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## ***Технології утримання, годівля, селекція тварин та переробка продукції тваринництва***

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### ***Technologies of housing, feeding, selection of animals and processing of livestock products***

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#### **RELATIONSHIP OF THE CULLING OF FIRST-BORN COWS FROM THE HERD WITH THE SCORE OF LINEAR TYPE TRAITS UNDER VARIOUS HOUSING**

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*The results obtained in breeding plants indicate the influence of the technology of housing primiparous cows on their score of linear type traits. The influence of this factor ranged from 0.4% ( $p < 0.01$ ) to 2.9% ( $p < 0.001$ ). It was found that when stall housing, animals with a high assessment of body depth, angularity, and rear legs had a lower percentage of abandonment from the herd compared to animals with an average and low assessment of these traits ( $p < 0.05$ ). The results obtained when determining the percentage of abandonment of cows with different score of fore udder attachment indicate a greater stayability of first-born cows with a high assessment of this trait, which was 7.5-11% higher compared to animals with a lower score. The assessment of the central ligament of the udder (1.3%;  $p < 0.05$ ) and the depth of the udder (1.3%;  $p < 0.05$ ) had a probable impact on the abandonment of first-born cows from the herd. The number of animals that left the herd during the study period with a low assessment of the central udder ligament was higher by 0.7-13% compared to animals with a high and average assessment. A similar relationship between the loss of animals was established with the assessment of the depth of the udder (by 8.8-17.1%). The culling of first-born cows depending on the assessment of body structure traits in free housing, according to our data, probably depended only on one factor - the slope of the sacrum. The strength of the impact of the assessment of this trait was 2.9%. It is important to note that the percentage of animals leaving the herd with an average assessment of the slope of the sacrum was the lowest. When comparing the trends in the influence of the body structure traits of first-born cows on the level of their loss from herds under different methods of maintenance, it was found that, unlike tied housing, under free housing, there was a tendency to increase the percentage of culling first-born cows with a high growth assessment. Also, cows with a wide chest and a deep body had the highest risk of culling compared to the group of animals with average linear score indicators. There was a slight decrease in the percentage of culling of first-born cows with an increase in the score for the dairy type in both housing methods, animals with the desired posture of the hind limbs and a high score of the central ligament of the udder and body condition.*

**Keywords:** *dairy cattle, housing technology, body type traits, survival, culling.*



## ЗВ'ЯЗОК ВИБУТТЯ КОРІВ-ПЕРВІСТОК ІЗ ЛІНІЙНОЮ ОЦІНКОЮ ТИПУ БУДОВИ ЇХ ТІЛА ЗА РІЗНИХ ТЕХНОЛОГІЙ УТРИМАННЯ

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*Отримані у племінних заводах результати вказують на наявність впливу технології утримання корів-первісток на будову їх тіла. Сила впливу цього чинника коливалась від 0,4 % ( $p < 0,01$ ) до 2,9 % ( $p < 0,001$ ).*

*Встановлено, що за прив'язного утримання тварини з високою оцінкою глибини тулуба, кутастості, постави задніх кінцівок мали менший відсоток вибуття зі стада у порівнянні з тваринами із середньою та низькою оцінкою цих ознак ( $p < 0,05$ ). Результати отримані при визначенні відсотка вибуття корів із різною оцінкою висоти прикріплення задніх часток вимені вказують на більшу збереженість первісток із високою оцінкою даної ознаки, яка була на 7,5-11 % вищою у порівнянні з тваринами, які мали нижчу оцінку. Вірогідний вплив на вибуття первісток зі стада мала оцінка центральної зв'язки вимені (1,3 %;  $p < 0,05$ ) та глибини вимені (1,3 %;  $p < 0,05$ ). Кількість тварин, які вибули зі стада в досліджуваній період, із низькою оцінкою центральної зв'язки вимені була вищою на 0,7-13 % у порівнянні з тваринами з високою та середньою оцінкою. Аналогічний зв'язок вибуття тварин встановлено з оцінкою глибини вимені (на 8,8-17,1 %). Вибуття первісток залежно від оцінки ознак будови тіла за безприв'язного утримання за нашими даними вірогідно залежало лише від однієї статі – нахилу крижів. Сила впливу оцінки цієї ознаки становила 2,9 %. Важливо відзначити, що відсоток вибуття тварин зі стада з середньою оцінкою нахилу крижів був найменший.*

*При порівнянні тенденцій впливу ознак будови тіла первісток на рівень їх вибуття зі стад за різних способів утримання встановлено, що на відміну від прив'язного за безприв'язного утримання спостерігалась тенденція збільшення відсотка вибракування первісток із високою оцінкою росту. Також найвищий ризик вибуття мали корови з широкою грудною клітиною та глибоким тулубом порівняно з групою тварин із середніми показниками лінійної оцінки. Спостерігалось незначне зменшення відсотка виракування первісток із підвищенням оцінки за молочний тип за обох способів утримання, тварин із бажаною поставою задніх кінцівок та високою оцінкою центральної зв'язки вимені та вгодованості.*

**Ключові слова:** молочна худоба, технологія утримання, ознаки типу будови тіла, збереженість, вибуття.

**Introduction.** The practice of using cattle has convincingly shown that the economic efficiency of milk production largely depends on the duration of its productive longevity (Pidpala T. and Zaitsev Ye., 2018). Intensification of dairy cattle breeding leads to a significant reduction in the life span of cows, as a result of which the average term of their use on dairy farms is limited to only 3-4 lactations (Karatieieva O. I., 2019). Therefore, the modern approach to improving dairy cattle is shifting from milk production traits to functional traits, such as longevity and body type traits (Tapki I. and Guzey, Y. Z. 2013; Stanojević D. et al., 2018). Although body type traits have low to medium



heritability (Bohlouli M. et al., 2015; Susanto A. et al., 2018), they are registered in a single database, which makes them reliable and relatively inexpensive for inclusion in national cattle breeding programs (Němcová E. et al., 2011). Therefore, the selection of animals by exterior type is of leading importance in the aspect of genetic improvement of herd productivity and longevity of cows (Camara Y. et al., 2019; Rodriguez-Bermudez R. et al., 2019). Only animals with good health, adapted to the harsh conditions of use in industrial complexes, can guarantee high lifetime productivity and longevity (Berry D. P. 2018; Kern E. L. et al., 2015). Improved lifespan of dairy cows is associated with increased profitability due to reduced need for replacement heifers to maintain herd size, higher average herd productivity (Williams M. et al., 2022) and greater opportunity for selective culling (van Pelt M. L., et al., 2015). In studies by foreign scientists, it was reported that the location of the front teats, udder depth, front udder attachment and location of the rear teats had a greater impact on cow culling compared to other linear type traits (Imbayarwo-Chikosi V. E. et al., 2016). Other researchers have estimated a higher risk factor for animals with deep udders and weak and loose anterior and posterior attachments. The relationship between the stature of the posterior udder attachment and longevity (Zavadilová L. and Štipková M., 2012) and between the anterior udder attachment and longevity (Dube B. et al., 2009) has also been confirmed by other authors.

Limb and hoof problems, as well as other musculoskeletal problems, are the third most common problem and cause of culling of dairy cows after reproductive problems and mastitis (Sulayeman M. and Fromsa A., 2012; Kerlake J. I. et al., 2018). In numerical terms, losses due to lameness were associated with a decrease in milk yields of affected cows, which accounted for 40% of total losses, 34% of treatment costs and a decrease in conception rate – 26% (Olechnowicz J. and Jaskowski J. M., 2011). Extremely saber-shaped legs and low hoof angle also increased culling (Morek-Kopec M. and Zarnecki A., 2012).

Since scientists have established a high positive correlation between linear traits and longevity traits (Novotný L. et al., 2017), studying the relationship between traits of the body type of animals and their duration of economic use is a relevant task.

In this regard, the aim of the work was to study the dependence of the culling of first-born cows on the traits of their body type under different housing technologies.

**Materials and methods of research.** The work was carried out at the State Enterprises of the State Farm "Kutuzivka" and "Hontarivka" of the Kharkiv region and the State Enterprises of the State Farm "Stepne" and the Decembrists of the Poltava region. The first three enterprises are breeding plants for the Ukrainian black-and-white dairy breed, the fourth is the Ayrshire. The milk yield for one cow in the specified period was more than 6000 kg of milk. At the dairy complex of the State Farm "Kutuzivka" the first-born cows are kept free on long-term straw bedding. At the farms of the State Farm "Hontarivka", "Stepne" and the Decembrists of the State Farm "Agroprogress" they are kept tied.

