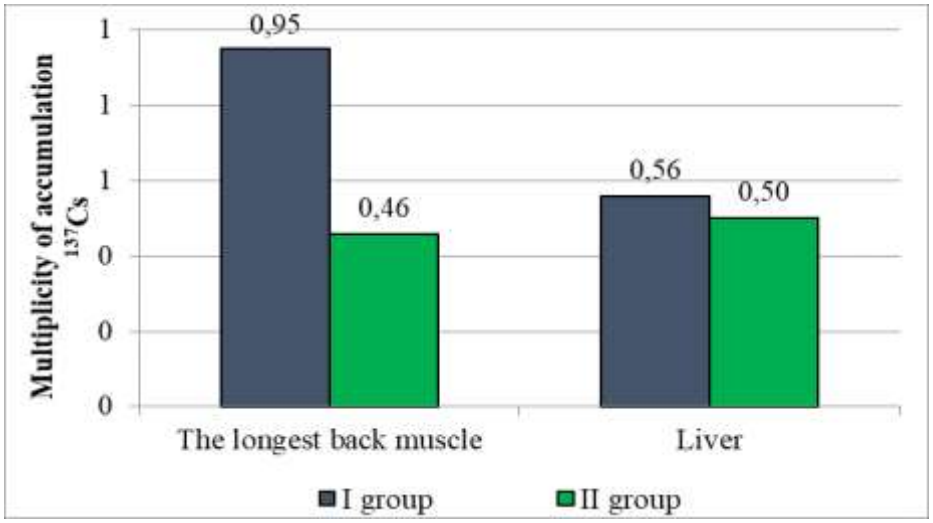

Fig. 1. **Multiplicity of ^{137}Cs accumulation in the longest back muscle and liver of experimental pigs**

The multiplicity of accumulation of ^{137}Cs in the liver of experimental animals varied between 0.50-0.56 and was 12.0% higher in piglets of the control group (I) compared to the II (experimental) group.

Taking into account the above, it can be asserted that the replacement of 60% of wheat grain and 10% (by mass) of sunflower and lupine cake with the appropriate amount of corn grain and GM soybeans for fattening young pigs in the Polissia zone of Ukraine leads to a reliable decrease in the specific activity of ^{137}Cs in the longest back muscle and insignificant in the liver.

According to the researchers' reports (Wang Y. et al, 2018), heavy metals of high toxicity Pb, Cd, Hg, As, Se, F, Zn pose a special danger to farm animals and humans. Moreover, according to WHO experts and other international organizations, one of the global and dangerous environmental pollutants is Pb. This metal has sufficiently large volumes of production and a wide scope of application, which causes its arrival and distribution in various objects of the surrounding natural environment. The ability to accumulate in organs and tissues, high biological activity of Pb pose a real threat to human and animal health. At its blood concentration of 200-400 μg/l, people may develop signs of poisoning (Yabe J. et al., 2015).

Experimental studies showed that a significant amount of Pb entered the body of experimental pigs with feed rations, the daily consumption of which by young pigs ranged from 2.464 to 3.641 mg and was 47.8% higher in the I (control) group than in II (experimental) group (Table 3).

Based on the results of the research, it was established that in the longest back muscle of pigs of both experimental groups, the accumulation of Pb was significantly lower than the MPC (0.50 mg/kg) and varied within 0.170-0.206 mg/kg. However, as a result of the introduction of 60% of corn grain and 10% (by mass) of GM soybeans into the grain mixture, the concentration of Pb in the muscle tissue of animals of II group decreased by 0.036 mg/kg, or by 17.5% ($P<0.95$) compared to the corresponding indicator for feeding young animals with grain-carrying mixture № 1.

The opposite pattern was observed in the accumulation of Pb in the liver of experimental young pigs. Thus, with the introduction of corn and GM soybeans into the composition of the grain mixture, the concentration of Pb in the liver of animals of the II (experimental) group compared to the I (control) group increased by 0.048 mg/kg, or

by 18.5% (P<0.95). It should be emphasized that the concentration of Pb in the liver of piglets of both groups was lower than TLK by 48.7.7-56.7%.

Table 3

Concentration of Pb in feed rations and products (n=3; M ± m)

Groups of pigs	Concentration of Pb			
	average daily ration, mg	production, mg/kg	± to control	
			mg/kg	%
The longest back muscle				
I - control	3.641	0.206 ± 0.018	-	-
II - experimental	2.464	0.170 ± 0.014	-0.036	-17.5
TLK	-	0.50	-	-
Liver				
I - control	3.641	0.260 ± 0.007	-	-
II - experimental	2.464	0.308 ± 0.029	+0.048	+18.5
TLK	-	0.60	-	-

As for the transition of Pb from ration fodder to the longest back muscle and liver of young pigs, it should be noted that this indicator was higher in animals of II (experimental) group by 1.24% and 5.36% abs. than in I (control) group (Fig. 2).

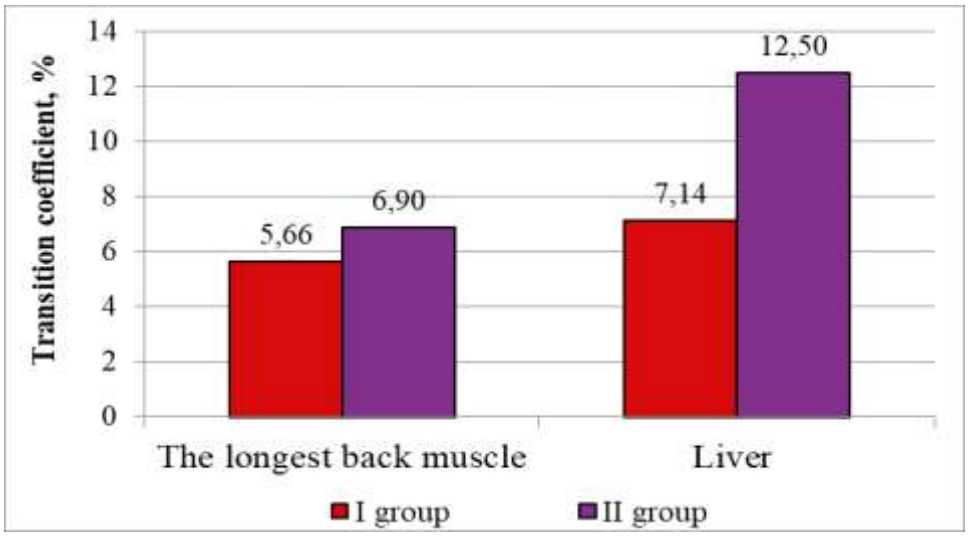

Fig. 2. **Coefficients of Pb transition into muscle tissue and liver of pigs**

Cadmium is one of the most toxic heavy metals, it belongs to the second class of danger as highly dangerous substances (Lavryshyn Y. et al., 2018). Like most other heavy metals, Cd has a clear tendency to accumulate in the body: its half-life is 10–35 years (Lavryshyn Y. et al., 2019). Cadmium has a harmful effect on the vital systems of human and animal bodies, causing pathological changes in tissues and organs (kidneys, lungs, bone tissue, organs of the reproductive and endocrine systems), suppressing the process of erythropoiesis (Peng L. et al., 2015). Cadmium and its compounds are immunotoxicants that lead to disturbances in the functioning of the body's immune system, reduce resistance to infections, and contribute to the formation of allergic, autoimmune, and oncological pathologies (Ostapyuk A.Y., GutyjB.V., 2020). The feature of the biological action of Cd is its ability to negatively affect the health of animals with

long-term exposure to low levels of pollution due to a high coefficient of biological accumulation (up to 40 years) (Lavryshyn Y., Gutyj B., 2019).

The content of Cd, which entered the body of experimental young pigs of the large white breed after being fed with different versions of grain mixtures, was significantly lower than Pb, which was 0.293-0.345 mg/day (Table 4).

Table 4

Concentration of Cd in feed rations and slaughter products of pigs (n=3; M ± m)

Groups of pigs	Concentration of Cd			
	average daily ration, mg	production, mg/kg	± to control	
			mg/kg	%
The longest back muscle				
I - control	0.345	0.072 ± 0.003	-	-
II - experimental	0.293	0.070 ± 0.004	-0.002	-2.8
TLK	-	0.05	-	-
Liver				
I - control	0.345	0.118 ± 0.009	-	-
II - experimental	0.293	0.098 ± 0.007	+0.020	-16.9
TLK	-	0.30	-	-

According to the research results, it can be stated that the concentration of Cd in the muscle tissue of experimental pigs varied in a small range of values 0.070 – 0.072 mg/kg. This indicator in animals of I and II groups was higher than the maximum permissible concentration by 1.44 and 1.40 times, respectively. The improbable intergroup difference in the content of Cd in the longest back muscle was established depending on the feeding of fattening pigs in rations of different composition of grain mixtures: in the meat of young animals of II (experimental) group relative to the I (control) group, this indicator was lower by 2.8% ($P<0.95$).

A much larger amount of Cd accumulates in the liver of experimental animals, 0.098-0.118 mg/kg, while this indicator did not exceed TLK (0.30 mg/kg). Instead, the smallest amount of Cd was contained in the liver of piglets of II group (0.098 mg/kg), which were fed with grain mixture № 2.

The transition coefficients of Cd in pork (longest muscle) and liver were quite high,20.87-23.89 and 33.45-34.20%, respectively (Fig. 3). When feeding young pigs with grain mixture № 2, the coefficient of Cd transition into the muscle tissue of animals of II group compared to I group increases by 3.02% abs., while using different variants of grain mixtures, the transition of Cd into the liver of young pigs of both groups did not differ significantly (33.45-34.20%).

The average daily intake of trace element Cu in the body of fattening animals with fodder rations did not differ significantly between the experimental groups and was in I group 27.2 mg/day, in II group 27.9 mg/day (Table 5). The concentration of Cu in the longest back muscle of young pigs of both groups was low (0.91-0.95 mg/kg) and did not exceed TLK (5.0 mg/kg). The use of grain mixtures of different composition for fattening pigs did not have a significant effect on the content of this element in meat, however, it was 4.2% lower in animals of II group than in the analogues of I group.

The main depot of Cu in the body of animals is the liver, the content of the element in which was 10.0-22.2 times higher than in the longest back muscle. When using the proposed variants of grain mixtures of corn and GM soybeans, the concentration of Cu in the liver relative to the control decreased by 10.70 mg/kg, or by 52.9% for a prob-

able difference (P>0.95). At the same time, in young pigs of I (control) group, the content of Cu in the liver was at the level of TLK (20.21 mg/kg).

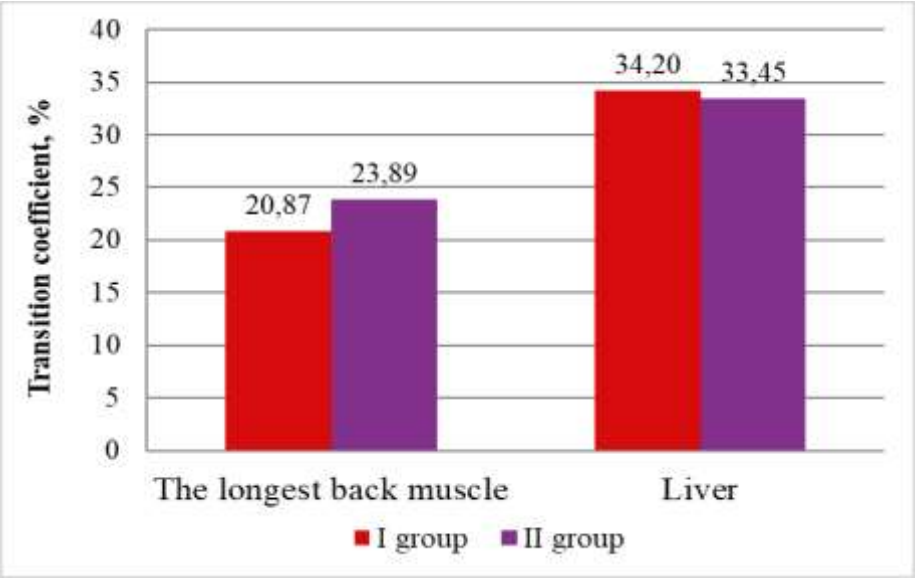

Fig. 3. **Coefficients of Cd transition into muscle tissue and liver of pigs**

Table 5

Concentration of Cu in feed rations and products (n=3; M ± m)

Groups of pigs	Concentration of Cu			
	average daily ration, mg	production, mg/kg	average daily ration, mg	
			mg/kg	%
The longest back muscle				
I - control	27.2	0.95 ± 0.06	-	-
II - experimental	27.9	0.91 ± 0.03	-0.04	-4.2
TLK	-	5.0	-	-
Liver				
I - control	27,2	20.21 ± 2.46	-	-
II - experimental	27,9	9.51 ± 0.62*	-10.70	-52.9
TLK	-	20.0	-	-

*Note. * - P>0.95*

The transition coefficients of Cu from the forages of the rations to the liver of animals were high, 34.09-74.30%, compared to 3.26-3.49% in the longest back muscle (Fig. 4). The positive trend was noted as with the introduction of corn and GM soybeans into the fodder grain mixture, the accumulation of Cu in both the longest back muscle and the liver of young pigs of II (experimental) group relative to the control decreased by 0.23% and 40.21% abs., respectively.

On the basis of research conducted on the fattening of young pigs in III zone of radioactive contamination, certain regularities of the transformation of heavy metals into the products of animal slaughter have been revealed. Thus, according to our data, the transition coefficients of individual metals were (%):

- in the longest back muscle: Cd–20.87-23.89>Pb–5.66-6.90>Cu-3.26-3.49;
- in the liver: Cu – 34.09-74.30 > Cd – 33.45-34.20 > Pb – 7.14-12.50.

This makes it possible to state that among the studied metals, Cd is characterized by significant accumulation properties in muscle tissue, and Cu in the liver. The transition coefficients of Cd into pig slaughter products were 2.7-4.8 times higher compared to Pb.

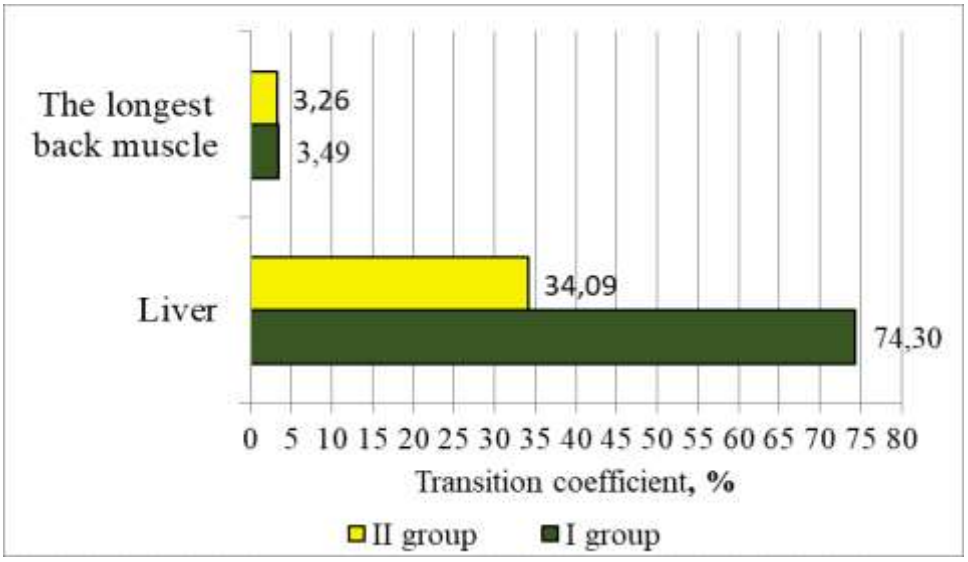

Fig. 4. **Transition coefficients of Cu into products**

Therefore, the use of grain mixture No. 2 (corn + triticale + GM soy + PMVA) for fattening young pigs in the accident zone at the Chernobyl NPP had a positive effect on the ecological quality of pig farming products, reducing the specific activity of ^{137}Cs and the concentration of heavy metals, in particular Pb, Cd and Cu.

Discussion. The replacement of 60% (by mass) of wheat grain, 10% of cake and lupine in the composition of the grain mixture with the appropriate amount of corn chaff and GM soybeans for fattening young pigs in III zone of radioactive contamination had a positive effect on the ecological quality of pork, reducing in the longest back muscle and the liver, the specific activity of ^{137}Cs and the concentration of heavy metals, in particular Cd, Pb and Cu. This can be explained by a slightly better provision of protein in the rations of young pigs of the research group and its better amino acid composition. Thus, the ratio of amino acids to crude protein in the rations of animals of groups I and II was (%): lysine – 4.26 and 5.33, methionine + cystine – 3.07 and 3.13, threonine – 3.07 and 4.80, respectively. Therefore, the content of essential amino acids in the feed of the ration can serve as one of the indicators of the overall protein quality.

According to research by both domestic and foreign authors (Mamenko O.M., Portiannik S.V., 2021; Savchuk I.M. et al., 2021; Chalabis-Mazurek A. et al., 2021), the transition of radionuclides and heavy metals from feed to products depends on ecological and technological conditions of production, type and degree of digestibility of feed, age and physiological state of animals, as well as to a large extent from the level and full value of feeding, balance of rations in terms of substances that have radio-protective properties. These substances increase the body's resistance to radiation, accelerate the removal of radionuclides and heavy metals, and reduce their content in products. Such substances include many amino acids (especially sulphur-containing ones), fibre, minerals, vitamins, especially A, E, groups B, C. (Hashemi S., 2018; Moharrery et al., 2015).

In addition, the selection of the optimal type of animal feed contributes to a significantly lower transition of radionuclides and heavy metals into livestock products, which is confirmed by studies conducted on dairy cows, young cattle and pigs

(O.M. Mamenko, S.V. Portyanik, 2019; Savchuk I. et al., 2021; Zhukorskyi O.M. et al., 2018).

Conclusions.

1. When various high-protein feeds were used to optimize the protein nutrition of young pigs, the specific activity of ^{137}Cs in the longest back muscle and liver of the experimental animals varied by group within the range of 3.7-10.3 Bq/kg and 4.0-6.0 Bq/kg, respectively, and did not exceed permissible levels (DR-2006 = 200 Bq/kg). The introduction of GM soybeans into the grain mixture, the concentration of radioesium in the muscle tissue of pigs of II (experimental) group relative to the control decreased by 6.6 Bq/kg, or by 64.1% with a statistically significant difference (P>0.95). Therefore, the multiplicity of accumulation of ^{137}Cs in the longest back muscle of piglets was 0.46-0.95 and was 2.1 times higher in young pigs that received grain mixture No. 1 without GM soy compared to the use of grain mixture № 2.

2. The concentration of Pb and Cu in the slaughter products of experimental animals was significantly lower than TLK, while the level of contamination of the longest back muscle of pigs Cd exceeded the regulatory requirements by 1.40-1.44 times. The introduction of 60% (by mass) of corn grain and 10% of GM soybeans into the grain mixture instead of a similar amount of wheat grain, lupine and sunflower cake for feeding animals in III zone of radioactive contamination contributes to a significantly lower accumulation of heavy metals in muscle tissue and liver (excluding the content of Pb in the liver): Pb – by 17.5%, Cd – by 2.8 and 16.9%, Cu – by 4.2 and 52.9%, respectively. Thus, the coefficients of transition of heavy metals into the products of pigs depended on their consumption with fodder in the rations with a higher content of Pb and Cd in the rations, their transition coefficients into the longest back muscle and liver decrease.

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POPULATION CHARACTERISTICS AND THE CONCEPT OF GENE POOL CONSERVATION FOR THE OF HORSES OF THE NEWLY CREATED UKRAINIAN TROTTING BREED GROUP

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Population characteristics were analyzed and specific features of the newly created Ukrainian trotting breed group of horses were revealed. The main conditions for stable preservation of the horse gene pool, risks and opportunities for the newly created Ukrainian trotting breed group are determined. The quantitative composition of the reproductive population of the Ukrainian trotting breed group of horses, which according to the FAO classification is in the status of "in a state of danger", has been established. The basic population characteristics of the newly created breed group are described. The share of the breed group in the total of trotting horses of Ukraine, which is 37.2%, was determined, the amount of interbreeding improving crossbreeding with the American Standardbred and French trotting breeds was determined, and the expediency of its use was justified. The maximum number of generations in the breed – 11 – was observed in the Scotland line, a branch of Speedy Crown. The use of indicators, one of which is based on the generation interval, and the second is based on the inbreeding coefficient, made it possible to analyze the population and identify specific features of the Ukrainian trotting breed group of horses. The average annual yield of foals and the number of repair young ones were calculated to preserve the gene pool of the breed group at the current level. The generation interval for the breed group was L=11.57±0.98 years. It was determined that the generation interval between sires and their male offsprings is greater than between dams and male offsprings, and the generation interval between dams and female offsprings is greater than between sires and female offsprings. The effective number of the population, according to which the new breed group will develop qualitatively, is 688.2 horses of reproductive age. The increase in the inbreeding coefficient for one generation is ΔF=0.0063, and therefore, to prevent the growth of inbreeding, the increase in the effective size of the population per generation should be 79.4 heads. If the quantitative composition of the breed group does not increase, the population will lose 2.36% of genetic diversity in 10 generations.

Key words: horses, Ukrainian trotting breed group, population characteristics, generation interval, inbreeding coefficient, preservation of the gene pool.

ПОПУЛЯЦІЙНІ ХАРАКТЕРИСТИКИ ТА КОНЦЕПЦІЯ ЗБЕРЕЖЕННЯ ГЕНОФОНДУ КОНЕЙ НОВОСТВОРЮВАНОЇ УКРАЇНСЬКОЇ РИСИСТОЇ ПОРОДНОЇ ГРУПИ

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Проаналізувано популяційні характеристики та виявлено специфічні особливості новостворюваної української рисистої породної групи коней. Визначено головні умови сталого збереження генофонду коней, ризики і можливості для новостворюваної української рисистої породної групи. Встановлено кількісний склад репродуктивного поголів'я української рисистої породної групи коней, що за класифікацією ФАО знаходиться у статусі «у стані небезпеки». Описано основіні популяційні характеристики новостворюваної породної групи. Визначено частку породної групи у масиві рисистих коней України, що складає 37,2 %, встановлено обсяги міжпорідного поліпшуючого схрещування з американською стандартбредною та французькою рисистою породами і обгрунтовано доцільність його використання. Максимальну кількість генерацій в породі – 11 – спостерігали в лінії Скотланда, гілка Спіді Крауна. Використання показників, один з яких заснований на генераційному інтервалі, а другий – на коефіцієнті інбридингу, дозволило проаналізувати популяцію та виявити специфічні особливості української рисистої породної групи коней. Розраховано середньорічний вихід лошат та кількість ремонтного молодняку для збереження генофонду породної групи на сучасному рівні. Генераційний інтервал для породної групи склав: L=11,57±0,98 років. Визначено, що генераційний інтервал між жеребцями та їх синами більший, ніж між матерями та синами, а генераційний інтервал між матерями і дочками більший ніж між батьками і дочками. Ефективна чисельність популяції, за якою нова породна група буде якісно розвиватись, становить 688,2 коней репродуктивного віку. Підвищення коефіцієнту інбридингу за одну генерацію становить ΔF=0,0063, а отже, для запобігання зростання інбридингу, приріст ефективної чисельності популяції на одну генерацію має складати 79,4 голів. Якщо кількісний склад породної групи не підвищуватиметься, популяція втрачатиме 2,36 % генетичного різноманіття за 10 генерацій.

Ключові слова: коні, українська рисиста породна група, популяційні характеристики, генераційний інтервал, коефіцієнт інбридингу, збереження генофонду.

For the first time, the issue of preservation of local and endangered breeds of agricultural animals was raised by the geneticist O.S. Serebrovskyi in 1928, and in 1946, at the first session of the Advisory Committee on Agriculture of the FAO, the need for the assessment and conservation of the world fund of animals and plants was discussed.

In June 1992, the Convention on Biological Diversity was adopted in Rio de Janeiro with the aim of sustainable use of its components, obtaining fairly distributed profits from the use of genetic resources, joint use of relevant technologies, taking into account the necessary financing (http://www.biodiv.org).

In September 2007, at the International Technical Conference on Animal Genetic Resources for Food Production and Agriculture, the Global Action Plan on Animal Genetic Resources was developed (FAO, 2007).

The second report on the state of animal genetic resources in the world, held in Rome in 2015, identified the main aspects of species extinction, namely, the decrease in animal numbers and the decrease in genetic diversity, which are closely related. This report also identified the most significant threats to the genetic resources of animals in different countries: unsystematic interbreeding, introduction of exotic breeds, ineffective policy of organizational structures, loss of profitability (competitiveness) of breeds, intensification of production systems, weak veterinary control, loss of pastures and production areas, weak inbreeding control (FAO, 2015).

An. Allier et al. (2020) emphasize the need for an effective strategy to expand the genetic base of commercial breeding programs, which includes the determination of optimal crossbreeding schemes that balance expected genetic value and diversity. Slowing down the development of fundamental knowledge, strategies and laws on the conservation of genetic resources hinders the formation of reliable mechanisms for the conservation and management of breed diversity.

The first draft of the framework, released in July 2021, is based on lessons learned from the Strategic Plan for Biodiversity Conservation 2011-2020 and its Biodiversity Targets adopted in Aichi. It recognizes that urgent policy action is needed at global, regional and national levels to transform economic, social and financial models so that trends that have exacerbated biodiversity loss are stabilized by 2030 and allow natural ecosystems to recover with net improvements by 2050 (http: //www.biodiv.org).

The gene pool preservation strategy should include: genetic monitoring, identification and passporting of animals, creation of databases, organization of gene pool farms, genetic banks, development of breeding programs, preservation and management of breeds, as well as introduction and protection of traditional agricultural management zones. In this regard, FAO proposes a strategy scheme for the control of animal genetic resources, presented in Figure 1.

This scheme also includes parameters for preserving the gene pool of domestically bred animal breeds. To do this, it is necessary to determine the value of each breed, the potential of breed development, the amount of purebred breeding and interbreeding with improving breeds.

In recent decades, the horse population in the world has been relatively stable – about 58 million heads (https://worldpopulationreview.com). Such stability of the industry is based on the multifaceted possibilities of using the horse in society. Depending on the level of economic development of the country, natural and climatic conditions, and the traditions of the population, one or another form of horse use dominates.

For horse breeds of world importance, geographically distributed and numbering several thousand breeding mares in different countries of the world, the factor of inbreeding depression is not a threat (thoroughbred horse, Arabian, American Standardbred, French trotter, etc.) (Babenko O., 2020). The analysis of the presence of pedigree (assessed) stock of horses of domestic selection showed (Table 1) that all of them, according to the number of stock capable of reproduction, according to the FAO classification, are "in a state of danger" (from 100 to 1000 females capable of reproduction). In particular, trotting breeds in Ukraine are represented by three populations, two of which have long been the subject of selection by domestic studs - the Orlov trotting breed (194 mares) and the newly created Ukrainian trotting breed group (182 breeding mares) (Tkachova I.V., 2023). Horses of the French trotting breed have been bred quite recently, currently their stock is concentrated in two breeding breeders in the amount of 43 individuals (Suprun I.O., 2020).

Table 1

The number of breeding horses of trotting breeds in breeder of Ukraine

Breeds	2023			
	total		in particular	
	heads	%	male	female
Orlov trotter	465	*57.5*	30	194
Newly created Ukrainian trotting breed group	301	*37.2*	15	182
French trotter	43	*5.3*	3	15

Horses of trotting breeds have been bred in Ukraine since the beginning of the 20s of the XIX century along with the popularization of prize-winning equestrian sports. The Dibriv Stud Farm was founded in 1888 in the Poltava province as the first recognized stud farm in Ukraine for breeding trotting horses. Since then and to this day, this stud farm maintains a leading position in the selection of horses of the Orlov trotting breed and the newly created Ukrainian trotting breed group (Strijak T.A., 2014).

At the beginning of the previous century, trotting horses underwent significant population changes, in particular, a part of horses that, in order to increase liveliness, were crossed with the precocious and lively American Standardbred trotter (bred from a thoroughbred riding breed) (Hered J., 2022). In further selection work with the local population of trotting horses with improved liveliness, reproductive interbreedings with the American Standardbred trotter were used. As a result of purposeful work, a lively type of Ukrainian trotter was selected, which has original phenotypic and genetic qualities. The interbreeding of trotters of the domestic gene pool with the French trotter breed began in Ukraine in 1965, when the Dibriv Stud Farm received several foals from the most lively French trotter of that time – Okapi'S 1.16.6 (2.08.6) min. s, 1958 (Fe Follie X – Jatian V). This interbreeding did not give significant results, the offspring of Okapi'S were inferior in liveliness to their dams and offspring of American Standardbred stallions, although they were on average livelier than their purebred peers. The best was the result of using the French trotting stallion Minden in 1994-2000 1.17 min. (Brutus Lucius – Made) – more than 80 offspring were obtained from it, of which 29.6% entered the liveliness class 2.10 and livelier, 6.2% entered the liveliness class 2.05 and livelier. Since 2004, artificial insemination of domestic prize trotters with the semen of stallions of the French trotting breed has been introduced in the Dibriv and Zaporizhzhya Stud Farms, as well as in the leading stud breeders, the importation of horses of this breed to the studs of Roda Trading House LLC and Komyshanske LLC. As a result of crossbreeding, local horses outperformed purebred peers (both Ukrainian and French), as well as descendants of American Standardbred stallions in liveliness and prize-winning speed.

Taking into account the separation of trotting horse selection directions, the stud farms of Ukraine – Dibriv, Lozivskyi, Zaporizhzhya and Limarivskyi, along with the preservation of the population of the original Orlov trotter, through long-term selection for liveliness, prize precociousness, endurance, distance, created their own type of trotting horse, and in 2016 there was the first stage of approbation of the newly created Ukrainian trotting breed group of horses was carried out (Tkachenko O.O., 2016). The main selection method of improving the new breed group is breeding in "self" with limited use of the gene pool of the American Standardbred and French trotting breeds for interbreeding at the level of "infusion of blood" to increase indicators of liveliness and prize maturity.

The main conditions for the sustainable preservation of the gene pool of horses are the presence of an organizational structure responsible for the preservation of national genetic resources, relevant laws on breeding matters, programs for the preservation of the gene pool of animals (Kruglyak O. V., 2016). The gene pool preservation strategy should include: genetic monitoring, identification and passporting of animals, creation of databases, organization of gene pool farms, genetic banks, development of breeding programs, preservation and management of breeds, as well as introduction and protection of traditional agricultural zones.

According to the recommendations of the Commission on genetic resources in the field of food and agriculture of FAO, the following strategy scheme for the control of genetic resources of animals is proposed (Fig. 1).