To determine the patterns of influence of body type traits, a linear assessment of animals was carried out using the ICAR scale (Khmelnychyi L. M. et al., 2008). According to the linear assessment method, each of the traits had its own value and was evaluated with scores from 1 to 9. Scores 1 and 9 are the extreme values of the traits. The assessment was carried out visually. In the period 2013-2021, 569 first-born cows were assessed for tethered maintenance and 501 for free-range maintenance. Depending on the results of the assessment of individual linear features of the body structure type of animals, they were divided into 3 groups: low score - 1-3 points, average score - 4-6 points and high score - 7-9 points.



The percentage of cows leaving the herd was calculated as the ratio of the number of animals that left the herd in the period from the assessment of the body structure type of animals to their second calving. Analysis of variance was used to process the experimental data.

**Research results.** At the beginning of the research, differences in the assessment of first-born cows by the characteristics of the body structure type of animals under different technologies of their maintenance were considered (Table 1).

*Table 1*

**Evaluation of first-borns by linear type traits and their culling rate under different housing ( $\bar{x} \pm SE$ ), points**

Indicators	Housing		Total	Effect size, %
	stall	free		
Stature	6,30±0,05	6,71±0,05	6,49±0,04	2,9***
Chest width	5,31±0,04	5,16±0,05	5,24±0,03	0,5**
Body depth	5,61±0,05	5,41±0,06	5,52±0,04	0,7**
Angularity	5,91±0,04	5,69±0,05	5,81±0,03	1,2***
Rump angle	5,09±0,04	5,37±0,04	5,22±0,03	2,2***
Rump width	5,23±0,04	5,36±0,05	5,29±0,03	0,4**
Rear legs	5,57±0,04	5,91±0,06	5,73±0,04	2,0***
Legs rear view	5,11±0,05	4,74±0,06	4,94±0,04	1,8***
Foot angle	3,99±0,04	4,25±0,05	4,11±0,03	1,5***
Fore udder attachment	4,92±0,07	5,38±0,08	5,14±0,05	1,8***
Rear udder height	5,06±0,05	4,69±0,05	4,88±0,04	2,6***
Central ligament of the udder	4,96±0,07	4,54±0,07	4,76±0,05	1,6***
Udder depth	5,78±0,06	5,44±0,06	5,62±0,04	1,6***
Front teat position	4,27±0,04	4,07±0,05	4,18±0,03	1,0***
Rear teat position	5,77±0,05	6,13±0,06	5,94±0,04	2,0***
Teat length	4,37±0,04	4,57±0,04	4,46±0,03	1,2***
Body condition	4,84±0,04	4,78±0,04	4,81±0,03	0,1
Number of cows that were eliminated before the second calving, %	0,23±0,02	0,15±0,02	0,19±0,01	1,0***

Note: \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

According to the results of the analysis of variance, only for the assessment of body condition, insignificant differences were observed in the score of the characteristics of animals kept using tethered and untethered technologies. For all other indicators of linear assessment of the exterior, the differences were significant ( $p < 0.05$ ). The influence of the method of keeping on these indicators of body structure ranged from **0.4% to 2.9%**.

First-born heifers that were kept on a leash had a higher score: for chest width by 0.15 points, body depth by 0.2 points, angularity by 0.22 points, pelvic limb posture by 0.37 points, posterior udder attachment by 0.37 points, central udder ligament by 0.42 points, udder depth by 0.34 points, and anterior teat placement by 0.2 points. Animals in free-range housing had a higher score on the linear scale: stature by 0.4 points, sacral slope by 0.28 points, sacral width by 0.13 points, pelvic limb



angle by 0.34 points, anterior udder attachment by 0.46 points, posterior teat placement by 0.36 points, and teat length by 0.2 points.

Next, it was examined how the culling of first-born cows depended on the assessment of linear features of their body structure in tethered housing (Table 2).

*Table 2*

**Culling of first-born cows from the herd with different assessments of body structure indicators in stall housing (x±SE), %**

Indicators	Score of linear type traits			Effect size, %
	1-3	4-6	7-9	
Stature	-	23,3±1,0	23,2±1,2	0,3
Chest width	16,7±5,7	23,3±0,8	21,7±2,2	0
Body depth	50,0±10,2	24,7±0,9	15,7±1,2	1,2*
Angularity	-	24,2±0,9	20,5±1,2	0,1
Rump angle	20,8±3,4	23,2±0,8	22,5±2,8	0
Rump width	18,2±3,2	23,4±0,8	22,0±2,4	0,1
Rear legs	55,6±8,2	22,7±0,8	21,9±1,6	0,9
Legs rear view	35,5±2,9	21,2±0,8	22,8±1,7	0,8
Foot angle	25,1±1,5	22,3±0,9	14,3±4,6	0,1
Fore udder attachment	28,1±1,7	20,7±1,0	23,1±1,6	0,5
Rear udder height	20,8±2,4	25,3±0,9	13,3±1,2	1,1*
Central ligament of the udder	26,0±2,0	25,3±1,0	13,0±1,1	1,3*
Udder depth	34,3±3,8	25,5±1,0	17,2±1,0	1,3*
Front teat position	23,6±1,6	23,1±0,8	-	0,2
Rear teat position	18,8±3,8	23,5±0,9	22,1±1,5	0,1
Teat length	23,2±1,8	22,7±0,8	36,4±7,0	0
Body condition	37,2±3,6	22,3±0,8	7,1±1,8	1,3*

Note: \*  $p < 0.05$

The results of the study showed that the number of animals that dropped out of the herd did not depend on the assessment of their stature and chest width. However, animals with a high assessment of body depth (7-9 points) had a lower percentage of dropping out of the herd by 9% and 34.3% (respectively) compared to the average (4-6 points) and low (1-3 points) assessment. Differences in the dropping out of animals with different body structure assessments for this factor were significant ( $p < 0.05$ ), and the influence of the factor "keeping method" was 1.2%. Differences in the number of cows that dropped out of the herd with different angularity assessments were insignificant. A slight decrease in the percentage of dropping out was observed with an increase in the assessment of angularity, which characterizes the dairy type of animals. The dependence of the dropping out of cows on the assessment of the slope and width of the sacrum was not determined.

Animals with an average and high assessment of the posture of the hind limbs had somewhat better survival. However, the differences between the groups were not significant. The influence of the factor "pelvic limb angle" was 0.9%, and the factor "pelvic limb posture" was 0.8% ( $p > 0.05$ ).

Important signs of the type of body structure, on which the milk productivity of animals depends, as well as the duration of their use, are the signs of the udder. A greater number of animals were removed from the herd with weak anterior attachment of the



udder (1–3 points), but the differences in the percentage of removal of 5-7.4% between these groups were not significant. The results obtained when determining the percentage of removal of cows with different assessments of the stature of attachment of the hind udder lobes indicate a greater survival of first-born cows with a high assessment of this sign. It was 7.5-11% higher compared to animals with an average and low assessment. The influence of this factor was 1.1% ( $p < 0.05$ ).

The highest influence on the withdrawal of first-born heifers from the herd was the central ligament of the udder (1.3%;  $p < 0.05$ ) and the depth of the udder (1.3%;  $p < 0.05$ ). The number of first-born heifers that were withdrawn from the herd during the studied period with a low assessment of the central ligament of the udder was higher by 0.7-13% than in animals with a low and medium assessment. A similar relationship between the withdrawal of animals was established with the assessment of the depth of the udder (by 8.8-17.1%).

The withdrawal of first-born heifers did not significantly depend on the assessment of the placement of the front and rear teats, as well as their length.

The percentage of animals that were withdrawn from groups with a low assessment of body condition was significantly higher than in first-born heifers with a medium and high assessment (by 14.9-20.1%). The influence of the body condition assessment was 1.3% ( $p < 0.05$ ).

The results of the first-born calves culling with different assessments of the linear indicators of their body type under free housing are given in Table 3.

Table 3

**First-born calves culling from the herd with different assessments of body structure traits under free housing ( $\bar{x} \pm SE$ ), %**

Indicators	Score of linear type traits			Effect size, %
	1-3	4-6	7-9	
Stature	-	13,8±0,8	15,9±0,8	0,1
Chest width	16,7±3,3	14,6±0,6	17,0±1,9	0,1
Body depth	13,3±3,0	14,2±0,6	17,8±1,4	0,2
Angularity	14,3±4,6	15,3±0,7	13,8±1,0	0
Rump angle	33,3±6,4	12,4±0,5	28,6±2,6	2,9***
Rump width	22,2±5,8	14,7±0,6	15,7±1,6	0,1
Rear legs	11,8±2,5	15,1±0,7	15,1±0,9	0
Legs rear view	17,0±1,4	14,3±0,7	15,3±1,7	0,1
Foot angle	13,2±1,0	16,2±0,7	-	0,7
Fore udder attachment	13,3±1,3	17,9±0,9	10,3±0,8	0,9
Rear udder height	16,9±1,8	14,6±0,6	16,7±2,8	0,1
Central ligament of the udder	17,7±1,5	14,4±0,6	13,0±2,4	0,1
Udder depth	11,9±1,6	15,2±0,7	15,2±1,2	0,1
Front teat position	15,3±1,1	14,6±0,7	50,0±17,7	0,4
Rear teat position	14,3±4,6	13,2±0,6	18,1±1,1	0,4
Teat length	13,0±1,4	14,7±0,6	29,4±5,0	0
Body condition	18,9±2,5	14,8±0,6	11,1±2,3	0,1

Note: \*\*\*  $p < 0.001$



According to the results of the analysis of variance of the firstborn's mortality depending on the assessment of body structure traits in free housing, a probable influence of only one factor was established - the slope of the sacrum. The strength of the influence of this trait was 2.9%. It is important to note that the percentage of animals leaving the herd with an average assessment of the slope of the sacrum was the lowest. With a low assessment of this trait, 19.9% more animals were left, and with a high assessment by 16.2%.

The impact of the assessment of other traits on the mortality of firstborns was unlikely. Therefore, we will further compare the trends in the impact of body structure traits of firstborns on the level of their mortality in free housing and compare them with the results obtained in tied housing.

Unlike tied housing, a tendency was observed to increase the percentage of culling of firstborns with a high growth assessment in free housing. Cows with a wide rib cage and a deep body had the highest risk of culling compared to the group of animals with average linear scores. In this case, the cows had an optimal body weight, and this combination had a greater impact on the joints and hooves, since a heavier body puts more pressure on the foot, which is an unfavorable factor for the preservation of livestock. Since the concrete stall floor is part of the typical housing system on Ukrainian farms with loose housing, animals with a higher body weight were more likely to be culled from the herd.

A slight decrease in the percentage of culling was observed with an increase in the score for the dairy type for both housing methods. Also, animals with the desired hindlimb posture were better preserved for both technologies. In loose housing, as in tied housing, there is a tendency to reduce the number of animals that were culled from the herd with a high score for the central ligament.

Regardless of the housing technology, the percentage of animals that were culled with a high score for body condition was lower compared to first-borns that had low and medium body condition.

**Discussion.** The housing technology has a significant impact on the longevity and survival of dairy cattle. The most important type traits for animal longevity were those that described body size and udder. According to our data, it was found that first-borns with a high score for body depth had a lower percentage of culling from the herd when tied. In contrast to tied, when free-ranged, there was a tendency for the risk of culling first-borns with a high score for growth, a wide chest and a deep body, which is associated with the conditions of animal housing. Our data are consistent with (Morek-Kopec M. and Zarnecki A., 2012), as they also reported a longer productive life of cows with a smaller body depth and chest width. Our studies found a slight decrease in the percentage of animals being culled with an increase in the score for the milk type. Foreign scientists found that the most angular cows had a shorter lifespan (Zavadilová L. and Němcová E. 2011). They were more sensitive to environmental conditions, which led to a shorter productive life. Despite this, cows with good angularity had better milk productivity for different populations of the Holstein breed, as was found by other researchers (Campos R. V. et al., 2015; Battagin M. et al., 2013). In contrast to tethered housing, there was a tendency for the percentage of first-born cows with a high growth score to be culled. This fact has been confirmed by other studies (Palii A. P. et al., 2020).

It was found that animals that had an average and high score for the posture of the hind limbs were more adapted to the harsh conditions of industrial complexes. This pattern is confirmed by numerous authors (Morek-Kopec M. and Zarnecki A., 2012; Kerlake J. I. et al., 2018; Sulayeman M. and Fromsa A., 2012). Our studies revealed a higher percentage of withdrawal from the herd of first-born cows with weak anterior



udder attachment and better survival of animals with a higher score for the stature of the attachment of the hind udder lobes and a well-pronounced central ligament. Other researchers also emphasize that cows with weak and poorly attached udders, as well as deep udders, had an increased risk of culling compared to cows that had average scores for these traits (Török E. et al., 2021; Zavadilová L. and Štipková M., 2012; Dube B. et al., 2009).

### Conclusions:

1. It was established that the percentage of first-born cows leaving the herd depends on the characteristics of their body type, as well as the technology of housing.

2. When stall housing, animals with a high body depth score had a lower percentage of leaving the herd by 9% and 34.3% than animals with an average and low score. The stayability of first-born cows with a high score of the stature of attachment of the posterior lobes of the udder was 7.5-11% higher compared to lower scores of this trait. The number of animals that left the herd with a low score of the central ligament of the udder was 0.7-13% higher compared to animals with a low and average score. A similar relationship between the loss of animals was established with the score of the udder depth (by 8.8-17.1%). The percentage of animals that dropped out of groups with a low score for body condition was significantly higher than in first-born heifers with an average and high score (by 14.9-20.1%).

3. According to our data, only the score of the rump angle (2.9%) significantly influenced the dropout of first-born cows in free housing. The percentage of culling cows from the herd with an average score of the rump angle was 19.9% lower than in animals with a low score of this trait and 16.2% lower than in animals with a high score.

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## **INFLUENCE OF LIVE WEIGHT OF FIRST-CALF COWS FROM INSEMINATION TO THE FIRST 100 DAYS OF LACTATION ON SUBSEQUENT MILK PRODUCTION**

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*The article presents the results of determining the influence of the live weight of primiparous cows from fertilization to the first 100 days of lactation on subsequent milk productivity.*

*The study was conducted on primiparous cows of the Ukrainian black-and-white dairy breed, which are kept at the breeding plant of the State Enterprise "Hontarivka" of the Chuguyiv district of the Kharkiv region. During the experiment, the influence of various technological regimes on changes in live body weight during the first 100 days of lactation was studied. The main parameters studied were the live weight of primiparous cows at the stages of fertilization, after calving and in the first 100 days of lactation, as well as their milk productivity for 305 days of the lactation period. The determination of milk productivity included accounting for the gross milk yield of natural fat content on the 100th, 200th and 305th days, as well as an estimate of the mass fraction of fat and protein in milk for the entire lactation period. For the analysis, a retrospective study of cow productivity over the past decade was conducted.*

*For the study, four groups of primiparous cows were formed depending on their live weight at the time of fertile insemination: Group I - up to 380 kg, Group II - 380–399 kg, Group III - 400–419 kg, Group IV - over 420 kg.*

*The average live weight at insemination was: in Group I -  $358.8 \pm 1.51$  kg, Group II -  $387.7 \pm 0.49$  kg, Group III -  $406.3 \pm 0.65$  kg, Group IV -  $445.5 \pm 3.66$  kg. Animals of Group IV exceeded their peers from other groups by 39.2–86.7 kg, which corresponded to an increase in weight by 8.8–19.5%. The live weight of cows after calving was: in group I –  $573.9 \pm 3.56$  kg, II –  $583.4 \pm 3.58$  kg, III –  $590.3 \pm 6.01$  kg, IV –  $591.1 \pm 5.51$  kg. However, after calving, the difference between the groups began to decrease: cows of group IV outweighed animals of group I by only 17.2 kg (2.9%), group II – by 7.7 kg (1.3%), group III – by 0.8 kg (0.1%).*

*On the 100th day after calving, the difference in live weight between group IV and other groups increased again. The average weight in group IV was  $561.7 \pm 4.54$  kg, which exceeded the indicators of group I by 5.5% ( $530.6 \pm 3.00$  kg), group II by 3.1% ( $544.1 \pm 2.95$  kg), group III by 1.6% ( $552.8 \pm 4.90$  kg).*

*Live weight losses from the moment of calving to the 100th day of lactation were: in group I -  $43.2 \pm 8.6$  kg (7.5%), in group II -  $39.3 \pm 8.3$  kg (6.7%), in group III -  $37.6 \pm 11.2$  kg (6.4%), in group IV -  $29.3 \pm 9.2$  kg (5.0%).*



*Analysis of the obtained data showed that the first-born cows of group III had better adaptation to further productive use. The implementation of these results in production will help optimize the feeding of heifers and cows in the post-calving period, which will increase the efficiency of herd management.*

**Keywords:** replacement heifers, first-born, analysis, live weight, milk productivity.

## **ВПЛИВ ЖИВОЇ МАСИ КОРІВ-ПЕРВІСТОК ВІД ЗАПЛІДНЕННЯ ДО ПЕРШИХ 100 ДНІВ ЛАКТАЦІЇ НА НАСТУПНУ МОЛОЧНУ ПРОДУКТИВНІСТЬ**

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*У статті наведено результати визначення впливу живої маси корів-первісток від запліднення до перших 100 днів лактації на послідуочу молочну продуктивність.*