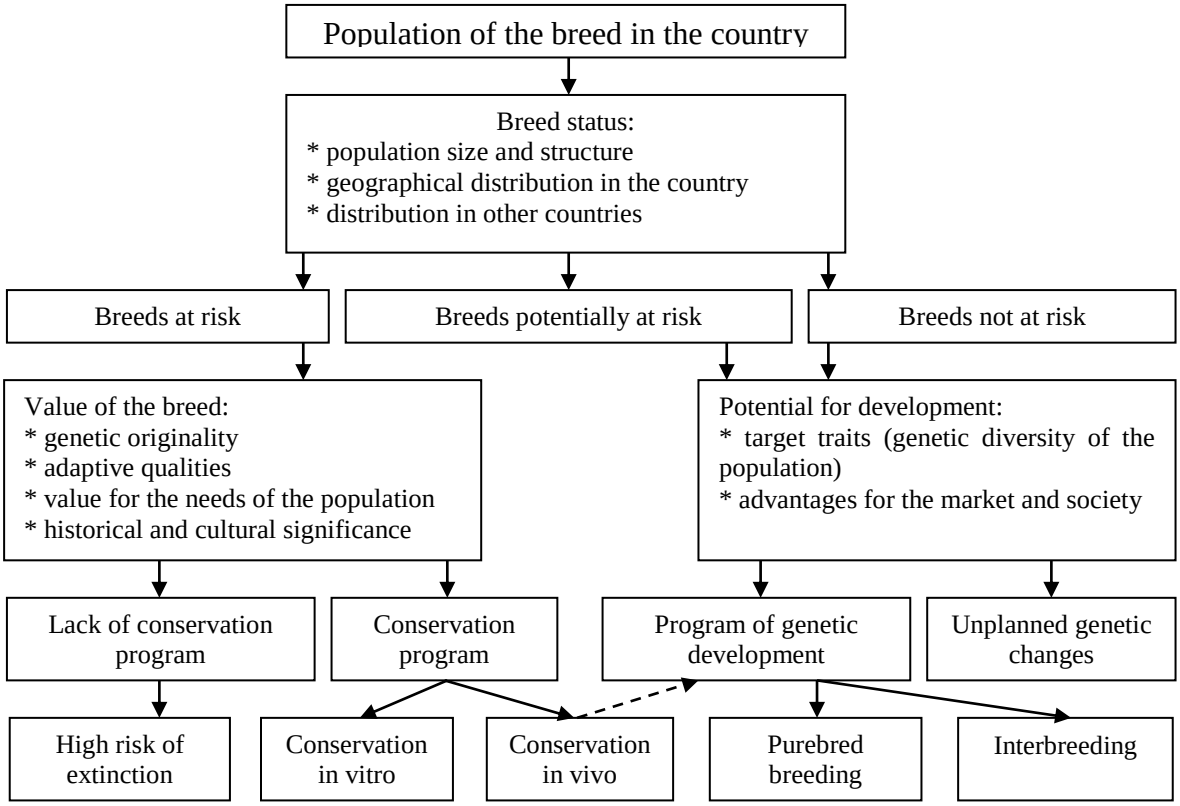

Fig. 1. **Scheme of the strategy of control over the genetic resources of animals**

The proposed scheme also includes parameters for preserving the gene pool of domestically bred horse breeds. To do this, it is necessary to determine population characteristics, breed development potential, the amount of purebred breeding and interbreeding with improving breeds. It was this goal that was set to determine the population characteristics and justify the concept of preserving the gene pool of the newly created Ukrainian trotting breed group of horses.

Materials and methods. The research material was the database of horses of the Ukrainian trotting breed group with confirmed origin (n=1973). Horses are evaluated based on a complex of breeding traits and population characteristics. The average annual yield of foals and the number of repair young ones were calculated. The share of breeding stallions of different breeds was determined and the amount of interbreeding was established.

The generational interval was defined as the average age of the parents at the birth of the offspring. The average age of the parents at the birth of the offspring was calculated according to the entries in the pedigree books. The final indicator was defined as the average for all years of birth (Bijma P., 2001):

$$L = \frac{Lmm + Lmf + Lfm + Lff}{4}, \qquad (4)$$

where L_{mm} – sire-male offspring generation interval;
L_{mf} – sire-female offspring generation interval;
L_{fm} – dam-male offspring generation interval;
L_{ff} – dam-female offspring generation interval.

The effective annual number of the population *(Na)* was determined as the increase over the years of the inbreeding coefficient *(Fx)* and the average population of related animals *(f_{xy})*:

$$Na = \frac{1}{2 \cdot (1 - e^{\beta})}, \qquad (5)$$

where Na is the effective annual population size; β is the regression coefficient, as ln(1-Ft) by year, where Ft is the average value of the inbreeding coefficient.

The variance drift deviation per generation can be described as:

$$Var(\delta q) = \frac{q(1-q)}{2Ne}, \qquad (6)$$

where Ne is the effective population size; q is the drift frequency of the dispersion.

For a known number of generations in a breed (breed group, line) (L), the variance drift will accumulate to:

$$\sum_{i=1}^{L} Var(\delta q)i = \frac{q_0(1-q_0)}{2Ne} + \frac{q_1(1-q_1)}{2Ne} + \cdots + \frac{q_{L-1}(1-q_{L-1})}{2Ne} \qquad (7)$$

Setting the ratio

$$q_1(1-q_i) = q_{i-1}(1-q_{i-1})\left(1 - \frac{1}{2Ne}\right), \qquad (8)$$

leads to the equation:

$$\sum_{i=1}^{L} Var(\delta q)i = \frac{q_0(1-q_0)}{2Ne}\left[1 + \left(1 - \frac{1}{2Na}\right) + \left(1 - \frac{1}{2Na}\right)^2 + \cdots + \left(1 - \frac{1}{2Na}\right)\right]. \qquad (9)$$

By replacing the geometric sequence with its sum in the limits, we get:

$$\sum_{i=1}^{L} Var(\delta q)i = \frac{q_0(1-q_0)}{2Na} \cdot \frac{1-(1-(1/2Na))^L}{1/2Na} = q_0(1-q_0)\left[1 - \left(1 - \frac{1}{2Na}\right)^L\right] \qquad (10)$$

By comparing the equations for both values, we get:

$$\frac{q(1-q)}{2Ne} = q(1-q)\left[1-\left(1-\frac{1}{2Na}\right)^{L}\right] \text{ and } \frac{1}{2Ne} = 1-\left(1-\frac{1}{2Na}\right)^{L}. \quad (11)$$

According to this method, the effective size of the population was estimated as:

$$Ne(generation) = \left[2\left(1-\left(1-\frac{1}{2Na}\right)\right)^{L}\right]^{-1}. \quad (12)$$

Statistical data processing was carried out in the Microsoft Excel software environment (Baranovskii, 2017).

Research results. According to herd monitoring and genealogical analysis, the active part of the population of the new breed group at the time of approval was represented by 204 mares and 27 breeding stallions.

On January 1, 2023, the number of horses decreased to 301 breeding horses were registered in the Ukrainian trotting breed group, including 182 mares of reproductive age (table 2), that is, the breed group is small in number, or, according to the FAO classification, "in a state of danger" . The ratio in the reproductive composition of stallions and mares is 7.6% and 92.4%.

Table 2

Quantitative composition of horses of the Ukrainian trotting breed group (on 01.01.2023)

The subject of the breeding case	n	Including:		
		breeding stallions	mares	Repair young horses 2019-2022 born
Zaporizhzhya Horse Breeding No. 86*	62	5	43	14
Lymarivsky Horse Breeding Plant No. 61*	64	4	43	17
Dibriv Stud Farm No. 62*	56	3	31	22
Total in stud farms:	182	12	117	53
PE "Roda" of the Kyiv region.	37	2	10	25
Individuals	82	1	55	26
Total:	301	15	182	104

*Note: * - horse studs are branches of SE "Konyarstvo Ukrainy"*

The average liveliness of mares of the reproductive age is 2.12.9 min. The liveliest mares are produced in the breeding stock of the branch of "Dibriv Stud Farm № 62" of the SE "Konyarstvo Ukrainy" (average liveliness 2.08.2±0.86 min.). In terms of prize precocity (liveliness at the age of two), fillies of the Zaporizhzhia Stud Farm No. 86 with a high degree of probability ($p<0.01$) prevail over fillies of other stud farms (average liveliness at the age of 2 – 2.24.9±1.47 min.).

Based on the current number of herds of the newly created breed group – 182 fillies/mares of reproductive age, according to the indicators of the average annual output

of foals 55.7%, from the actual herd of fillies/mares, an average of 101.4±5.19 foals are obtained per year. Assuming that the ratio of newborn male and female foals by breed is almost the same, the average annual number of female foals will be 50 individuals. Taking into account 10% culling, 45 three-year-old fillies per year should be included in the breeding nucleus.

Considering the fact that the Ukrainian trotting breed group was created using the method of backinterbreeding the Orlov trotter, American Standardbred and French trotter breeds, therefore, in order to improve the lively characteristics and refresh the gene pool of the population, the breeding program provides for limited interbreeding with the American Standardbred and French trotter breeds. So, in addition to stallions of the breed group, 3 breeding stallions of the American Standardbred and 3 of the French trotter breeds were involved in the breeding work. French trotter mares, American Standardbred mares and crossbred mares are also involved in breeding. The average liveliness of stallions is 2.00.8 min. The stallions belong mainly to the American lines of Speedy Crown and Victory Song. The share of horses from purebred stallions and those obtained from stallions of other breeds (American Standardbred, French Trotter) in the studied breeding periods is presented in Figure 1.

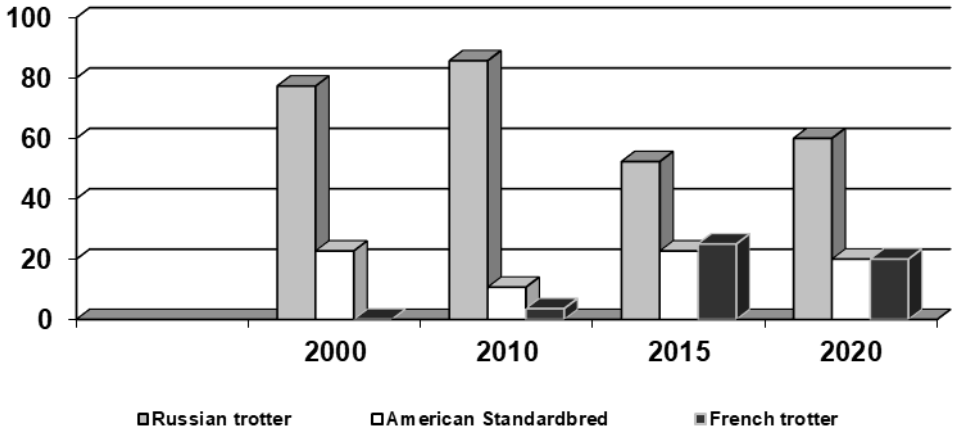

Fig. 1. **Share of horses (%) that come from purebred stallions and from stallions of improving breeds**

In the period 2010-2015, 10 stallions of the American Standardbred breed and 11 stallions of the French trotter breed were used. In 2015, the share of improving breed stallions was 47.7%, which exceeds the parameters recommended by the breeding program, while the share of breeding stallions of the French trotter breed increased significantly. Considering this, the number of stallions of other breeds was reduced and currently only 40.0% of them are used, which corresponds to the parameters recommended by the breeding program.

Breeding use of trotting colts is allowed at the age of 3, but most of the stallions planned for breeding use (outstanding for prize performance) perform on racetracks up to 5-7 years and older, while fillies, with rare exceptions, perform on racetracks up to 3-4 years old, after which they enter the breeding stock. Nevertheless, from the majority of colts planned for breeding use, based on the results of tests at the age of 2-4, breeders begin to receive offspring (or sperm production) during the tests. The generation change interval depends on these indicators. The maximum number of generations in the breed group – 11 – was observed in the Speedy Crown line.

The main parameters of the population are presented in Table 3.

Table 3

Main parameters characterizing the horse population of the Ukrainian trotting breed group

Population parameters	Indicators
Number of horses of reproductive composition	301
Number of stallions	15
Number of mares	182
The maximum number of generations	11

The main population characteristics of the newly created Ukrainian trotting breed group are presented in Table 4.

Table 4

Population characteristics of fillies/mares of reproductive age of the Ukrainian trotting breed group

Population characteristics	Indicators
Record (lifetime) liveliness at 1600 m, min.	128.9±0.59
Prize precociousness (liveliness at the age of 2), min.	146.4±0.90
Share of fillies/mares in the liveliness class 2.05 min. and livelier, %	12.0
Share of fillies/mares of liveliness class 2.10 min. and livelier, %	36.8
Body measurements, cm:	
height at the withers	159.8±0.32
oblique body length	162.2±0.36
chest circumference	181.4±0.42
cannon bone circumference	19.91±0.06
Credit rating, score	
origin	9.15±0.05
type	8.37±0.06
exterior	8.14±0.06
measurements	8.15±0.10
working capacity	7.37±0.16

Determination of the generation interval and the inbreeding coefficient made it possible to analyze the population and identify specific features of the Ukrainian trotting breed group of horses.

The result of the calculation of the generation interval is shown in Figure 2.

The generation interval calculated for the Ukrainian trotting breed group of the domestic part of the population was: L=11.57±0.98 years. This indicator is somewhat lower than that of the French trotter breed according to the results of research by Moureaux S. (Moureaux S. et al, 1996), who established a generation interval for the French trotter breed – 11.8 years. It is also determined that the generation interval between sires and their male offsprings is bigger than between dams and male offsprings, and the generation interval between dams and female offsprings is bigger than between sires and female offsprings.

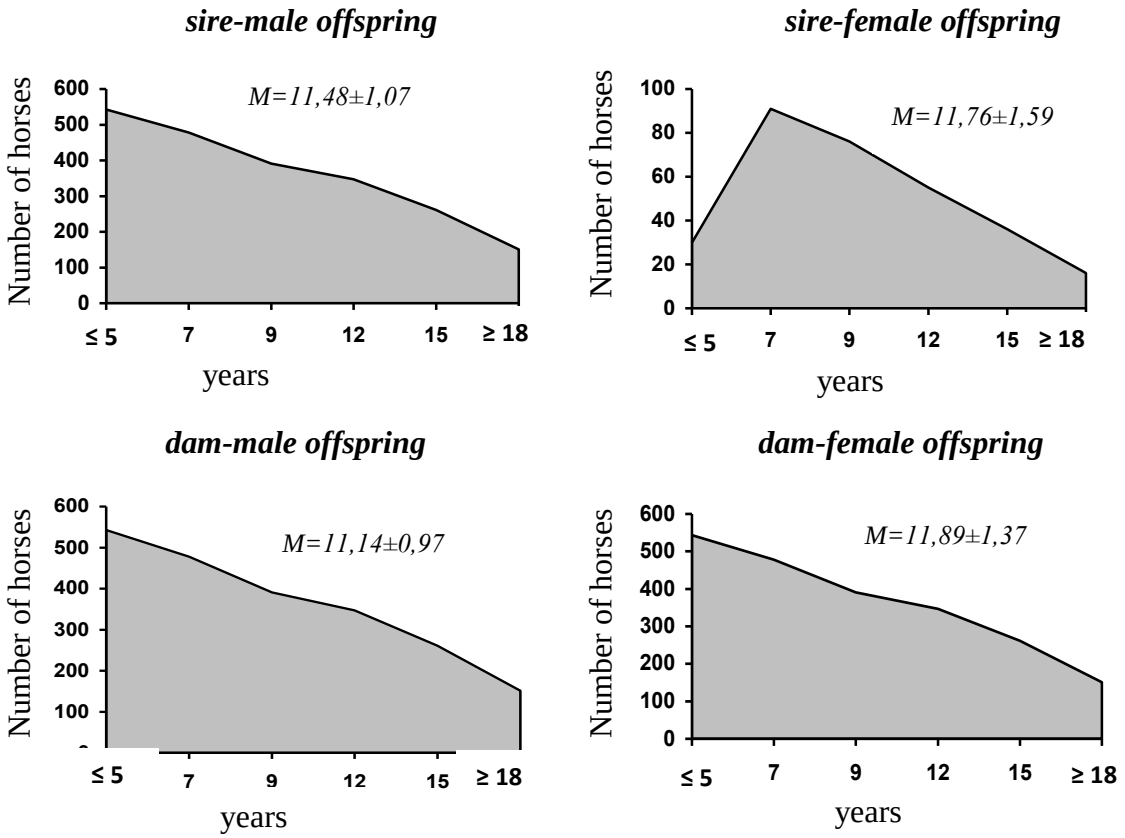

Fig. 2. **Generation interval (L) between family groups of horses of the Ukrainian trotting breed group**

The effective number of the population, according to which the new breed group will develop qualitatively, is 688 fillies/mares of reproductive age.