*Дослідження було проведено на коровах-первістках української чорно-рябої молочної породи, які утримуються на племінному заводі ДП ДГ «Гонтарівка» Чугуївського району Харківської області. У ході експерименту вивчали вплив різних технологічних режимів змін живої маси тіла протягом перших 100 днів лактації. Основними досліджуваними параметрами були жива маса корів-первісток на етапах запліднення, після отелення та в перші 100 днів лактації, а також їх молочна продуктивність за 305 днів лактаційного періоду. Визначення молочної продуктивності включало облік валового надою молока натуральної жирності на 100-й, 200-й і 305-й дні, а також оцінку масової частки жиру та білка в молоці за повний лактаційний період. Для аналізу було проведено ретроспективне вивчення продуктивності корів за останнє десятиріччя.*

*Для дослідження сформували чотири групи первісток залежно від їх живої маси під час плідного осіменіння: I група – до 380 кг, II – 380–399 кг, III – 400–419 кг, IV – понад 420 кг.*

*Середні показники живої маси при осіменінні становили: у I групі –  $358,8 \pm 1,51$  кг, II –  $387,7 \pm 0,49$  кг, III –  $406,3 \pm 0,65$  кг, IV –  $445,5 \pm 3,66$  кг. Тварини IV групи перевищували своїх ровесниць з інших груп на  $39,2$ – $86,7$  кг, що відповідало збільшенню маси на  $8,8$ – $19,5$  %. Жива маса корів після отелення становила: у I групі –  $573,9 \pm 3,56$  кг, II –  $583,4 \pm 3,58$  кг, III –  $590,3 \pm 6,01$  кг, IV –  $591,1 \pm 5,51$  кг. Проте після отелення різниця між групами почала зменшуватися: корови IV групи переважали тварин I групи лише на  $17,2$  кг ( $2,9$  %), II групи – на  $7,7$  кг ( $1,3$  %), III групи – на  $0,8$  кг ( $0,1$  %).*

*На 100-й день після отелення різниця у живій масі між IV групою та іншими групами знову збільшилася. Середня маса у IV групі становила  $561,7 \pm 4,54$  кг, що перевищувало показники I групи на  $5,5$  % ( $530,6 \pm 3,00$  кг), II групи – на  $3,1$  % ( $544,1 \pm 2,95$  кг), III групи – на  $1,6$  % ( $552,8 \pm 4,90$  кг).*

*Втрати живої маси від моменту отелення до 100-го дня лактації склали: у I групі –  $43,2 \pm 8,6$  кг ( $7,5$  %), у II групі –  $39,3 \pm 8,3$  кг ( $6,7$  %), у III групі –  $37,6 \pm 11,2$*



кг (6,4 %), у IV групі –  $29,3 \pm 9,2$  кг (5,0 %).

*Аналіз отриманих даних показав, що корови-первістки III групи мали кращу адаптацію до подальшого продуктивного використання. Впровадження цих результатів у виробництво сприятиме оптимізації годівлі нетелів та корів у післяотільний період, що дозволить підвищити ефективність управління стадом.*

**Ключові слова:** *ремонтні телиці, первістки, аналіз, жива маса, молочна продуктивність.*

**Introduction.** One of the main modern global problems that humanity needs to solve is the shortage of food in certain regions. The latest report of the FAO (2023) “State of Food Security and Nutrition in the World” states that in 2022 from 691 to 783 million people faced the problem of hunger. This is 122 million people more than in 2019, before the start of the COVID-19 pandemic.

One of the ways to solve this problem is to increase the production of livestock products, in particular milk and beef.

In modern conditions, it is possible to increase the profitability of agricultural enterprises producing milk only by increasing animal productivity. A special role in the formation of milk productivity of cows and the manifestation of its genetic potential is given to the improvement of milk production technology (Voytenko S.L., Zheliznyak I.M., 2019).

Studies by Khmelnychy and Bardash (2019) have proven that different environmental conditions in which animals are during their growth and development can both contribute to high milk productivity and suppress it. According to Kostenko V. (2020), low and very high levels of feeding when raising dairy cows are inappropriate, as they negatively affect further milk productivity and reproductive ability. Scientists have found that when heifers are overfed and inseminated at an older age, they have increased fat deposition in the body, reproductive functions develop worse, and milk productivity subsequently decreases. In underdeveloped dairy cows, the economic value decreases sharply, since they have a low manifestation of almost all economic useful traits, and animals with excessive mass do not fully pay for the feed spent on its production with products (mainly milk). Milk productivity of cows is the result of the interaction of many factors, among which the live weight of primiparous cows in the first 100 days of lactation is singled out as one of the key indicators. This period is critical for the formation of both the productivity of the animal and its health. Many studies confirm that maintaining optimal body weight at this time is an important condition for achieving high results in subsequent lactation (Dymchuk A. V., Ponko L. P., 2022).

Also, the age of the first insemination (L. Karlova et al., 2018), body weight at fertile insemination (Vedmedenko O. V., 2019) have a significant impact on the subsequent milk productivity of animals.

According to the results of their own studies, Pidubna et al. (2021) concluded that with increasing calving age, milk productivity and milk quality indicators tend to decrease.

At the same time, according to Turiello et al. (2020), first-born cows with an earlier calving age had lower milk productivity in the first 5 months of lactation.

According to the studies of Kusaka et al. (2022) found that the frequency of difficult calvings and the prevalence of stillbirths were significantly higher in animals with low body weight compared to heifers with medium and high body weight.

At the same time, scientists and practitioners have long been interested in the impact of post-calving weight loss on the milk production of cows and primiparous cows. Unfortunately, such studies have been conducted quite rarely, especially in Ukraine. This



was due to the complexity of recording the necessary indicators. But recently, with the introduction of modern automatic accounting systems on farms, these studies can provide more interesting information.

For example, according to the results of a study by Berry et al. (2017), cows that lost more weight at the beginning of lactation had higher milk production with an increased content of fat and protein. However, this trend changed in those cows that significantly reduced their condition after calving, while cows with higher weight demonstrated an inverse relationship. Milk production was observed to increase with increasing body weight, but this effect decreased as the animals recovered.

Zahout and Moallem (2017) found in their study that cows that experienced significant body weight loss (7% to 17%) during the first five weeks of lactation had an average 30-day milk yield of 1.4 kg/day more compared to animals whose weight loss was only 3–6%. This suggests that more intense body weight loss after calving may be associated with increased milk production in the early stages of lactation.

According to Wathes et al. (2008), heifers that calve with higher fatness (3.8–3.9 points) subsequently mobilize more tissues for milk production and recovery, which also has detrimental consequences for fertility.

This relationship requires further research to clarify the causes and possible consequences for animal health. This and the small amount of information on this problem in Ukraine served as an impetus for our research.

The purpose of the work is to establish the influence of heifer live weight and its decrease in the first 100 days after calving on the milk productivity of first-born heifers.

To achieve this goal, the following tasks were set:

- to analyze the dynamics of live weight of primiparous cows during their rearing period, after calving, in the first 100 days of lactation;
- to determine the dynamics of live weight, milk productivity and milk quality indicators of primiparous cows for 305 days of lactation.

Materials and methods of the study. The study was conducted on primiparous cows of the Ukrainian black and white dairy breed of the breeding plant of the State Enterprise “Hontarivka” of the Chuguyiv district of the Kharkiv region using different technological regimes of changes in live body weight during the first 100 days of lactation. The key paratypic factors were the live weight of primiparous cows from the moment of fertilization, after calving and in the first 100 days of lactation, as well as milk productivity during 305 days of lactation. Milk accounting was carried out taking into account the gross milk yield of natural fat content on the 100th, 200th and 305th days and the mass fraction of fat and protein content for 305 days of lactation. For this purpose, a retrospective analysis of the productive indicators of the livestock over the past 10 years was conducted.

The implementation of the tasks of the work at their various stages involved the use of standard and generally accepted research methods: zootechnical (growth and development of heifers, milk productivity of primiparous cows), abstract-logical (theoretical generalizations, critical analysis of publications by domestic and foreign scientists, formation of conclusions); retrospective, analytical and probabilistic methods; variational statistics (biometrics of the reliability of the conducted research); statistical and economic (for processing and analysis of statistical data), multi-criteria analysis.

Four technological groups of primiparous cows were formed depending on the live weight at fertile insemination according to the scheme (Table 1).



Table 1

**Scheme for forming groups of primiparous cows**

Indicators	Group			
	I	II	III	IV
Number of animals in the sample, head	149	150	96	52
Live weight at fertile insemination, kg	to 380	380-399	400-419	420 and more

The following factors were taken into account in the process of conducting the study:

- change in live weight of heifers (according to data from monthly control individual weighings) and primiparous cows (according to data from weighings and calculation of live weight using measurements). Based on the data obtained, the dynamics of live weight of experimental animals was calculated;
- level of milk productivity of primiparous cows – according to individual data from monthly control milkings with the selection of average milk samples to determine its quality indicators;
- quality indicators of milk (mass fraction of fat and protein) – according to individual data from zootechnical accounting;
- statistical processing of the research results was carried out using biometric methods with determination of the probability level (Ibatullin I.I. et al., 2017).

Multicriteria analysis was performed using the method of evaluating the integral criterion of the distance to the target using the approach of collapsing all criteria to one N using normalization (Piskun, V., 2020). The relative distance  $N(C_k)$  will be found for each alternative solution from the expression:

$$N(C_k) = \frac{\sum_{i=1}^n u_{ij}^N - \sum_{i=1}^n u_{i_0}^N}{\sum_{i=1}^n u_{i_0}^N},$$

where  $N(C_k)$  – the efficiency of each of the options under study compared to the idealized one;

$u_{ij}^N$  – the normalized j-th indicator of the option under study;

$u_{i_0}^N$  – the normalized 0-th indicator of the idealized option;

n – the number of evaluated criteria.

Digital material of experimental research was processed by biometric methods using the MS Excel 2003 application package.

**Results of research.** During the research period, initial data were obtained on establishing patterns and features of the dynamics of live weight indicators, milk productivity of first-born cows for the first 100 days of lactation, 200 days and 305 days of lactation and milk quality indicators (Fig. 1).

It was established that the live weight at fertile insemination was on average in group I –  $358.8 \pm 1.51$  kg, II –  $387.7 \pm 0.49$  kg, III –  $406.3 \pm 0.65$  kg and IV –  $445.5 \pm 3.66$  kg. According to this indicator, it is clear that animals of group IV outweighed animals of other groups by 86.7-39.2 kg or 19.5-8.8% ( $P > 0.999$ ).

The same picture was observed after calving. The live weight of primiparous cows of group I was  $573.9 \pm 3.56$  kg, group II –  $583.4 \pm 3.58$  kg, III –  $590.3 \pm 6.01$  kg and IV –  $591.1 \pm 5.51$  kg. However, it was found that the live weight in the first days had a



tendency to decrease the difference between the groups: animals of group IV outweighed the firstborn from group I by an average of 17.2 kg (or 2.9%), group II - by 7.7 kg (1.3%), group III - by only 0.8 kg (0.1%) ( $P>0.99$  with the first group, with groups II and III there is no significant difference).

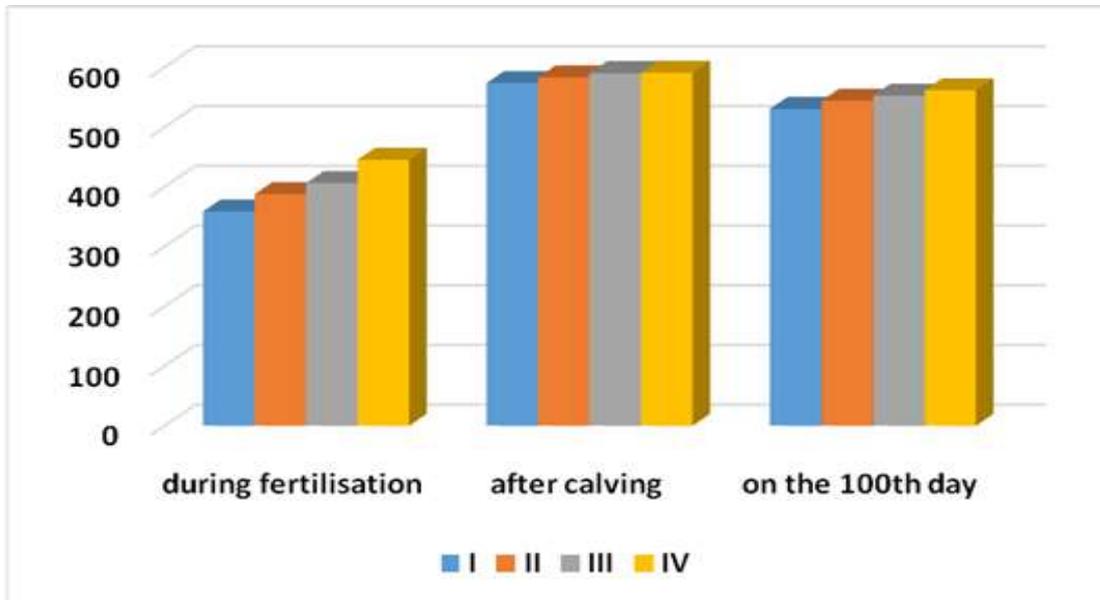


Fig. 1. Dynamics of live weight of heifers and primiparous cows.

And according to the results of monitoring live weight on the 100th day after calving, an increase in the difference in live weight of cows of group IV compared to animals of other groups was again noted -  $561.7 \pm 4.54$  kg versus  $530.6 \pm 3.00$  kg (or + 5.5 %) in group I ( $P>0.999$ ),  $544.1 \pm 2.95$  kg (+ 3.1 %) in group II ( $P>0.99$ ) and  $552.8 \pm 4.90$  (+ 1.6 %) in group III ( $P<0.90$ ).

An analysis of milk productivity of primiparous cows during 305 days of lactation was also conducted (Table 2).

Table 2

**Indicators of milk productivity and milk quality of primiparous cows per lactation**

Indicators	Group			
	I	II	III	IV
Number of heads	149	150	96	52
Milk yield for 1-100 days of lactation, kg	2106,3±36,29	2462,2±36,41	2589,4±45,93	2503,1±56,22
Milk yield on 101-200 days of lactation, kg	1693,0±27,43	1952,5±27,93	2046,8±33,04	1992,4±43,07
Milk yield on 201-305 days of lactation, kg	1366,5±23,19	1605,7±24,20	1671,4±28,73	1605,5±33,59
Milk yield on 305 days of lactation, kg	5165,8±85,74	6020,3±87,19	6307,5±105,53	6101,0±129,68
Fat content by mass, %	4,07 ±0,31	4,06 ±0,32	4,04 ±0,33	4,00 ±0,33
Milk fat, kg	210,5	244,4	254,7	244,2
Protein content by mass, %	3,26 ±0,23	3,25 ±0,20	3,24 ±0,29	3,16 ±0,24
Milk protein, kg	168,6	195,4	204,6	192,6



According to the results of the assessment, it was found that during the first 100 days of lactation, the largest amount of milk with natural fat content was obtained from cows of group III - 2589.4 ± 45.93 kg, from first-born cows of group IV - less by 86.3 kg (3.3%), group II - less by 127.3 kg (4.9%), and group I - less by 483.1 kg (18.7%) (Fig. 2).

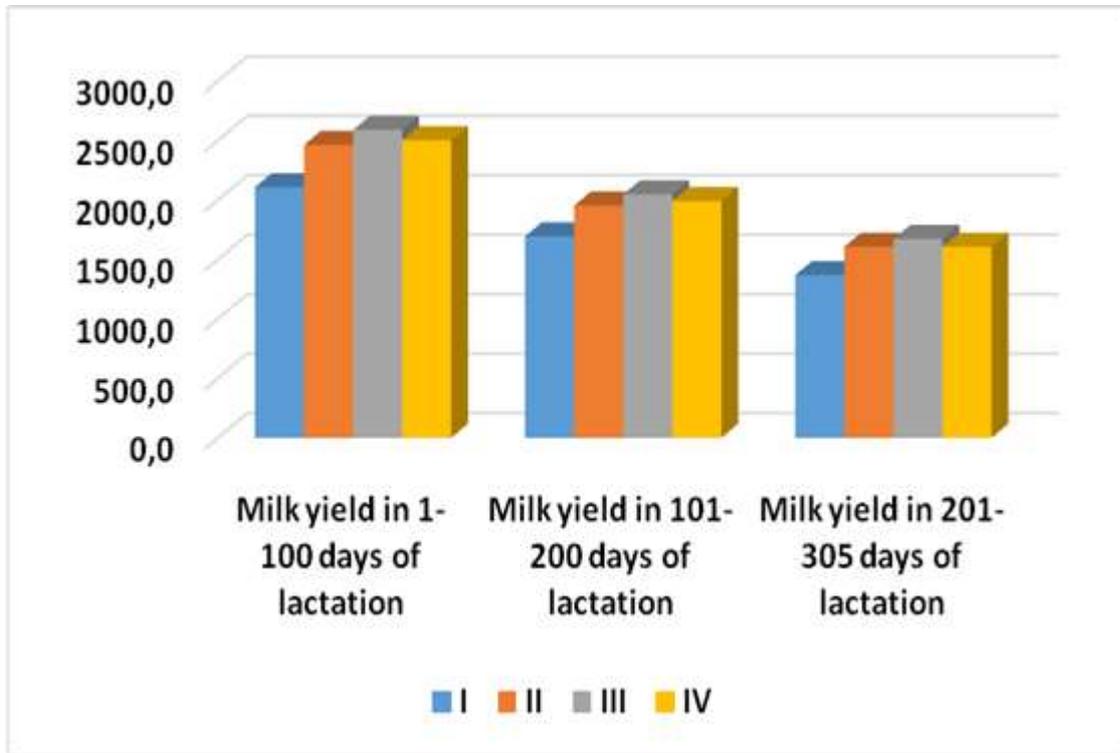


Fig. 2. Dynamics of milk productivity of primiparous cows depending on live weight.