The increase in the inbreeding coefficient for one generation, calculated according to formula 3, is $\Delta F=0.0063$, and therefore, to prevent the growth of inbreeding, the increase in the effective size of the population per generation should be 79.4 heads.

If the Ukrainian trotting breed group remains in the number of livestock that it is today – 182 fillies/mares of reproductive age, which is 318 heads below the lower limit of genetic diversity proposed by I.R. Franklin (1980) for small populations and for 838 heads below the limit determined for the number of female individuals (Scherf B., 2009), the population will lose 2.36% of genetic diversity in 10 generations.

Discussion. The main problem of preserving animal breeds of local (national, regional) significance is that their maintenance is often not economically profitable, and therefore the population needs support to ensure their survival. If these breeds are historical and traditional, closely related to demographic processes in different geographical regions, or carry in their genotype unique properties that cannot be reproduced in caset of extinction, then society bears responsibility for these breeds beyond commercial profit (Chaudhary P., 2020). That is why national horse breeding is supported in leading European countries at the expense of the state (France) or public organizations (Great Britain). Countries with the largest number of horses in the world: the USA (about 10 million), China, Mexico, Brazil. However, the number of horses in these countries does not exceed the consumer needs of the population, remaining at the level of active reproduction. Even in Great Britain, where sport horse racing is a cult industry, it is shrinking every year due to the needs of the population (British-Racing-Factsheet-October-2019.pdf). Nevertheless, in Great Britain, the share of income from equestrian sports is about £8 billion per year and the industry creates 85,000 jobs (Equine Devel-

opment, 2017). In total, according to the calculations of Steve Elliott from the company Alltech, the annual economic impact of the horse breeding industry is about 300 billion US dollars and 1.6 million jobs. In the US, the horse business has a greater economic impact than the film industry (EQUO, 2017). Considering the fact that Ukrainian horse breeding is much more modest (about 200,000 horses), in accordance with today's political and economic realities, a decrease in the number of horses according to the needs of the population can be seen.

However, the process of preserving the breeds and types of horses traditional for Ukraine continues. It is known that the preservation of the gene pool of animals requires the solution of a number of issues: identification of useful traits, selection of methods for their identification and evaluation, substantiation of the breeding system, establishment of effective and optimal population size, development of methods for preserving genetic variability, methods of control at genetic processes, assessment of erosion of the gene pool (Stolpovsky Yu. A., 2017). The selection process for breeds of local origin is aimed not so much at genetic improvement as at preserving the originality of the traits inherent in this population in given environmental conditions and their constant reproduction in generations. And in these conditions, the optimization of the reproductive population of animals, which is necessary for reproduction without the threat of inbreeding depression, becomes especially important. The calculation of the effective size of the population is complicated by the fact that, where selection is limited by the number of animals, and the duration of the intervals between generations. In addition, the number of breeding stock is closely related to economic indicators, namely, the population's need for horses of a certain direction of economic use. The important step in the preparation of conservation programs for animals with a depleted gene pool is the calculation of the effective population size *(Ne)*, which depends on genetic drift and the inbreeding coefficient in the population (Arden W. R., 2003). Determining the effective number of livestock for active reproduction is necessary to optimize breeding programs, and for this it is necessary to identify the risk status and development trends of breeds in modern conditions, as well as their role, direction of use, cultural and economic value. The concept of preserving the gene pool of animals in the context of population genetics and planning the selection process consists of analyzing the population structure, determining the generation interval, effective population size, the contribution of improving breeds and justifying their use for interbreeding (Haberland A. M., 2012). Recently, the process of population genetics of horses has been accelerated with the use of modern research methods (Petersen, J.L., 2020).

At a low level of effective population size, the rate of loss of genetic diversity increases (Buffalo V., 2021). Thus, when the value of *Ne* is lower than 500, 250, 125, 50, 25 individuals, in 10 generations the population loses 0.8, 1.6, 4, 10, 18% of genetic diversity, respectively. For the newly created Ukrainian trotting horse breed group, we have proven that it will lose 2.36% of its genetic diversity in 10 generations, if it remains at the current level of the gene pool of 182 fillies/mares capable of reproduction. As the horse population tends to decline, the proportion of genetic diversity that will be lost will increase proportionally.

Franklin I.R. (1980) proposed an effective population size of 500 individuals as the lower limit of genetic diversity and the possibility of its evolution in a changing environment. However, other researchers believe that this limit should be higher (Rizzato, F., 2019). The criteria proposed by the working group "Rare Breeds Survival Fund" (RBST, Great Britain) for the survival of horse breeds as endangered are the number of fillies/mares capable of reproduction of at least 1000 individuals (Maijala K., (19 80). Our research established a criterion for the full preservation and development

of the newly created Ukrainian trotting breed group at the level of 688 fillies/mares capable of reproduction. However, the number of colts/stallions is not of critical importance as the diversity of their genotype is more important due to belonging to different genealogical lines, branches, as well as the involvement in the reproductive process of stallions of improving breeds (American Standardbred, French trotter).

The decrease in the number of animals of the breeding nucleus invariably causes the increase in the level of inbreeding, and therefore, it is necessary to manage the selection process of the entire population of animals kept by the population in order to minimize inbreeding. Hence, the main task of breeding programs is to optimize the genetic trend while simultaneously reducing the level of inbreeding, for which modern software tools are created to control (Schierenbeck S., 2011). In order to substantiate the genetic aspects of gene pool preservation, it is important to consider that even a small degree of inbreeding reduces the reproductive function of the population, especially for low-fertile species of animals with a long period of generation change, to which horses belong. Thus, Chujie Chen and colleagues (2023) confirm that a 10% increase in homozygosity in a horse population reduces reproductive function.

The effective size of the population was used by Bijma R. et al. (2001) as a key parameter in the development of strategies for the identification and conservation of endangered animal species. Thus, the Federal Ministry of Food, Agriculture and Consumer Protection of Germany determined the effective size of a closed population of Trakehner horses, which depends on the number of animals selected for the breeding nucleus, the dispersion of the family size and the average generation interval (Hill W.G., 2000). We came to the same conclusion in our research.

The decrease in the number of animals of the breeding nucleus invariably causes the increase in the level of inbreeding, and therefore, it is necessary to manage the selection process of the entire reproductive stock of animals in order to minimize inbreeding (Fuping Zhao, 2023). In such conditions, branching of the population with the involvement of the best typical representatives of related breeds and types of animals is desirable, which is also proven by our research.

The calculated generation interval and effective population size for the newly created Ukrainian trotting breed group practically coincide with the researches of Moureaux S. et al. (1996) on the French trotter breed.

Modern breeding programs do not use the indicator of the generation interval and effective population size when evaluating animal populations, particularly horses. These indicators were determined by us for the first time for the Ukrainian trotting breed group of horses, and their further use in gene pool conservation programs is reasonable.

Thus, the concept of preserving the gene pool of animals in the context of population genetics and planning the selection process consists of an analysis of the population structure, determination of the generation interval, effective population size, the contribution of improving breeds and justification of their use for interbreeding.

Conclusions. The newly created Ukrainian trotter breed group of horses is a separate population with a limited gene pool, which according to the FAO classification is characterized as "in a state of danger". The reproductive composition of the population consists of 182 fillies/mares of reproductive age, in this state the population will lose 2.36% of its genetic diversity in 10 generations.

According to the results of the evaluation of the breeding stock, a high level of average liveliness of fillies/mares was established, 2.12.9 min. The liveliest fillies/mares are produced in the breeding stock of the branch of "Dibriv Stud Farm No. 62" of the SE "Konyarstvo Ukrainy" (average liveliness 2.08.2±0.86 min.). In terms of prize pre-

cocity (liveliness at the age of two), fillies of the Zaporizhzhia Stud Farm No. 86 with a high degree of probability ($p<0.01$) prevail over fillies of other stud farms (average liveliness at the age of 2 – 2.24.9±1.47 min.) .

In order to avoid inbreeding depression and increase the vitality and prize-winning speed of the newly created Ukrainian trotting breed group of horses, a limited (no more than 40%) crossbreeding with American Standardbred and French trotter breeds was substantiated and included in the selection program, which was justified by the positive results of the previous program. The stallions belong mainly to the American lines of Speedy Crown and Victory Song, which requires further branching of the genealogical structure of the breed group. The maximum number of generations in the breed group – 11 – was observed in the Speedy Crown line.

The established population characteristics of the newly created Ukrainian trotting breed group: record liveliness for a distance of 1600 m – 2.08.9±0.59 min., prize precociousness (liveliness at the age of 2) - 2.26.4±0.90 min., share of mares liveliness class 2.05 min. and livelier - 12.0%, the share of mares of liveliness class 2.10 min. and more lively - 36.8%, height at the withers 159.8±0.32 cm, oblique length of the body - 162.2±0.36 cm, chest circumference – 181.4±0.42 cm, cannon bone circumference – 19.91±0.06 cm, origin assessment – 9.15±0.05 points, type – 8.37±0.06 points, exterior – 8.14±0.06 points, measurements – 8.15±0.10 points, work capacity – 7.37±0.16 points.

The specific features of the newly created Ukrainian trotting breed group of horses have been revealed. Thus, the generation interval (L) was 11.57±0.98 years. It is also determined that the generation interval between sires and their male offsprings is greater than between dams and male offsprings, and the generation interval between dams and female offsprings is greater than between sires and their female offsprings. The effective size of the population, according to which the newly created Ukrainian trotting breed group will develop qualitatively, is 688.2 horses of reproductive age.

The increase in the inbreeding coefficient for one generation is $\Delta F=0.0063$, therefore, to prevent the growth of inbreeding, the increase in the effective number of the Ukrainian trotting breed group per generation should be 79.4 fillies/mares of reproductive age. This indicator should be consistent with the consumer needs of the population and the development of the healthy lifestyle, sports and leisure sector using horses as a historical and traditional heritage.

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OXIDATIVE STRESS BIOMARKERS IN THE CARDIAC AND HEPATIC TISSUES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) FED THE B-GLUCAN-SUPPLEMENTED DIET

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Treatment with β-glucans has been found to stimulate various aspects of immune responses such as resistance to infections and resistance to environmental stress. The effects of dietary β-glucans on the general health status of rainbow trout, as well as oxidative stress biomarkers in different tissues specifically should be explored. This prompted us to investigate the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout (Oncorhynchus mykiss Walbaum) after a 14-day period of oral supplementation with β-glucans. Thirty healthy rainbow trout weighing 55.9 ± 2.1 g were used in the experiments. The fish were fed with a commercial basal diet at a rate of 1.5% body weight four times a day. After acclimation, the fish were randomly divided into two groups. The groups were fed for 14 days as follows: the control group comprising rainbow trout (n = 15) received a control basal diet and the β-glucan group (n = 15) was fed with the Yestimun® food product at a dose of 1% of the basal feed (with 85% of β-1.3/1.6-glucans, Leiber GmbH, Bramsche, Germany). The basal feed was supplemented with 1% of Yestimun® powder (dose: 1 kg per 99 kg, w/w). This insoluble and highly purified preparation contains natural polysaccharides, e.g. β-1,3/1,6-D-glucans derived from Spent Brewers' Yeast (Saccharomyces cerevisiae). Yeast cell walls typically contain approximately 30% of β-glucans of dry weight. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout. Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls. In conclusion, our results unambiguously showed that β-glucan did not induce oxidative stress in the hepatic and cardiac tissues of rainbow trout.

Keywords: β-glucans, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity, liver, heart, Oncorhynchus mykiss.

БІОМАРКЕРИ ОКИСНОГО СТРЕСУ В СЕРЦЕВІЙ І ПЕЧІНКОВІЙ ТКАНИНАХ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM), ЯКУ ГОДУВАЛИ ДІЄТОЮ З ДОБАВКАМИ В-ГЛЮКАНУ

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Було встановлено, що лікування β-глюканами стимулює різні аспекти імунної відповіді, такі як стійкість до інфекцій і стійкість до стресу навколишнього середовища. Вплив β-глюканів на загальний стан здоров'я райдужної форелі, а також на біомаркери окисного стресу в різних тканинах слід вивчити окремо. Це спонукало нас дослідити вплив β-1,3/1,6-D-глюканів дріжджів, доданих протягом 14-денного періоду годування, на функцію печінки та серця та окиснювальні механізми, що лежать в основі цих ефектів. Ми оцінювали рівні перекисного окиснення ліпідів, похідних окиснювально модифікованих білків (ОМБ) і загальну антиоксидантну активність (ТАС) у тканинах печінки та серця райдужної форелі (Oncorhynchus mykiss Walbaum) після 14-денного періоду перорального прийому добавок з β-глюканами. В дослідах використовували 30 здорових особин райдужної форелі вагою 55,9 ± 2,1 г. Рибу годували комерційною дієтою з розрахунку 1,5% маси тіла чотири рази на день. Після акліматизації рибу випадково розділили на дві групи. Групи годували протягом 14 днів наступним чином: контрольна група райдужної форелі (n = 15) отримувала контрольну основну дієту, а група β-глюканів (n = 15) отримувала харчовий продукт Yestimun® у дозі 1% основного корму (складається з 85% β-1,3/1,6-глюканів, Leiber GmbH, Bramsche, Німеччина). Базальний корм доповнювали 1% порошком Yestimun® (доза: 1 кг на 99 кг маси). Цей нерозчинний і високоочищений препарат містить природні полісахариди, β-1,3/1,6-D-глюкани, отримані з відпрацьованих пивних дріжджів (Saccharomyces cerevisiae). Клітинні стінки дріжджів зазвичай містять приблизно 30% β-глюканів. Наші результати показали, що годування низькими дозами β-глюканів викликало статистично незначуще зниження рівня TBARS у тканинах печінки та серця райдужної форелі. Годування низькими дозами β-глюканів викликало незначні зміни рівнів ТАС як у печінковій, так і серцевій тканинах. Рівні альдегідних і кетонових похідних ОМБ у серцевій і печінковій тканинах райдужної форелі, яку годували дієтою з добавками β-глюканів, були на тому ж рівні, що й у контрольних групах. На закінчення, наші результати однозначно показали, що β-глюкан не викликає окисного стресу в печінкових і серцевих тканинах райдужної форелі.