In the next 100 days of lactation, this trend was preserved. The productivity of first-born cows of group III was higher than that of yearlings from group IV by 54.3 kg (2.6%) ( $P < 0.90$ ), group II - by 94.2 kg (4.6%) ( $P > 0.95$ ), and group I - by 353.8 kg (17.3%) ( $P > 0.999$ ).

In the third phase of lactation (201-305 days), the dominance of animals of group III over others in terms of milk productivity was also observed. They prevailed in this indicator over cows from group IV by 65.8 kg (3.9%) ( $P < 0.90$ ), group II by 65.7 kg (3.9%) ( $0.90 < P < 0.95$ ), and group I by 304.8 kg (18.2%) ( $P > 0.999$ ).

It was established that the highest milk productivity for 305 days of lactation according to the primary accounting data was observed in cows that had a live weight of 400-419 kg (group III) at the time of fertile insemination, - 6307.5 ± 105.53 kg of milk of natural fat content. This indicator turned out to be greater than that of animals of group I by 1141.7 kg or 18.1% ( $P > 0.999$ ), group II - by 287.2 kg or 4.6% ( $P > 0.95$ ) and group IV - by 206.5 kg or 3.3% ( $P < 0.90$ ).

At the same time, a different pattern is observed with respect to the qualitative indicators of milk (mass fraction of fat and protein) - in cows with lower productivity, these indicators turned out to be higher. Thus, the fat content in milk in group I turned out to be higher than in group II by 0.01% (0.36 abs. %), compared to group III - by 0.04% (0.86 abs. %) and IV - by 0.07% (1.17 abs. %).

The same trend was observed with the protein content. In group I, this indicator was higher compared to other groups by 0.02% (0.53 abs. %), 0.02% (0.62 abs. %) and 0.11% (3.28 abs. %), respectively.



Based on the results of determining the indicators of live weight at fertile insemination, live weight at calving, on the 100th day of lactation, milk productivity and milk quality of first-born cows per lactation, a multi-criteria analysis was performed (Table 3).

Table 3

**Multicriteria analysis of the dynamics of live weight and milk productivity of primiparous cows**

Indicators	Group			
	I	II	III	IV
Live weight at fertile mating, kg	1,0805	1	1,0478	1,1491
Live weight after calving	1,0166	1	1,0118	1,0132
On the 100th day of lactation	1,0586	1,0323	1,0161	1
Milk yield for 1-100 days of lactation, kg	1,2293	1,0517	1	1,0345
Milk yield on 101-200 days of lactation, kg	1,2089	1,0483	1	1,0273
Milk yield on 201-305 days of lactation, kg	1,2231	1,0409	1	1,0410
Milk yield on 305 days of lactation, kg	1,2210	1,0477	1	1,0338
Mass fraction of fat content %	1	1,0025	1,0074	1,0175
Mass fraction of protein content, %	1	1,0031	1,0062	1,0316
Σ	10,038	9,2265	9,0894	9,3480
Objective function (SC)	0,1153	0,0252	0,0099	0,00387
Difference, times	11,6465	2,5455	-	3,9091

The results of the comparative analysis by the complex efficiency indicator of each of the alternative options N(Ck) in comparison with the idealized one allow us to note the significant advantage of the third group, for which the objective function according to the considered criteria is the smallest and is 0.0099. At the same time, the objective function for the first group turned out to be 11.65 times worse, and the second and fourth groups were 2.55 and 3.91 times worse, respectively. That is, by the complex indicator, the efficiency of animals of group III by economic characteristics turned out to be higher than in other groups.

And to establish the relationship between paratypic factors - milk yield for 305 days of lactation, kg (Y); and live weight at fertilization, kg (X1); live weight after calving, kg (X2); live weight 100 days after calving, kg (X3); average mass fraction of fat in milk, % (X4); average mass fraction of protein in milk, % (X5) mathematical models were developed. The following were developed and analyzed: linear, incomplete quadratic and complete quadratic models.

Linear model:

$$Y = 165,337 + 16,213 X_1 + 5,242X_2 - 7,934X_3 - 412,412X_4 + 685,676X_5$$

R-squared = 0.168

Incomplete quadratic model:

$$Y = -18534.660 + 109.107 X_1 - 8.407 X_2 + 20.053X_3 - 3724.916X_4 + 2981.336X_5 - 0.118X_1^2 + 0.010X_2^2 - 0.023X_3^2 + 385.264X_4^2 - 337.956X_5^2$$

R-squared = 0.220



Full quadratic model:

$$Y = 28346.410 + 7.230 X_1 - 10.757X_2 + 33.814X_3 - 10976.278X_4 - 6632.054X_5 - 0.113X_1^2 + 0.024X_2^2 - 0.042X_3^2 + 496.708X_4^2 - 336.888X_5^2 + 0.004X_1 * X_2 - 5.666 X_1 * X_4 + 22.482 X_1 * X_5 - 5.141 X_2 * X_5 + 1.826 X_3 * X_4 + 970.384X_4 * X_5$$

$$R\text{-squared} = 0.249$$

Analysis of the obtained models showed that the R-squared value of the full quadratic model is the largest, that is, this model most adequately describes the relationship between paratypical factors.

**Discussion.** The results of this study show that primiparous heifers that were inseminated at a body weight of 400–419 kg (slightly above optimal) and had an average body weight loss at day 100 of lactation ( $37.6 \pm 11.2$  kg) outperformed their peers in terms of milk production.

According to Handcock et al. (2018), the increase in milk yield due to an increase in the percentage of target body weight achieved is consistent with the finding that heavier heifers (especially after puberty and at calving) have more milk solids during the first lactation. Body weight at calving influences the body reserves available to the primiparous heifer to support lactation, the growth requirements during lactation, and the heifer's likely hierarchical position in the herd, so it is not surprising that this would affect lactation.

According to Martín et al. (2018), first-born cows that reached the target live weight (for fertilization) at the age of 15 months produced more milk, protein and total milk solids than their peers that did not reach this at the same age.

At the same time, as noted by V. Liskovich (2023), a different trend was observed in Ukrainian red-and-white cows - the minimum milk yield (4715 kg) was obtained from animals that had the lowest live weight at the first insemination - 374 kg and age 17.5 months, and the maximum milk yield - 5794 kg was obtained from first-born cows that had the maximum indicators of both age and live weight at the first insemination.

And the studies of Shulyar et al. (2020) found that the best milk yields and the amount of milk fat and protein, their total production were noted by first-born cows with a live weight at the time of the first insemination of 391–400 kg. First-born cows, whose live weight at the time of the first calving was within 511–530 kg, were noted for the highest milk yields, the amount of milk fat, protein, their total production. They significantly exceeded cows weighing 471–490 kg in milk yield by 309 kg, but were insignificantly inferior to them in fat and protein milk content. At the same time, they were inferior to cows with a live weight at the time of the first calving of 531 kg and more in milk protein content by 0.04%.

Shevchenko O.B. et al. (2023) found that there is a direct relationship between the live weight of cows and milk productivity indicators in the 1st lactation - with an increase in the live weight of a cow, its milk yield also increases. Thus, if the productivity of cows of the 1st gradation was 7389 kg, then the milk yield of the same-age cows of the 2nd gradation was 156 kg higher (by 2.1%,  $P < 0.50$ ), of the 3rd gradation - by 15 kg, of the 4th gradation - by 118 kg (by 1.4%), of the 5th gradation - by 181 kg (by 2.4%,  $P > 0.95$ ). The average fat content in milk also tends to increase - the difference between the 1st and 5th gradations was 0.07%, but this difference was not significant. At the same time, it was noted that the average mass fraction of fat in the milk of cows of the 4th gradation is 0.4-0.5% lower than this indicator of peers of adjacent gradations. The amount of milk fat is directly proportional to milk yield, therefore, with an increase in the value of the gradation of the body weight of cows, in accordance with milk yield, the amount of milk fat also increases - when comparing the values of the 1st and 5th gradations, the difference was 22.5 kg at  $P > 0.999$ . A similar pattern was established for the average content and



amount of milk protein - with an increase in the value of the gradation of the live weight of cows, their values also increase: the average mass fraction of protein by 0.08%, and milk protein - by 20.4 kg at  $P > 0.999$ .

Berry et al. (2007) noted that cows that lost 100 kg of weight from calving to calving weight produced 139 kg more milk in the first 60 days of lactation than cows that lost only 50 kg during the same period.

In their own study, Carvalho et al. (2014) noted that cows that maintained their body weight (BW) or lost up to 10% of their BW during the 21-day postpartum transition period were more productive during early lactation. The authors noted that multiparous cows with extreme BW changes of more than approximately  $\pm 10\%$  during the 21-day postpartum period experienced significant declines in their gross milk yield at 90 days. For example, cows that lost approximately 21.4% of their initial BW during the 21-day postpartum period had a 90-day milk yield of  $3603 \pm 162.0$  kg, while cows in the same parity group with a BW gain of 16.7% had an estimated 90-day milk yield of  $3037 \pm 149.8$  kg. When these figures are compared with  $4548 \pm 52.2$  kg in cows of the other group with a 21-day body weight loss of 4.5%, a serious deterioration in the milk production of the cows becomes evident. The maximum estimated milk yield in 90 days for cows of this group was reached when they had lost 7.4% of their initial body weight, and the large body weight loss had no significant effect on milk yield in 90 days. On the other hand, primiparous cows that gained body weight in 21 days showed a significant decrease in milk yield in 3 months. For example, cows with a 21-day body weight gain of 16.8% had an estimated milk yield in 90 days of  $1932 \pm 134.4$  kg, in contrast to  $3123 \pm 52.6$  kg for those who had lost 7.4% of their body weight in the same period. The effect of nutrient allocation on recovery rather than milk production in cows may contribute to this finding and warrants further study. The authors suggest that high-producing cows mobilize large body reserves to meet the demands of producing more milk at the expense of reproductive function.

As Berry et al. (2007) suggest, significant body weight loss early in lactation was associated with increased lactation persistence and higher and earlier peak milk yields.

Therefore, in part, our results are consistent with studies in which cows with early lactation weight loss are those that produce more milk. We believe that primiparous cows with significant post-calving body weight loss may experience more severe negative nutrient balance and potentially health problems, which may also affect dry matter intake, ultimately negatively affecting their performance.

The results of the study by Peiter et al. (2023) show that body weight change during the first 21 days after calving had strong quadratic associations with total milk production over 90 lactations, with differences between groups by insemination. It was found that primiparous cows with the highest productivity lost 7.4% of their body weight during the first 21 days of lactation, while cows with extreme body weight gain had much lower productivity during the first 90 days. In addition, second lactation and older cows with the highest productivity lost an average of about 5% per 21 days. These results demonstrate the usefulness of data from automated systems on the farm to improve the management of dairy cows during the transition period.

This is partially consistent with our data, where primiparous cows with an average post-calving loss of 6.4% (Group III) were the most productive, followed by animals with a loss of 5.0% (Group IV). The lowest productivity was in group I, where live weight decreased by 7.5%.

According to Wathes et al. (2008), heifers that calved with higher fatness (3.8–3.9 points) subsequently mobilize more tissues for milk production and recovery, which also has detrimental consequences for fertility.



### **Conclusions:**

1. It is proven that the highest milk productivity potential for the first 100 days of lactation of 2589.4 kg and more of milk was found in primiparous cows of group III, which exceeded their counterparts from groups I, II and IV by 18.7-3.3%. The same trend was also maintained during the second and third lactation periods.

2. It is noted that the live weight at fertile insemination was on average in group I -  $358.8 \pm 1.51$  kg, II -  $387.7 \pm 0.49$  kg, III -  $406.3 \pm 0.65$  kg and IV -  $445.5 \pm 3.66$  kg. According to this indicator, it is clear that animals of group IV exceeded animals of other groups by 86.7-39.2 kg or 19.5-8.8%. The live weight after calving of the firstborns of group I was  $573.9 \pm 3.56$  kg, of group II –  $583.4 \pm 3.58$  kg, of group III –  $590.3 \pm 6.01$  kg and of group IV –  $591.1 \pm 5.51$  kg. However, it was found that the live weight in the first days had a tendency to decrease the difference between the groups: animals of group IV outweighed the firstborns of group I by an average of 17.2 kg (or 2.9%), of group II – by 7.7 kg (1.3%), of group III – by only 0.8 kg (0.1%).

On the 100th day after calving, an increase in the difference in live weight of cows of group IV compared to animals of other groups was noted -  $561.7 \pm 4.54$  kg versus  $530.6 \pm 3.00$  kg (or + 5.5 %) in group I,  $544.1 \pm 2.95$  kg (+ 3.1 %) in group II and  $552.8 \pm 4.90$  (+ 1.6 %) in group III.

It was found that the loss of live weight of cows from calving to day 100 of lactation was  $43.2 \pm 8.6$  kg (7.5 %) in group I,  $39.3 \pm 8.3$  kg (6.7 %),  $37.6 \pm 11.2$  kg (6.4 %), and  $29.3 \pm 9.2$  kg (5.0 %).

3. As a result of the multi-criteria analysis, it was found that for group III the objective function according to the considered criteria is the smallest and is 0.0099. At the same time, the objective function for the first group turned out to be 11.65 times worse, and for the second and fourth groups it was 2.55 and 3.91 times worse, respectively.

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## FACTORS INFLUENCE ON THE MILK QUALITY INDICATORS OF NOVOOLEKSANDRIVSKIA HEAVY-DUTY BREED MARES

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*The article presents the results of studies of milk productivity and milk quality of mares of the Novooleksandrivka heavy draft breed. The daily milk yield of mares and milk quality indicators in samples taken in June and August were determined, and it was proven that all indicators vary significantly. It was established that the indicators of dry matter, protein, lactose and dry non-fat milk residue were higher in June, and the protein content, fat-protein ratio, freezing point and somatic cell count were higher in August. Differences in milk quality indicators from the first and second milking were revealed, and a higher content of almost all studied indicators was established in milk samples taken from the first milking in June and August, in August - with a much smaller difference. Significant correlations were found between milk quality indicators - dry matter ( $r=0.856$ ), fat ( $r=0.728$ ), fat-protein ratio ( $r=0.861$ ) in milk samples taken in June and August from the first, second milking and daily milk yield. In milk milked in August, these relationships. In all samples, a negative relationship was established between the amount of milk and the content of somatic cells.*

*The influence of the lactation period on milk productivity and milk quality indicators of experimental mares was established - the highest daily milk yield, dry matter and fat content in milk were characterized by mares at the lowest lactation periods. The influence of the age of experimental mares on their daily milk yield and milk quality indicators was established, the superiority of mares aged 9-13 years in June ( $r=0.431$ ), and older ones in August ( $r=0.352$ ) was proven in terms of daily milk yield. It was determined that the age of mares affects the duration of their foaling ( $r=0.396$ ). The advantage in daily milk yield of mares with a foaling duration of over 350 days was proven according to the results of control milking in both June and August. In terms of dry matter, fat and fat-protein ratio in milk samples of experimental mares with a foaling duration of over 340 days according to the results of control milking in both June and August, as well as the highest lactose content and freezing point index in samples taken in June and protein - in samples taken in August.*

*It was established that mares that foaled with foals, with a high degree of probability ( $p<0.01$ ) had a higher daily milk yield, as well as milk yields for the first and second milking, a higher dry matter, fat, fat-protein ratio.*

**Keywords:** horses, mares, Novoaleksandrivka heavy-duty breed, milk productivity, milk quality, influencing factors



## **ФАКТОРИ ВПЛИВУ НА ПОКАЗНИКИ ЯКОСТІ МОЛОКА КОБИЛ НОВООЛЕКСАНДРІВСЬКОЇ ВАГОВОЗНОЇ ПОРОДИ**

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*У статті викладено результати досліджень молочної продуктивності і якості молока кобил новоолександрівської вагОВОЗНОЇ породи. Визначено добовий надій кобил та показники якості молока у зразках, відібраних у червні та серпні, доведено, що усі показники значно варіюють. Встановлено, що показники вмісту сухої речовини, білку, лактози і сухого знежиреного залишку молока були вищими у червні, а вміст білку, співвідношення жиру і білку, точка замерзання і кількість соматичних клітин – у серпні. Виявлено відмінності показників якості молока від першого і другого доїння, встановлено вищий вміст майже усіх досліджених показників у зразках молока, відібраного від першого доїння у червні і серпні, у серпні – зі значно меншою різницею. Виявлено значні кореляційні зв'язки між показниками якості молока – сухої речовини ( $r=0,856$ ), жиру ( $r=0,728$ ), співвідношенням жиру і білку ( $r=0,861$ ) у зразках молока відібраних у червні і серпні від першого, другого доїння і добовому надої. У молоці, надоєному у серпні, ці зв'язки. В усіх зразках встановлено негативний зв'язок між кількістю молока і вмістом соматичних клітин.*

*Встановлено вплив терміну лактації на показники молочної продуктивності і якості молока дослідних кобил – найвищим добовим надоєм, вмістом сухої речовини і жиру у молоці характеризувалися кобили на найменших термінах лактації. Встановлено вплив віку дослідних кобил на їх добовий надій і показники якості молока, доведено перевагу за добовим надоєм кобил у віці 9-13 років у червні ( $r=0,431$ ), та старшого віку – у серпні ( $r=0,352$ ). Визначено, що вік кобил впливає на тривалість їх жеребності ( $r=0,396$ ). Доведено перевагу за добовим надоєм кобил з тривалістю жеребності понад 350 діб за результатами контрольного доїння як у червні, так і у серпні. За вмістом сухої речовини, жиру та співвідношення жиру і білка у зразках молока дослідних кобил з тривалістю жеребності понад 340 діб за результатами контрольного доїння як у червні, так і в серпні, а також найвищий вміст лактози і показник точки замерзання у зразках, відібраних у червні та білка – у зразках, відібраних у серпні.*

*Встановлено, що кобили, які ожеребилися жеребчиками, з високим ступенем вірогідності ( $p<0,01$ ) мали вищий добовий надій, а також надої за перше і друге доїння, вищий вміст сухої речовини, жиру, співвідношення жиру і білку.*

**Ключові слова:** коні, кобили, новоолександрівська вагОВОЗНА порода, молочна продуктивність, якість молока, фактори впливу.