Ключові слова: β-глюкани, перекисне окиснення ліпідів, окиснювально модифіковані білки, загальна антиоксидантна активність, печінка, серце, Oncorhynchus mykiss

Aquaculture is a rapidly growing part of agriculture worldwide, accounting for about 44 percent of the world's total fish production. Today, preventive and management measures in aquaculture are central to dealing with outbreaks of various diseases (Assefa, A., & Abunna, F., 2018). Immunostimulators are considered an effective means

of increasing the immune status of cultivated organisms. Among the various immunostimulants used in aquaculture practice, one of the promising immunostimulants is β-glucan, which is a homopolysaccharide of a glucose molecule linked by a glycosidic bond (Meena at al., 2013). β-glucans are a group of β-D-glucose polysaccharides that form the major constituents of the cell wall of some plants, fungi, bacteria, mushrooms, yeast, and seaweeds (Singh, R. P., & Bhardwaj, A. 2023). β-glucans from different sources differ in their structure, conformation, physical properties, receptor binding affinity, and therefore biological functions (Han, at al., 2020). They are known for their metabolic and immunomodulatory properties, including anticancer, immunomodulatory, antimicrobial, antiviral, antinociception, antiinflammatory, prebiotic, antioxidant, antidiabetic, etc. (Dalonso, at al., 2015); Mirończuk-Chodakowska, I., at al., 2021). The immunostimulatory activity of β-glucan results from its attachment to specific receptors present on the surface of immune cells (Ciecierska, A. at al.,2019). In addition, β-D-glucans are also suitable for use in nanomedicine to prepare natural nanocarriers for the delivery of drugs or biological molecules [Lehtovaara, B. C., & Gu, F. X. 2011; Soto, E. R. at al., 2012; Vannucci, L., at al., 2013)

Dietary β-glucans also exert immunostimulatory and antitumor effects by activating mucosal immune system cells through β-glucan receptors (Nakashima, A., at al., 2018). Currently, the following β-glucan receptors have been identified: dendritic cell (DC)-associated C-type lectin-1 (Dectin-1), complement receptor 3 (CR3), differentiation cluster 11b (CD11b)/CD18, αMβ2-integrin, macrophage differentiation antigen-1 (Mac-1), lactosylceramide (LacCer), and scavenger receptors (SRs) (Zhong, X., at al., 2023). β-glucan and mannan from yeast cell walls can replace antibiotics for the prevention and treatment of animal diseases, and reduce the development and spread of antibiotic-resistant bacterial pathogens (Liu, Y., at al.,2021).

Orally administered β-glucans are absorbed through the gastrointestinal tract and taken up by tissue macrophages (Barton, C., at al., 2016). Here they are fragmented, transported to the bone marrow and the reticuloendothelial system, and eventually released and taken up by other immune cells, leading to various immunological effects (Chan, G. C., at al., 2009). Based on these properties, β-glucan particles have been evaluated as potential vaccines against invasive fungal diseases (Edwards J. E. 2012), and beta-glucan particles have been proposed as a delivery system for oral vaccines, acting both as a carrier and as an adjuvant (Huang, H., at al., 2013). Beta-glucans are a group of polysaccharides with inherent immunostimulatory properties, which makes it very promising to create new adjuvants for vaccines based on β-glucans (Colaço, M. at al., 2022). Carbohydrate structures – polygalactans, fructans, β-D-glucans, α-D-glucans, D-galactose, and D-glucose – are desirable candidates for vaccine adjuvants and immunomodulators because they perform important functions in nature and are often biocompatible, safe, and well tolerated (Abbasi, A. at al., 2022). The size of the particles and the loading method of the antigen encapsulated in the particles or adsorbed on their surface will influence the toxicological and adjuvant properties of the adjuvant powder (Colaço, M., at al., 2022).

Experimental studies have largely elucidated the underlying mechanisms involved in immune stimulation induced by β-D-glucans, especially with regard to the involvement of dectin-1 and C3-iCR3. A clear definition of biologically active molecules and a more detailed chemical and biological characterization of glucans from various sources seem necessary in order to better define the rationale for their use in the therapy of relevant pathologies (Vannucci, L., at al., 2013). The main physicochemical properties of β-glucans include their antioxidant properties, which are responsible for

scavenging reactive oxygen species (ROS), as well as their role as a dietary fiber to prevent cholesterol absorption, improve digestion, and produce short-chain fatty acids in the intestines (Nakashima, A., at al., 2018).

Little is known about the biochemical changes in various tissues of salmonids after oral administration of β-glucans. The effects of dietary β-glucans on the general health status of rainbow trout, as well as oxidative stress biomarkers in different tissues specifically should be explored. This prompted us to investigate the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after a 14-day period of oral supplementation with β-glucans.

Materials and methods.

Fish and experimental design. Thirty healthy rainbow trout (*Oncorhynchus mykiss*) weighing 55.9 ± 2.1 g were used in the experiments. The fish were kept in an indoor system with a supply of freshwater with adequate aeration and internal power filter. The water quality parameters were as follows: a temperature of 16 ± 2 °C, 12 ± 0.5 ppm of dissolved oxygen, and a pH value of 7.4–7.6. During the acclimation period (14 days), the fish were fed with a commercial basal diet at a rate of 1.5% body weight (BW) four times a day. After acclimation, the fish were randomly divided into two groups kept in aerated 250-L square tanks containing dechlorinated tap water (70 fish per tank). One tank comprised one group. Natural photoperiod conditions were maintained throughout the feeding trial. The experimental part of the study was carried out in the Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute (Rutki, Poland).

The groups were fed for 14 days as follows: the control group comprising rainbow trout (n = 15) received a control basal diet and the β-glucan group (n = 15) was fed with the Yestimun® food product at a dose of 1% of the basal feed (with 85% of β-1.3/1.6-glucans, Leiber GmbH, Bramsche, Germany). The basal feed was supplemented with 1% of Yestimun® powder (dose: 1 kg per 99 kg, w/w). This insoluble and highly purified preparation contains natural polysaccharides, e.g. β-1,3/1,6-D-glucans derived from Spent Brewers' Yeast (*Saccharomyces cerevisiae*). Yeast cell walls typically contain approximately 30% of β-glucans of dry weight (Stier, H., at al., (2014).

The survival rate of fish in the different treatment groups was recorded during the feeding trial. An increase in fish weight was observed as well. At the end of the 14-day feeding period, the fish were decapitated, and the liver and heart were dissected. Blood was sampled with plastic syringes from the caudal vein. The experiments were performed in duplicate.

Hepatic and cardiac tissue isolation. Tissue samples were removed from fish after decapitation. One fish was used for each homogenate preparation. Briefly, the liver and heart were excised, weighed, and washed in the ice-cold buffer. The minced tissue was rinsed clear of blood with ice-cold 100 mM Tris-HCl isolation buffer, homogenized in 10 vol. (v/w) in isolation buffer, and centrifuged at 3,000 × g at 4 °C for 10 min. The resulting supernatant was stored in a refrigerator at −22 °C and used for analyses of enzyme activities and biomarkers of oxidative stress. The isolation buffer contained 100 mM Tris-HCl; the pH value was adjusted to 7.2 with HCl.

Biochemical assays. All enzymatic assays were carried out at 24 ± 0.5°C with the use of a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The homogenate suspension was added to start the enzymatic reactions. The specific assay conditions are

presented below. Each sample was analyzed in duplicate. The protein concentration in each sample was determined as in Bradford (1976) using bovine serum albumin as a standard.

2-Thiobarbituric acid reactive substances (TBARS). Lipid peroxidation was determined in aliquots of 10% hepatic and cardiac tissue homogenates from the treated and control groups with the procedure developed by Kamyshnikov (2004). The absorbance of each aliquot was measured at 540 nm, and the lipid peroxidation level was expressed as nanomoles of TBARS formed per milligram of protein (nmol MDA·mg^{-1} protein) using a molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ (Kamyshnikov, V.S. (2004).

The carbonyl derivatives of oxidatively modified proteins (OMP) assay. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995) (Dubinina, E. at al., 1995; Levine, R. L.,at al., 1990). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC). The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova, L. P., at al.,1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis. The basic statistical analysis was performed using the Statistica 13.3 package (TIBCO Software Inc.). The data were tested for homogeneity of variance using Levene's test of equality of error variances. Normality was checked with the Kolmogorov-Smirnov test. The results are expressed as mean ± S.D. Significant differences among the means were measured with the use of a multiple-range test at min. $P < 0.05$. Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at $P < 0.05$. Non-normally distributed data were log-transformed (Zar, J.H.,1999).

Results.

Levels of TBARS (nmol MDA·mg^{-1} protein) and TAC (%) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet were presented in Fig. 1.

Our results showed that feeding with low doses of β-glucans induced a decrease in TBARS levels in the hepatic tissue of rainbow trout to (132.41 ± 26.65 nmol·mg^{-1} protein) compared to the untreated trout (147.03 ± 32.33 nmol·mg^{-1} protein) (by 9.9%, $p > 0.05$). In the cardiac tissue, TBARS level was also decreased to (39 ± 19.55 nmol·mg^{-1} protein) compared to the untreated trout (76.51 ± 30.29 nmol·mg^{-1} protein) (by 49%, $p > 0.05$) (Fig. 1A). The feeding with low doses of β-glucans induced non-significant changes in the TAC levels (Fig. 1B). In the hepatic tissue of rainbow trout, TAC level was at the same level (25.88 ± 2.35 %) compared to the untreated trout (25.90 ± 2.52 %). In the cardiac tissue, the TAC level was decreased to (27.01 ± 3.68 %) compared to the untreated trout (28.69 ± 3.91 %) (by 5.9%, $p > 0.05$) (Fig. 1A).

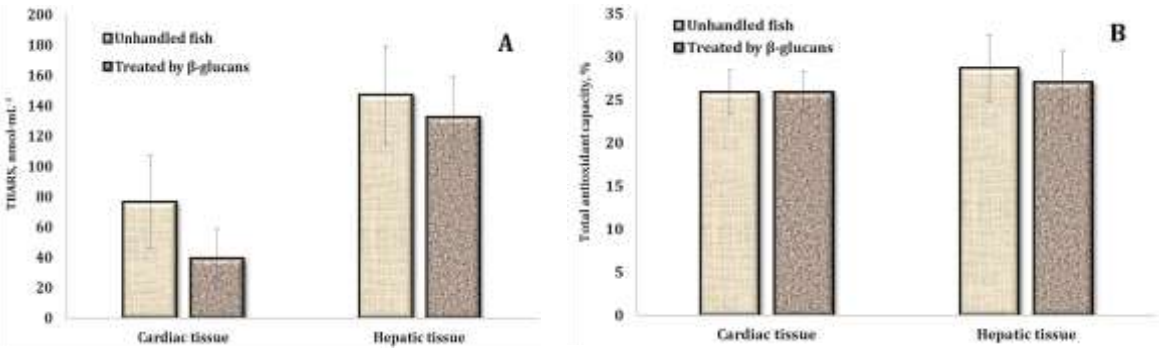

Fig. 1. **Levels of TBARS (nmol MDA·mg^{-1} protein) and TAC (%) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet.**

The results are expressed as mean ± S.D. Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at P < 0.05.

Levels of aldehydic and ketonic derivatives of OMP (nmol·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet were presented in Fig. 2.

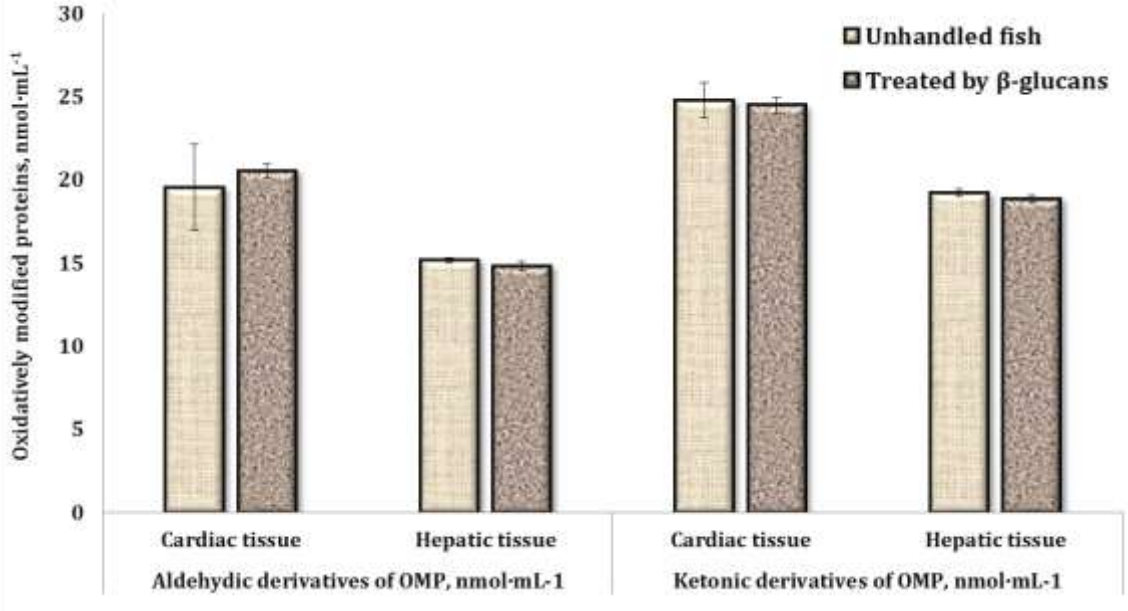

Fig. 2. **Levels of aldehydic and ketonic derivatives of OMP (nmol·mg^{-1} protein) in the cardiac and hepatic tissues of rainbow trout *(O. mykiss)* fed the β-glucan-supplemented diet.**

The results are expressed as mean ± S.D. Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at P < 0.05.

Our results revealed that feeding with low doses of β-glucans induced an increase in aldehydic derivatives of OMP level in the hepatic tissue of rainbow trout to (20.52 ± 0.44 nmol·mg^{-1} protein) compared to the untreated trout (19.54 ± 2.60 nmol·mg^{-1} protein) (by 5%, p > 0.05). In the cardiac tissue, level of aldehydic

derivatives of OMP was at the same level (14.82 ± 0.27 nmol·mg^{-1} protein) compared to the untreated trout (15.17 ± 0.15 nmol·mg^{-1} protein) (Fig. 2). The feeding with low doses of β-glucans induced non-significant changes in the levels of ketonic derivatives of OMP (Fig. 2). In the hepatic tissue of rainbow trout, level of ketonic derivatives of OMP was at the same level (24.49 ± 0.50 nmol·mg^{-1} protein) compared to the untreated trout (24.78 ± 1.08 nmol·mg^{-1} protein). In the cardiac tissue, the level of ketonic derivatives of OMP was decreased to (18.86 ± 0.24 nmol·mg^{-1} protein) compared to the untreated trout (19.23 ± 0.22 nmol·mg^{-1} protein) (by 1.9%, $p > 0.05$) (Fig. 2).

Discussion. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout (Fig. 1). Membrane destruction as a pathological phenomenon is primarily due to the involvement of cell membrane lipids in the processes of lipid peroxidation (LPO), which leads to a change in lipid-protein bonds, the stability of the enzyme complexes and other membrane macromolecules, damage to nucleic acids, and disruption of cellular metabolism (Gaschler, M. M., & Stockwell, B. R., 2017; van Ginkel, G., & Sevanian, A., 1994). The classic biochemical indicators of the state of animals, which are used in assessing the effect of various medicals and toxicants on the body, include products of lipid peroxidation (LPO), which can be assessed by the accumulation of primary LPO products as diene conjugates (DC) and diene ketones (DK), as well as one of end metabolites of lipid peroxidation – malonic dialdehyde (MDA) (Gaweł, S., at al., 2004, Lefèvre, G., at al., 1998). Total antioxidant capacity (TAC) is a measure of the ability of a food or substance to reduce oxidative stress in the body. Determination of antioxidant activity plays an important role in assessing the body's defense against oxidative stress (Rubio, C. P., at al., 2016).

Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls (Fig. 2). To date, the concept of oxidative degradation of proteins as the earliest and most reliable indicator of oxidative stress has already been clearly and voluminously formed. Since proteins are present in all tissues and organs, it is their modification that can be a reliable indicator of pathological processes both at the local and general body levels. Since proteins perform specific functions (often having a clearly visible and easily fixed manifestation), the assessment of the qualitative and quantitative aspects of oxidative modification of proteins has a number of advantages in the diagnosis of pathological conditions Hawkins, C. L., & Davies, M. J. 2019; Squier T. C. 2001); Stadtman E. R., 2001).

It has been shown that in a wide range of pathologies of the most diverse etiologies, it is an oxidative modification specifically proteins (and not lipids or nucleic acids) that is one of the earliest and most reliable markers of their presence and even just their occurrence. In addition, it has been established that oxidatively modified proteins can exist in living organisms for a long time (hours, days, and even years; for example, lipofuscin), while the primary intermediates of oxidative stress (free radicals, lipid peroxidation products) exist in a free state for much shorter (usually a few minutes, maximum a few hours). This circumstance also makes it possible to consider the phenomenon of oxidatively modified proteins in living organisms as relatively stable diagnostic parameters of their structural and functional state, which is of great importance in clinical practice (Kehm, R., at al., 2021).; Squier, T. C., & Bigelow, D. J., 2000).

Similar to our results, several authors have reported that β-glucans incorporated

in fish feed increase the non-specific resistance mechanisms and raise the counts of intestinal immune cells [Akhtar, M. S.,at al.,2021); Cornet, V.,at al., 2021; Kazuń, B., at al., 2020; Koch, J. F. A., at al., 2021). For example, Kazuń and co-workers (2020) compared the effect of dietary supplementation of β-1,3/1,6-glucan, Lactobacillus plantarum bacteria or their mixture on the growth performance, selected parameters of the immune system, as well as the liver and intestinal histology of roach. Fish were fed for 14 days with four different diets, each treatment was carried out in triplicate. In the control group, fish were fed dry commercial starter feed Aller Performa 2 (Aller Aqua, Denmark). The other experimental fish groups received the same commercial starter feed supplemented with 1% β-1,3/1,6-glucan (Leiber® Beta-S) in group G; 10^8 CFU *L. plantarum* g^{-1} in group L; 1% β-1,3/1,6-glucan + 10^8 CFU *L. plantarum* g^{-1} in group G + L. The stimulating effect of the tested preparations was evaluated once the feeding with commercial feed exclusively was resumed and 2 weeks afterward. No effect on the survivability and growth performance of the fish was observed in any of the groups. Supplementation of feed with β-1,3/1,6-glucan improved selected parameters of innate humoral immunity and the pinocytotic activity of phagocytes. Increased respiratory burst activity of head kidney phagocytes (RBA) was observed in groups L and G + L, and the effect persisted for 2 weeks after the commercial feed regime was resumed. An analogous tendency was determined for the killing activity of phagocytes (PKA) of the head kidney with respect to *Aeromonas hydrophila*, although this effect appeared only during the feed supplementation period. Supplying roach with β-1,3/1,6-glucan, singly or with L. plantarum, had no effect on the proliferation of mitogen-activated lymphocytes. However, an increase in the number of CD3-positive cells and goblet cells was noticed in the digestive system of the L-group fish (Kazuń, B., at al.,2020).

An eight-week feeding trial was performed by Akhtar and co-workers (2021) to assess the effect of different dietary levels (0, 0.5, 1.0, and 1.5%) of β-glucan (sourced from *Saccharomyces cerevisiae*) on growth, survival, immunological parameters (immune gene expression, lysozyme, and antiprotease), total antioxidant status, thermal tolerance, and disease resistance of *Tor putitora* fry (Akhtar, M. S., at al.,2021). Feeding of moderate doses (0.5 and 1.0%) of β-glucan significantly improved survival but not weight gain percentage as compared to that received unsupplemented control and the highest dose (1.5%) of glucan. Supplementation of β-glucan in diets differentially influenced the mRNA expression of cytokine and other immune genes. On the other hand, the dietary inclusion of β-glucan markedly improved total antioxidant levels and extended the thermal tolerance limits at both ends. After feeding β-glucan for eight weeks, the fish were bath-challenged with a bacterial pathogen, *Aeromonas salmonicida*. The challenge study results revealed that β-glucan intake improved most of the studied immune parameters, resulting in lower mortality. Overall, dietary inclusion of β-glucan (0.5-1.0%) was efficient in improving the immune responses, thermal tolerance, and disease resistance of *T. putitora* fry (Akhtar, M. S., at al.,2021).

In the study conducted by Valérie Cornet and co-workers (2021), the immunostimulant effects of two β-glucan types extracted from wild-type baker's yeast (*S. cerevisiae*) and its null-mutant Gas1 were investigated. Gas1 has a β-1,3-glucanosyltransferase activity necessary for cell wall assembly. Using a positive (commercial product MacroGard®) and negative control (a diet without glucans), these researchers evaluated the immune responses and disease resistance of rainbow trout juveniles (mean weight, ~44 g) fed control, low (0.2%) and high (0.5%) doses of Macrogard®, Gas1, and Wild type-β-glucan after a short-term (15 days, D15) or mid-term (36 days, D36) feeding periods (Cornet, V., at al., 2021). These researchers found that β-glucan supplemented diets did not affect growth performance, mortality, splenic

index, or leukocyte respiratory burst activity on D15 nor D36. Each β-glucan triggered different immune effectors, depending on the doses or length of exposure compared to others and the negative control. Indeed, a high dose of MacroGard® significantly increased lysozyme activities at D15 compared with the control and other diets. At D36, MacroGard β-glucan enhanced the production of lymphocytes in comparison with the control diet. Regarding WT β-glucan, at D36, WT-β-glucan, especially the high dose, provided the highest enzymatic activities (lysozyme and ACH50) and Ig level. On D36, Gas1 also increased lysozyme activity, Ig proportion, and some immune genes (mcsfra, hepcidin) compared with MacroGard®. Besides, both doses of Gas1-β-glucans increased the resistance of juveniles to bacterial infection highlighted by a higher survival rate at 14 days post-challenge compared with the control and other types and doses of β-glucans (Cornet, V., at al., 2021).

Dietary β-glucan (MacroGard®) improves innate immune responses and disease resistance in Nile tilapia regardless of the administration period. Koch and co-workers (2021) demonstrated that β-glucan improved the innate immune responses and the tilapia's resistance to disease, and this protection could be observed up to 10 days post-feeding trial, adding *in vivo* evidence that β-glucan may contribute to a trained innate immunity. Additionally, these researchers showed that a longer period of administration did not cause immunosuppression as previously hypothesized but promoted further growth and immune performance (Kehm, R., at al., 2021). Sabioni and co-workers (2020) demonstrated that β-glucan enhances the respiratory activity of leukocytes suppressed by stress and modulates blood glucose levels in pacu (*Piaractus mesopotamicus*). These findings confirm the immunomodulatory action of glucan and add evidence showing that glucan can have a role in stress response (Sabioni, R. E., at al., 2020). . Also, Do Huu and co-workers (2016) revealed that dietary β-glucan improved growth performance, *Vibrio* counts, hematological parameters, and stress resistance of pompano fish, *Trachinotus ovatus* L. (Do Huu, H., at al., 2016).

The dietary intake of 0.75% β-glucan improved resistance to ammonia stress to a certain degree by activating the anti-oxidative system and reducing brachial ammonia uptake as shown by Ciji and co-workers (2023). The study investigated the effects of dietary administration of β-glucan on aquaporins and antioxidative & immune gene expression in endangered golden mahseer, *Tor putitora* juveniles, exposed to ammonia stress. For that, fish were fed experimental diets having 0 (control/basal), 0.25, 0.5, and 0.75% β-d-glucan for five weeks and then exposed to ammonia (10 mgL-1 total ammonia nitrogen) for 96 h. Administration of β-glucan differentially influenced the mRNA expression of aquaporins, anti-oxidative, and immune genes in ammonia-exposed fish. For instance, the transcript abundance of catalase and glutathione-s-transferase in gill varied significantly among the treatment groups, with the lowest levels in 0.75% β-glucan fed groups. At the same time, their hepatic mRNA expression was similar (Ciji, A., AT AL., 2023).

Zeng and co-workers (2018) evaluated and investigated the effects of β-glucan on oxidative stress, inflammation, and copper transport in two intestinal regions of a large yellow croaker (*Larimichthys crocea*) under acute copper stress. Biochemical indicators (MDA, Cu content, MTs protein levels, Cu,Zn-SOD, CAT and iNOS activities), gene expressions of oxidative stresses (Cu,Zn-SOD, CAT, Nrf2, MTs and MTF-1), inflammatory responses (NF-κB, iNOS, IL-1β, IL-6 and TNF-α) and Cu transporters (ATP7A, ATP7B and CTR1) were determined. In the anterior intestine, β-glucan increased MT levels, activities of Cu,Zn-SOD, CAT, and iNOS, mRNA levels of MTs, CAT, iNOS, ATP7A, and ATP7B, and reduced Cu content and CTR1 gene expression to inhibit Cu-induced MDA. However, β-glucan had no effect on

inflammatory gene expressions. In the mid intestine, β-glucan increased activities of Cu,Zn-SOD and iNOS, mRNA levels of Cu,Zn-SOD, CAT, and iNOS to maintain MDA content. However, unlike the anterior intestine, β-glucan had no effect on Cu transporter gene expressions. Furthermore, transcription factors (Nrf2, NF-κB, and MTF-1) paralleled with their target genes in the mid-intestine, but no correlation was observed between NF-κB and IL-1β and TNF-α gene expressions in the anterior intestine (Zeng, L.,at al., 2018)..

In our previous study (Tkachenko H., at al., 2023), we assessed relevant lipid peroxidation in the hepatic and cardiac tissue of rainbow trout (*Oncorhynchus mykiss*), European whitefish (*Coregonus lavaretus*), and graylings (*Thymallus thymallus*) after a 14-day period of supplementation with β-glucans. Our results showed that feeding with low doses of β-glucans induced a decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout, and European whitefish. Similarly, 14 days of feeding graylings with low doses of β-glucans resulted in a decrease in the TBARS levels both in the hepatic and cardiac tissues. This study confirms that dietary β-glucan is beneficial for promoting growth and enhancing antioxidant capacity against oxidative stress in rainbow trout, European whitefish, and graylings. Indeed, we cautiously hypothesized that feeding low β-glucans doses may help to boost antioxidant function, especially by the decrease of biomarkers of lipid peroxidation in the hepatic and cardiac tissues of these fish.

Conclusions. The effects of dietary β-glucans on the oxidative stress biomarkers in different tissues specifically were explored. We investigated the effects of dietary yeast β-1,3/1,6-D-glucans supplemented for a 14-day feeding period on liver and heart function and the oxidative mechanisms underlying these effects. We assessed the levels of lipid peroxidation, derivatives of the oxidatively modified proteins (OMP), and the total antioxidant capacity (TAC) in the hepatic and cardiac tissue of rainbow trout after a 14-day period of oral supplementation with β-glucans. Our results showed that feeding with low doses of β-glucans induced a statistically non-significant decrease in TBARS levels in the hepatic and cardiac tissues of rainbow trout. The feeding with low doses of β-glucans induced non-significant changes in the TAC levels both in the hepatic and cardiac tissues of rainbow trout. Levels of aldehydic and ketonic derivatives of OMP in the cardiac and hepatic tissues of rainbow trout fed the β-glucan-supplemented diet were at the same levels as in the untreated controls. In conclusion, our results unambiguously showed that β-glucan did not induce oxidative stress in the hepatic and cardiac tissues of rainbow trout.

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ANTIMICROBIAL POTENTIAL OF EXTRACT DERIVED FROM THE LEAVES OF *FICUS VILLOSA* BLUME AGAINST SOME FISH PATHOGENIC STRAINS

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The use of natural compounds can be found in many species of plants, and this holds much antimicrobial potential against fish pathogens in animal husbandry and fish farmers. In the current study, we studied the antimicrobial activity of the ethanolic extract derived from the leaves of F. villosa against the Aeromonas species: A. sobria, A. hydrophila, and A. salmonicida subsp. salmonicida, as well as Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens to evaluate the possible use of this plant in preventing infections caused by these fish pathogens in aquaculture. The current study was conducted as a part of an ongoing project between five universities undertaken in the frame of a cooperation program aimed at the assessment of medicinal properties of tropical and subtropical plants. The leaves of F. villosa, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. The Aeromonas strains used in our studies were Aeromonas hydrophila (K886), Aeromonas sobria (K825) and Aeromonas salmonicida subsp. salmonicida (St30). These microorganisms originated from the bacterial strain collection of the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, and had been isolated from fish of two farmed freshwater species exhibiting clinical signs of disease: common carp (Cyprinus carpio L.) (K886 and K825) and rainbow trout (Oncorhynchus mykiss Walbaum) (St30). Bacteria Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens were isolated both from apparently healthy rainbow trout (Oncorhynchus mykiss Walbaum), as well as from individuals exhibiting clinical signs of the diseases, according to the procedure developed at the Department of Fish Disease of the National Veterinary Research Institute in Poland. Antimicrobial susceptibility of the tested strains was performed by the Kirby-Bauer disc diffusion method (1966) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014), with our some modifications. Results of the antimicrobial screening revealed, that F. villosa possessed different antibacterial properties against fish pathogens. The ethanolic extract derived from leaves of F. villosa exhibited the maximum antimicrobial activity against Shewanella putrefaciens, Aeromonas sobria, and Pseudomonas fluorescens. The percentage of increase in the diameters of inhibition zones was 122.7% for Shewanella putrefaciens,

54.9% for Aeromonas sobria, and 48.5% for Pseudomonas fluorescens, respectively. F. villosa exhibited mild antibacterial properties against Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida. Thus, the Shewanella putrefaciens strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of F. villosa, while Serratia liquefaciens and Yersinia ruckeri strains were resistant to this extract. These findings demonstrate that plant extracts derived from plants belonging to the Ficus genus are potential sources of botanical drugs for controlling bacterial infection in aquaculture.

Keywords: Ficus villosa Blume, extract, antimicrobial efficacy, Kirby-Bauer disk diffusion technique, fish pathogens, susceptibility, resistance

АНТИМІКРОБНИЙ ПОТЕНЦІАЛ ЕКСТРАКТУ, ОТРИМАНОГО З ЛИСТЯ *FICUS VILLOSA* BLUME, ЩОДО ДЕЯКИХ ПАТОГЕННИХ ШТАМІВ РИБ

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Використання природних сполук, які можна знайти в багатьох видах рослин, має великий антимікробний потенціал щодо збудників хвороб риб у тваринництві та аквакультурі. У цьому дослідженні ми вивчали антимікробну активність етанольного екстракту, отриманого із листя Ficus villosa Blume, щодо 3 видів Aeromonas: A. sobria, A. hydrophila та A. salmonicida subsp. salmonicida, а також Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens, з метою оцінки можливого використання цієї рослини для запобігання інфекціям, викликаними цими збудниками захворювань риб в аквакультурі. Це дослідження було проведено в рамках поточного проекту між п'ятьма університетами, який здійснюється в рамках програми співпраці, спрямованої на оцінку лікувальних властивостей тропічних і субтропічних рослин. Зразки листя F. villosa, культивованого в тепличних умовах, відбирали в Національному ботанічному саду імені М.М. Гришка (НБС) НАН України. Штами Aeromonas, використані в наших дослідженнях, були Aeromonas hydrophila (K886), Aeromonas sobria (K825) і Aeromonas salmonicida subsp. salmonicida (St30). Ці мікроорганізми походять із колекції штамів бактерій Відділу хвороб риб Національного ветеринарного науково-дослідного інституту в Пулавах (Польща), і були виділені з риб двох прісноводних видів, що вирощуються, і мали клінічні ознаки захворювання: короп звичайний (Cyprinus carpio L.) (K886 і K825) і райдужна форель (Oncorhynchus mykiss Walbaum) (St30). Бактерії Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens виділяли як від умовно здорової райдужної форелі (Oncorhynchus mykiss Walbaum), так і від особин із клінічними ознаками захворювань за методикою, розробленою у Відділі хвороб риб Національного ветеринарного науково-дослідного інституту в Пулавах (Польща). Антимікробну чутливість досліджуваних штамів проводили методом дискової дифузії Кірбі-Бауера (1966) згідно з рекомендаціями Інституту

клінічних і лабораторних стандартів (CLSI, 2014) з нашими деякими модифікаціями. Результати антимікробного скринінгу показали, що екстракт з листя F. villosa проявляв різні антибактеріальні властивості щодо патогенів риб. Спиртовий екстракт, отриманий із листя F. villosa, виявляв максимальну антимікробну активність щодо Shewanella putrefaciens, Aeromonas sobria та Pseudomonas fluorescens. Відсоток збільшення діаметрів зон інгібування становив 122,7% для Shewanella putrefaciens, 54,9% – для Aeromonas sobria та 48,5% – для Pseudomonas fluorescens, відповідно. F. villosa виявляв помірні антибактеріальні властивості щодо Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida. Отже, штам Shewanella putrefaciens виявив найбільшу чутливість до спиртового екстракту, отриманого з листя F. villosa, тоді як штами Serratia liquefaciens і Yersinia ruckeri були найбільш стійкими до цього екстракту. Подібні дослідження демонструють, що рослинні екстракти, отримані з рослин роду Ficus, є потенційними джерелами ботанічних препаратів для боротьби з бактеріальними інфекціями в аквакультурі.

Ключові слова: Ficus villosa Blume, екстракти, антимікробна ефективність, методика дискової дифузії Кірбі-Бауера, патогени риб, чутливість, резистентність

Introduction. An intensive farming system puts fish at risk of disease outbreaks, with economic consequences. Antibiotics are commonly used to control the health of aquaculture species, but this has several disadvantages, including increasing antibiotic resistance in pathogenic bacteria and introducing antibiotic residues into the human food chain, which is a public health and environmental problem (Wei, L. S. at al., 2022). Medicinal plants and their use represent a fast, readily available, cost-effective, efficient, and environmentally friendly approach to the socio-economic and sustainable development of modern aquaculture practices (Semwal, A. at al.,2023). The potential of herbal medicines as alternatives to antibiotics for maintaining the health of aquaculture species has been explored in numerous studies (Wei, L. S. at al., 2022). Medicinal plants and their use represent a fast, readily available, cost-effective, efficient, and environmentally friendly approach to the socio-economic and sustainable development of modern aquaculture practices (Semwal, A. at al., 2023).

A wide range of medicinal plants such as herbs, seeds, and spices in various forms such as crude, extracts, blends, and active compounds are used as immunostimulants resulting in a marked enhancement of the fish's immune system to prevent and control microbial diseases (Awad, E., & Awaad, A., 2017). For example, most *in vitro* studies of plant extracts against *Streptococcus iniae* and S. agalactiae have shown antibacterial activity, and essential oils, especially those containing eugenol, carvacrol, or thymol, are more effective. Although the essential oils showed the best anti-streptococcal activity in *in vitro* assays, extracts tested *in vivo* showed moderate efficacy in increasing the survival of infected fish, probably by enhancing immunity (Van Doan, H. at al., 2022). Similar trends were observed in the study with herbal extractives performed against *Lactococcus garvieae* with no strong antibacterial activity (Soltani, M. at al., 2021).

Phytotherapy, either by immersion in water or by incorporation into the diet, is an alternative to synthetic pharmaceuticals to reduce the pathogenicity of pathogens in aquatic environments due to the presence of remarkable phytocomponents such as flavonoids, alkaloids, pigments, terpenoids, steroids, and essential oils, the medicinal plant exhibits antimicrobial, appetite-stimulating, anti-stress, growth-stimulating, and immunostimulating activity (Semwal, A. at al.,2023).

The genus *Ficus* belongs to the family Moraceae (Berg, C. C. 2003) and includes a variety of plants, from trees over 30 m tall in their countries of origin to dwarf, creeping species (Salehi, B. at al., 2021). It is one of the largest genera of angiosperms, numbering more than 800 species of moderately woody plants or trees, epiphytes, and shrubs (Singh, D. at al., 2011). They form an important part of biodiversity in many tropical regions of the world, including Indo-Australasia, Neotropics, and Afrotropics (Mon, A. M. at al., 2020). Figs have been used by humans for nutrition, healthcare, and other functional and cultural purposes throughout their distribution (Mon, A. M. at al., 2020).

Ficus villosa Blume is a (gyno)dioecious evergreen root-climber distributed in continental southern and eastern Asia to Malesia. Leaves are distichous, 9-30 cm long and 4.5-11 cm wide, (sub)coriaceous, puberulous to sub-hirsute or strigillose (entirely on only on the main veins), ovate to oblong or elliptic, symmetric, with subacuminate apex and cordate to obtuse base. Figs are axillary, in pairs or clustered (up to 8 in one cluster), also on up to 1 cm long spurs on the older wood, 0.8-1.8 cm in diameter, pedunculate or sessile, subglobose to ovoid, puberulous to glabrous, at maturity yellow to orange or crimson (Berg C.C., Corner E.J.H. 2005).

In the current study, we studied the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. villosa* against the *Aeromonas* species: *A. sobria*, *A. hydrophila*, and *A. salmonicida* subsp. *salmonicida*, as well as *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. The current study was conducted as a part of an ongoing project between the Institute of Biology and Earth Sciences (Pomeranian University in Słupsk, Poland), Faculty of Veterinary Medicine and Animal Sciences, University of Life Sciences (Poznań, Poland), M.M. Gryshko National Botanic Gardens of National Academy of Sciences of Ukraine (Kyiv, Ukraine), and Ivan Franko National University in Lviv (Lviv, Ukraine) undertaken in the frame of cooperation program aimed at assessment of medicinal properties of tropical and subtropical plants (Pękala-Safińska, A., 2021; Tkachenko, H. at al., 2021; 2016 (a); 2016 (b); 2016 (c); 2016 (d); 2016 (e); 2023 (a); 2022; 2016 (f); 2023 (b).

Materials and methods.

Collection of plant material and preparing plant extract. The leaves of *F. villosa*, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. Specifically, the leaves of *F. villosa* were sampled for our study.

The sampled leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, crushed, and homogenized in 96% ethanol (in proportion 1:10) at room temperature, and centrifuged at 3,000 g for 5 minutes. Supernatants were stored at -20°C in bottles protected with laminated paper until required.

Bacterial strains for antimicrobial activity assay. The *Aeromonas* strains used in our studies were *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30). These microorganisms originated from the bacterial strain collection of the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, and had been isolated from fish of two farmed freshwater species exhibiting clinical signs of disease: common carp (*Cyprinus carpio* L.) (K886 and K825) and rainbow trout (*Oncorhynchus mykiss* Walbaum) (St30). Each isolate was inoculated onto trypticase soy agar (TSA) (BioMérieux Polska Sp. z o.o.) and incubated at 27°C ± 2°C for 24 h. Pure colonies were used for biochemical

identifications, according to the manufacturer´s instructions, except the temperature of incubation, which was at 27°C ± 1ºC. The following identification systems were used in the study: API 20E, API 20NE, API 50CH (BioMérieux Polska Sp. z o.o.). Presumptive *Aeromonas* isolates were further identified to the species level by restriction analysis of 16S rDNA genes amplified by polymerase chain reactions (PCR) Kozińska A. 2007).

Bacteria *Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens* were isolated both from apparently healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), as well as from individuals exhibiting clinical signs of the diseases, according to the procedure developed at the Department of Fish Disease of the National Veterinary Research Institute in Poland. Collected bacteria were morphologically, physiologically, and biochemically characterized by conventional methods (Austin, B., Austin, D. A., 2016). All isolates were preliminarily identified using the API system (bioMérieux, France) according to the manufacturer's instructions, except for the incubation temperature, which was 27 ± 2°C. The results were interpreted using the "apiweb" system (bioMérieux). In order to confirm the correctness of biochemical identification, sequencing was performed according to the procedures described previously (Pękala-Safińska, A. at al., (2021).

Bacterial growth inhibition test of plant extracts by the disk diffusion method. Antimicrobial susceptibility of the tested strains was performed by the Kirby-Bauer disc diffusion method (1966) (Bauer, A. W.,1966) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (2014; 2006) with some modifications. Each inoculum of particular bacteria species in the density of 0.5 McFarland was cultured on Mueller-Hinton agar. After inoculation of bacteria, a maximum of 5 wells per Petri dish with a diameter of 6 mm each was cut into the medium, and plant extracts were added to them. Plates were incubated for 24 h at 28 ± 2°C and the inhibition zones for each well were measured. For each extract, eight replicates were assayed. The plates were observed and photographs were taken. Zone diameters were determined and averaged. Ethanol (at 96% strength, POCH, Poland) as used to prepare the extracts was also used as the negative control for the microbiological study.

***Statistical analysis*.** Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). To find significant differences (significance level, $p<0.05$) between groups, the Kruskal-Wallis test by ranks was applied to the data (Zar, J.H., 1999). All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland). The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm (Okoth, D. A., at al., 2013).

Results. Results on *in vitro* antimicrobial activity assessment of ethanolic extracts derived from leaves of *F. villosa* against some strains of fish pathogens expressed as a mean of diameters of inhibition zone is presented in Figure 1.

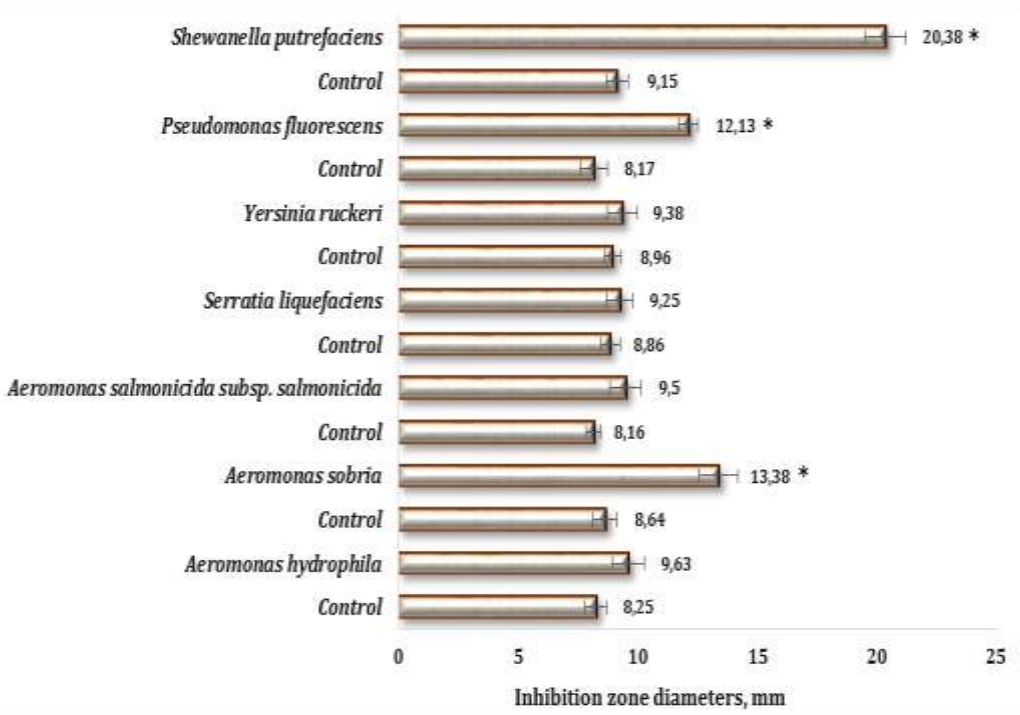

Fig. 1. **The mean inhibition zone diameters induced by ethanolic extract derived from leaves of *F. villosa* against some strains of fish pathogens such as *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens* (1000 µL inoculum) (M ± m, n = 8).**
**– changes are statistically significant compared to the 96% ethanol.*

The ethanolic extract derived from leaves of *F. villosa* exhibited the maximum antimicrobial activity against *Shewanella putrefaciens* (the mean of inhibition zone diameters was 20.38 ± 0.86 mm), *Aeromonas sobria* (13.38 ± 0.82 mm), and *Pseudomonas fluorescens* (12.13 ± 0.40 mm). The percentage of increase in the diameters of inhibition zones was 122.7% for *Shewanella putrefaciens*, 54.9% for *Aeromonas sobria*, and 48.5% for *Pseudomonas fluorescens*, respectively. *F. villosa* exhibited mild antibacterial properties against *Serratia liquefaciens* (the mean of inhibition zone diameters was 9.25 ± 0.56 mm), *Yersinia ruckeri* (9.38 ± 0.63 mm), *Aeromonas hydrophila* (9.63 ± 0.67 mm), *Aeromonas salmonicida* subsp. *salmonicida* (9.50 ± 0. 65 mm). The percentage of increase in the diameters of inhibition zones was 4.41% for *Serratia liquefaciens*, 4.69% for *Yersinia ruckeri*, 16.7% for *Aeromonas hydrophila*, and 16.4% for *Aeromonas salmonicida* subsp. *salmonicida*. Thus, the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and *Yersinia ruckeri* strains were resistant to this extract (Fig. 1).

Discussion. Our results of the antimicrobial screening revealed, that *F. villosa* possessed antibacterial properties against some strains of fish pathogens such as *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*. Results of our study revealed that the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and *Yersinia ruckeri* strains were resistant to this extract (Fig. 1).

Aeromonas hydrophila is a Gram-negative, rod-shaped, facultatively anaerobic,

non-spore-forming bacteria that is autochthonous and widely distributed in aquatic environments (Yu, H. B.,at al., 2004). Around the globe in warm water fisheries, bacteria belonging to this genus cause the most common diseases (Chowdhury, H., at al., 2023). Plant extracts and essential oils (EO) exhibited different antimicrobial activity against *Aeromonas hydrophila*, and the results of these studies were documented in many works (Al Laham, S. A., & Al Fadel, F. M. 2014) ;Chowdhury, H., at al., 2023; Sutili, F. J., at al., 2015). For example, Sutili and co-workers (2015) investigated the *in vitro* antibacterial activity of the essential oils (EOs) of *Hesperozygis ringens* (Benth.) Epling (HREO), popularly known as 'espanta-pulga' and two different species of basil, *Ocimum gratissimum* L. (OGEO) and *Ocimum americanum* L. (OAEO), as well as, the potential of these products to be used in silver catfish (*Rhamdia quelen*) infected with *Aeromonas hydrophila*. All tested EOs showed *in vitro* antibacterial properties against *A. hydrophila*; HREO and OAEO showed potential to be used in the treatment of infected fish. These products can be used in aquaculture as therapeutic and prophylactic agents against fish pathogens, with antimicrobial and immunostimulant properties (Sutili, F. J., at al., 2015). Essential oil of *Cymbopogon flexuosus* (Nees ex Steud.) Will.Watson (Poaceae family) was studied by Chowdhury and co-workers (2023) *in vitro* for its antibacterial efficacy against two oxytetracycline (OTC) resistant and one sensitive strain of *Aeromonas hydrophila*. This study, based on both *in vitro* and *in silico* methods, indicates the essential oil of *Cymbopogon flexuosus* as a potential antibacterial agent in fish disease control caused by the Aeromonads in aquaculture. The study also illustrates the favorable physicochemical and pharmacokinetic properties and drug-likeness of the essential oil components. However, the toxicological properties of the molecules did not conform with the literature reports necessitating further *in vitro* and *in vivo* toxicological validations (Chowdhury, H. at al., 2023). Al Laham and Al Fadel (2014) investigated the anti-bacterial activity shown by the extracts prepared from different parts of *Olea europea* L., *Myrtus communis* L., *Thymus vulgaris* L., *Rosmarinus officinalis* L., and *Achillea falcata* L. that grow in Syria against *A. hydrophila*. Ethanolic extracts of the studied plants had different antibacterial effects against antibiotic-resistant *A. hydrophila*. *T. vulgaris* had the highest activity, *R. officinalis* had the second, and *M. communis* and *A. falcate* were in the third place, while *O. europea* had the weakest antibacterial activity (Al Laham, S. A., & Al Fadel, F. M., 2014).

Medicinal plant-derived essential oils provide a virtually safer alternative to chemotherapeutics for fish, consumers, and ecosystems. For example, Abd El-Hamid and co-workers (2016) verified the *in vitro* antibacterial activities as well as the *in vivo* potential values of clove oil and ciprofloxacin against *Aeromonas sobria* in African catfish (*Clarias gariepinus*) (Abd El-Hamid, M. I. at al., (2016). Apparent lower mortality rates were correlated well with both decrescent bacterial burden and significant down-regulated transcripts of representative genes in the treated groups with clove oil, followed by ciprofloxacin as a prophylactic use for 15 days; however, the essential oil apart from ciprofloxacin significantly enhanced different hematological parameters (Abd El-Hamid, M. I. at al., (2016).

Non-motile *A. salmonicida* subsp. *salmonicida* is a ubiquitous gram-negative bacterium that is the causative agent of furunculosis in salmonid fish causing hemorrhagic sepsis, ulcerative lesions, pointed bleeding, and death (Dallaire-Dufresne, S., at al., 2014); Lim, J., & Hong, S., 2020). Appiah and co-workers (2022) evaluated the antimicrobial activities of the medicinal plant *Mallotus japonicus* (L.f.) Müll.Arg. against the fish pathogenic bacteria, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardisella tarda*, and *Vibrio anguillarum*, and also describes the antimicrobial

activities of the major and minor active compounds present within the plant extract. The synergistic effects by way of a combination of these compounds were also evaluated and described. Results of the study by Appiah and co-workers (2022) revealed that *Mallotus japonicus* could be used as a prophylaxis to treat bacterial disease infections of fishes and its diethyl ether-extract has the potential of an alternative to antibiotic treatment in aquaculture (Appiah, E. K. at al., 2022). Also, Hayatgheib and co-workers (2020) evaluated *in vitro* efficacy of essential oils and their compounds alone or in combination against *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of furunculosis in salmonid fish. Cinnamon, oregano, clove, and thyme oils and their major phytochemical compounds showed strong activities against *A. salmonicida* subsp. *salmonicida* strains (Hayatgheib, N. at al.,2020).

Serratia liquefaciens is considered a pathogenic bacterium of fish, and it causes infection leading to heavy mortalities in Atlantic salmon populations (McIntosh, D., & Austin, B.,1990). *Serratia liquefaciens* infections must be taken into consideration in the aquaculture industry due to the fact that the disease can cause economic losses however low the number of infection cases is (Aydin, S., at al., 2001). The antimicrobial activities of lemon oil-based nanoemulsion and two different concentrations of lemon essential oil (100% and 10%) on food-borne pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Salmonella paratyphi* A) and fish spoilage bacteria (*Photobacterium damselae*, *Enterococcus faecalis*, *Vibrio vulnificus*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas luteola*) were compared by Yazgan and co-workers (2019) in terms of disc diffusion, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). It was found that lemon nanoemulsion was more effective on food-borne pathogens except *K. pneumoniae* than 100% lemon essential oil. 10% lemon essential oil showed the highest inhibition effect on *S. paratyphi* A. The conversion of the essential oil into nanoemulsion improved antimicrobial activity. According to the value of MIC, both nanoemulsion and 100% essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective in inhibition of fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens (Yazgan, H. at al., 2019).

Enteric redmouth disease (ERM) is a severe septic bacterial disease of salmon species caused by the Gram-negative rod-shaped enterobacterium *Yersinia ruckeri*. It has a wide host range and wide geographic distribution. The disease got its name from subcutaneous hemorrhages; it can occur in the corners of the mouth, on the gums, and on the tongue (Kumar, G. at al., 2015). The disease mainly affects salmonids, and outbreaks have a significant economic impact on fish farms and the fish aquaculture industry around the world (Wrobel, A. at al., 2019). In the 21-day study, Terzi and co-workers (2021) examined the effects of the aqueous methanolic extract of thin-skinned plum (*Prunus domestica*) on growth, immune response, and resistance to a pathogenic bacterium *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). The thin-skinned plum aqueous methanolic extract could improve innate immunity, and survival against *Y. ruckeri* and decrease the feed conversion ratio level (Terzi, E. at al., 2021). The effects of dietary Myrtle (*Myrtus communis* L.) on non-specific immune parameters and bactericidal activity of skin mucus in rainbow trout (*Oncorhynchus mykiss*) fingerlings were conducted by Mansouri Taee and co-workers (2017). No antibacterial activity was detected against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* in all treatments and control group, whereas skin mucus of rainbow trout showed antimicrobial activity against fish pathogens (*Aeromonas hydrophila* and *Yersinia ruckeri*) in 1 and 1.5% Myrtle treatments. These results indicated the beneficial effects

of dietary Myrtle on the mucosal immune parameters of fingerling rainbow trout (Mansouri at al., 2017).

A 56-day research was performed by Hoseinifar and co-workers (2020) to examine the influence of graded levels [0 (control), 0.5, 1, and 2%] of Fern (*Adiantum capillus-veneris*) leaves powder (FLP) in the diet on immune competence and growth of common carp (*Cyprinus carpio*) (Hoseinifar, S. H. at al., 2020). The serum bactericidal activity against human and fish pathogens increased with enhancing the FLP level in diet against *Staphylococcus aureus*, *Escherichia coli* (EHEC ATCC 43895), *Escherichia coli* (CI), *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Aeromonas hydrophila*. The serum antibacterial activity against *Yersinia ruckeri* in the 2% FLP group was higher than the other treatments. Furthermore, the serum bactericidal activity against *P. aeruginosa* (ATCC 27853) was only observed in fish fed on the 1 and 2% FLP-supplemented diets. The skin mucosal bactericidal activity and inhibitory effects increased with enhancing the FLP level in the diet against *E. coli*, *K. pneumonia*, *Y. ruckeri*, and *A. hydrophila* in a dose-response manner. Moreover, the skin mucosal bactericidal activity against *S. aureus* was only observed in fish fed on 1 and 2% FLP-supplemented diets. Thus, by considering serum and mucosal bactericidal activities against different pathogenic bacteria, the supplementation of 2% FLP in the diet is recommended for *C. carpio* during the grow-out phase (Hoseinifar, S. H. at al., 2020). Baba and co-workers (2018) investigated the effects of olive leaf (*Olea europea* L.) extract (OLE) on the control of *Yersinia ruckeri* infection in rainbow (*Oncorhynchus mykiss*) trout and assessed the impact on the expression of immune-related genes in the spleen and serum biochemical parameters of rainbow trout. These researchers revealed that OLE/ especially at 0.1% added feed may effectively enhance the serum biochemical parameters, survival rate, and immune gene expression in rainbow trout (Baba, E. at al., 2018).

Pseudomonas fluorescens is one of the main causes of septicemic diseases among freshwater fish, causing severe economic losses and decreasing farm efficiency (Shabana, B. M., at al., 2022). Moreover, this strain is a specific spoilage microorganism of refrigerated marine fish and is highly adapted to low temperatures (Xu, J. at al.,2021). Hardi and co-workers (2018) described the antibacterial and immunostimulant abilities of *Boesenbergia pandurata* (Roxb.) Schltr. (BP), *Solanum ferox* Mill. ex Dunal (SF), and *Zingiber zerumbet* (L.) Sm. (ZZ) plant extracts to treat and prevent *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection on Tilapia (*Oreochromis niloticus*). Various extract combinations were 60 mg SF extract/kg feed with 40 mg ZZ/kg feed (SF60/ZZ40), SF50/ZZ50, BP90/SF10, and BP50/SF50. The number of pathogenic bacteria in fish-fed combined extracts was lower than the control at week 4. In both trials, the percentage of survival rate and relative percent survival of tilapia fed SF 50/ZZ 50, showed the optimum results compared to the other combinations. Thus, the combined extract in feed, especially SF50/ZZ50 has a positive effect on the tilapia's innate immune system of tilapia to treat and prevent bacterial infections (Hardi, E. H. at al., 2018).

The *Shewanella putrefaciens* group are ubiquitous microorganisms recently isolated from different freshwater fish species and causing serious health disorders, as well as a typical spoilage bacteria organism in seafood (Paździor, E., at al., 2019; Zhang, W., at al., 2022). Wu and co-workers (2018) explored the effect of adding traditional Chinese medicine (TCM) in feed on the intestinal microbiota of gibel carp (*Carassius auratus gibelio*). The results showed that the composition and structure of the bacterial community were significantly altered by the TCM feeding. At the genus level, the addition of TCM tended to reduce the incidence of potential pathogens

(*Aeromonas*, *Acinetobacter*, and *Shewanella*), while stimulating the emergence of some potential probiotics (*Lactobacillus*, *Lactococcus*, *Bacillus*, and *Pseudomonas*). These data suggested that the feed additive could regulate the fish intestinal microbiota by reinforcing the microbial balance (Wu, Z. B. at al., 2018)).

Therapeutic potential for the use of various plants of the *Ficus* genus in the control of bacterial diseases was evaluated against fish pathogens in *in vitro* study with promising results (Pękala-Safińska, A. at al., 2021; Tkachenko, H. at al., 2021; 2016 (a); 2016 (b); 2016 (c); 2016 (d); 2016 (e); 2023 (a); 2022; 2016 (f); 2023 (b). In our previous study, the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* species against *Citrobacter freundii* was evaluated (Tkachenko, H., at al., 2016 (b). The results proved that the extracts from *F. drupacea*, *F. septica, F. deltoidea*, as well as *F. hispida, F. mucuso, F. pumila, F. craterostoma*, exhibit favorable antibacterial activity against *C. freundii* (200 μL of standardized inoculum) (Tkachenko, H., at al., 2016 (b). Our results also proved that the ethanolic extracts obtained from *F. pumila, F. binnendijkii* 'Amstel Gold', *F. carica, F. erecta, F. hispida, F. mucuso, F. palmeri, F. religiosa* possess considerably sufficient antibacterial potential against *C. freundii* (Tkachenko, H. at al., 2016 (b). Among various species of *Ficus* screened ethanolic extracts of the leaves of ten *Ficus* species: *F. hispida, F. binnendijkii, F. pumila, F. rubiginosa, F. erecta, F. erecta* var. *sieboldii, F. sur, F. benjamina, F. craterostoma, F. lyrata, F. palmeri* (the species are listed in the order of effectiveness against pathogen tested) were the most effective against *P. fluorescens* (200 μL of standardized inoculum) (Tkachenko, H. at al., 2016). Moreover, previous investigation has shown that the most effective against *P. fluorescens* (400 μL of standardized inoculum) were the ethanolic extracts obtained from leaves of ten *Ficus* species: *F. craterostoma, F. cyathistipula, F. drupacea* 'Black Velvet', *F. hispida, F. macrophylla, F. mucuso, F. pumila, F. villosa (*Tkachenko, H. at al., 2016 (e). In our study, most ethanolic extracts derived from *Ficus* spp. proved effective against the bacterial strain of Gram-negative *A. hydrophila* tested, with 10-12 mm zones of inhibition being observed. *A. hydrophila* demonstrated the highest susceptibility to *F. pumila*. The highest antibacterial activity against *A. hydrophila* (200 μL of standardized inoculum) was displayed by *F. benghalensis, F. benjamina, F. deltoidea, F. hispida, F. lyrata* leaf extracts (Tkachenko, H. at al., 2016(c). Among various species of *Ficus* genus exhibiting moderate activity against *A. hydrophila* (400 μL of standardized inoculum), the highest antibacterial activity was displayed by *F. benghalensis, F. benjamina, F. deltoidea, F. hispida, F. lyrata* leaf extracts (Tkachenko, H., at al., 2016 (d); Tkachenko, H., at al., 2016 (f).

Conclusions. In the current study, we studied the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. villosa* against the *Aeromonas* species: *A. sobria, A. hydrophila*, and *A. salmonicida* subsp. *salmonicida*, as well as *Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. Results of the antimicrobial screening revealed, that *F. villosa* possessed different antibacterial properties against fish pathogens. The ethanolic extract derived from leaves of *F. villosa* exhibited the maximum antimicrobial activity against *Shewanella putrefaciens*, *Aeromonas sobria*, and *Pseudomonas fluorescens*. The percentage of increase in the diameters of inhibition zones was 122.7% for *Shewanella putrefaciens*, 54.9% for *Aeromonas sobria*, and 48.5% for *Pseudomonas fluorescens*, respectively. *F. villosa* exhibited mild antibacterial properties against *Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida* subsp. *salmonicida*. Thus, the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and

Yersinia ruckeri strains were resistant to this extract.

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	місця проведення дослідження, основні результати та їх практична цінність, НЕ вказується мета та методи роботи. Не можна в анотацію копіювати речення та абзаци з розділів статті.
Ключові слова	Курсивом, 12 pt, 5-7 слів або словосполучень.
Вступ	Виключка по ширині, 12 pt 1-2 стор. У розділі висвітлюється сучасний стан розглянутої проблеми, наукова новизна роботи, аналізуються дослідження та публікації за останні 5 років, обґрунтовується актуальність, напівжирним виділяється **мета дослідження.** В кожному посиланні коротко розкрити суть та результати досліджень автора (-ів), які цитуються. Посилання на літературу необхідно подавати у круглих дужках (…), наприклад: (Ivanov A. V., 2023; Zlobin S. E. et al., 2022a). Якщо потрібно зазначити посилання всередині рядка, варто вказувати тільки рік у (), наприклад: Тимошин М. (2023) у своїй роботі ....". В одному посиланні не варто цитувати більше 3-ох джерел.
Матеріали та методи досліджень	Зазначається об’єкт дослідження та експериментальна база. Викладена методологія повинна дати можливість повністю відтворити Ваші дослідження.
Результати досліджень	Розділ експериментальної статті повинен складати не менше 70 % від обсягу статті (не враховуючи анотацій та бібліографії) з повним обґрунтуванням отриманих наукових результатів. Таблиці, рисунки та формули повинні бути пронумеровані та подані після посилання на них у тексті. Слово “*Таблиця* __” писати справа курсивом з номером арабськими цифрами. **Заголовок таблиці** – напівжирним, виключка по центру. Посилання на таблицю у тексті вказують скорочено перед розміщенням таблиці. Примітки до таблиці – курсивом, шрифт 10 pt, без абзацного відступу. Формули – створювати у редакторі формул, виключка по центру. За наявності у тексті посилання на формулу, її нумерують арабською цифрою у круглих дужках з правого краю в межах форматування сторінки. Заголовок рисунка (Приклад: *Рис. 1.* **Назва**.) – напівжирним, виключка по ширині, без абзацного відступу. На кожен рисунок потрібно робити в тексті посилання – (рис. __). Умовні позначення та підписи на рисунку – шрифт 10 pt.
Обговорення	У цьому розділі необхідно зіставити результати власних досліджень з результатами робіт інших авторів, яких цитували у вступній частині, що найбільш близькі до теми дослідження.

Висновки	Рекомендується наводити пронумеровані висновки.
Подяки	Розділ не є обов'язковим, у ньому висловлюється подяка організаціям за посильну технічну допомогу; ідеї; фінансову підтримку, завдяки якому дослідження стало можливим.
Бібліографічний список	Джерела розташовувати в алфавітному порядку, без нумерації. В експериментальній статті повинно бути не менше 20 джерел, в оглядовій – не менше 40, переважно з наукових видань, які індексуються в наукометричних базах Scopus та Web of Science за останні 5 років, 90% джерел повинні мати DOI або електронні посилання. Не можна посилатись на ненаукову літературу (національні стандарти, технічні умови, конспекти лекцій тощо). За потреби посилань на патенти, стандарти, технічну документацію, їх робити як зноски внизу сторінки, але НЕ включати в список літератури. Слід уникати посилань на свої роботи - не більше 10 %. Вказуються прізвище та ініціали всіх (!) авторів. Ініціали пишуться після прізвища. Список літератури оформлюється лише латиницею. Англомовні посилання оформлюються згідно з зарубіжними бібліографічними стандартами, стиль APA https://apastyle.apa.org/ Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується Паспортний (КМУ 2010), а з російської – стандарт: BGN. Слід уникати посилань на літературу країни-агресора.

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