Mares' milk as a food product is traditionally produced in Asian countries: North China, Mongolia, Kazakhstan, Bashkortostan, Kyrgyzstan, Uzbekistan, Buryatia, Kalmykia (Pieszka M., 2016; Blanco-Doval A., 2024). In these countries, mares' milk accounts for more than 8% of total milk production (Gall C. F., 2013), and it is traditionally used for the production of fermented milk drinks (ayran, koumiss, etc.). At the same time, the consumption of mares' milk has spread in recent decades in the



countries of the European Union: France, Belgium, Germany, the Netherlands, Norway, Austria, Hungary, Bulgaria. In European countries, the share of mares' milk in total milk production is only 0.1%, which is about 1300 tons.

Horse breeding is well developed, and productive – in particular, in Kazakhstan, where there are 2,862,600 horses (as of June 1, 2018), while it is reported that the annual increase in the livestock is about 100 heads (Naimanov D. K. et al. 2018). The development of productive horse breeding in Kazakhstan is facilitated by the presence of large areas of natural pastures (in Northern Kazakhstan alone there are 1.5 million hectares). Kazakhstan can be an example of the boundless centuries-old love of the people for horses, as well as the traditional significant demand for koumiss and other horse breeding products, which ensures the development of the horse breeding industry.

More than 30 million people in the world regularly consume mare's milk and its processed products (Doreau M., 2011). Mare's milk has high nutritional value and practically does not contain allergenic proteins, unlike cow's milk. This factor makes it possible to use mares' milk in children's and dietary nutrition (Kunying L., 2024). Organoleptically, mares' and women's milk are very similar, but mares' milk is more transparent, whiter and sweeter due to the higher lactose content. The peculiarities of the morphology of the mammary gland of mares determine the low milk productivity of mares, however, for horses of the Mongolian, Bashkir, Yakut, Kyrgyz, Kazakh (with the Kabyn meat type and Adai breed), Kushum, Mughalzhaz, and Kustanai breeds, this feature is of selection (Naymanov D. K. et al., 2018). It is known that the highest amount of milk is produced by mares 30-60 days after foaling, when daily milk production can vary from 12 to 15 liters, which is about 21-25% of the foal's body weight (Morresey, P.R., 2012). However, there are more productive breeds: Mughalzhaz (2200-2500 l of milk per lactation) (Seleuova, 2018), Kushum (2000 l of milk per 3 months of lactation), Jabe (up to 20 l per day) (Naymanov D. K. et al., 2018).

Quite high milk productivity is characteristic of mares of heavy draft breeds, which is due to the greater body weight and intensity of foal development. A unique breed of heavy draft horses, Novoaleksandrivska, has been bred in Ukraine, which is characterized by relatively high milk productivity (2500-3000 kg of milk per lactation). Mares' milk has high nutritional value and practically does not contain allergenic proteins, unlike cow's milk (Reiter, 2023), which determines the relevance of the research. Based on the relevance, the purpose of the study was to determine the quality indicators of milk of mares of the Novoaleksandrivska heavy draft breed.

**Materials and methods of research.** In the conditions of the branch of the Dibrivsky stud farm No. 62 of the State Enterprise "Equestrianism of Ukraine" of the Poltava region, experiments were conducted in 2024 to study the milk productivity and quality of milk of mares of the Novoaleksandrivka heavy draft breed. 16 healthy mares were selected for the experiment, which successfully foaled healthy foals and produced mature milk. The beginning of milking of the mares took place a month after their foaling. Gross milk collection for the production of koumiss was carried out during May-October. Lactation dates were established according to primary documents, in particular, the data of the foaling-mating record, from which the term of fertilization of each mare, the term of foaling, and the sex of the foal were established. At the end of the season, gross milk yield was determined.

Control milkings were carried out using dual-mode milking machines DDU-2 in June and August. Sanitary and hygienic treatment of udders and teats in mares of all groups was carried out using an antiseptic solution. Mares were milked twice in the morning and evening, milking duration – up to 2 minutes. Milking was carried out in a specially equipped milking shop in separate stalls, foals during milking were in close



visibility of mares, which contributed to the milk ejection reflex and nervous balance of mares and foals. Foals on the day of control milking received the necessary amount of milk through artificial drinking, which was not reflected in their condition and live weight gain. After each milking, the amount of milk in the bucket was measured, the total daily yield was determined as the sum of both milkings per day. For statistical calculations, only the indicator of milked milk (excluding milk consumed by foals) was used.

Milk samples were collected in sterile containers with a preservative. When collecting milk samples, the European requirements of Codex Alimentarius, vol. 13 "Methods of analysis and sampling" and DSTU ISO 707:2002<sup>i</sup> Milk and dairy products, instructions for sampling were followed. Samples were collected with a sterile syringe in proportion to the first and second milkings per day, cooled to 10°C and immediately transported to the laboratory in a thermobox. Only milk samples that were visually determined to be whole, clean, and in consistency represented a white liquid with a slight blue tint, without sediment and clots, with a delicate milky odor were analyzed.

The physical and technological properties of milk were studied according to DSTU 8009:2015. Biochemical indicators of milk were determined in a certified laboratory for assessing the quality of livestock products. The chemical composition of milk (fat, protein, lactose, dry matter, protein, dry non-fat milk residue (SNFRM) content in %) and freezing point (°C) were determined according to ISO 9001:2000 instrumentally on the device "Bentley-150" (certificate IDA 0001461-1 dated 16.12.2004). The content of somatic cells (thousands/cm<sup>3</sup>) was determined according to ISO 9001:2000 instrumentally on the device "Somacount-150" (certificate IDA 0001461-1 dated 16.12.2004).

Daily milk yields and milk quality were determined in June and August, including for the first and second milking per day depending on the lactation period, age of mares, duration of pregnancy, sex of foals.

The relationships between the studied indicators were determined by calculating the correlation coefficient ( $r$ ).

All experimental studies were conducted in accordance with modern methodological approaches, requirements and standards (DSTU ISO/IEC 17025:2019, 2021), Directive 2010/63/EU (2010), the Procedure for conducting animal experiments in scientific research institutions (Law of Ukraine No. 249, 2012) and in accordance with the Regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985).

Research results. Analysis of milk productivity based on the results of control milkings of experimental mares showed that the daily milk yield with double milking in June was on average higher by the milking group than in August by 0.381 l (Table 1). at the same time, in both June and August, more milk was obtained from the second milking (by 0.587 l in June and 0.300 l in August). All the studied indicators of daily milk yield are characterized by high coefficients of variation.

A difference was established between the quality indicators of the milk of the studied mares in the samples taken in June and August. Thus, the indicators of dry matter, protein, lactose and SPPW were higher in June, and the protein content (and, accordingly, the ratio of fat to protein), freezing point and the number of somatic cells were higher in August. In general, almost no somatic cells were detected in the milk of mares, which is explained by its high antibacterial properties.

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<sup>ii</sup> DSTU ISO 707:2002 Milk and dairy products. Guidelines for sampling. (2003). Retrieved from [https://online.budstandart.com/ua/catalog/doc-page?id\\_doc=67272](https://online.budstandart.com/ua/catalog/doc-page?id_doc=67272)



Table 1

**Indicators of milk productivity and quality of daily milk yield of mares depending on the month of milking**

Milk quality indicators	June				August			
	M±m	Cv, %	min	max	M±m	Cv, %	min	max
Daily milk yield, l	2,78±0,24	34,5	1,6	5,9	2,46±0,21	33,2	1,1	4,9
1st milking	1,10±0,08	28,6	0,5	1,9	1,08±0,13	47,1	0,6	2,7
2nd milking	1,68±0,18	42,8	1,1	4,0	1,38±0,10	28,4	0,4	2,2
Content, %: dry matter	9,39±0,04	1,44	9,22	9,58	8,97±0,08	3,78	8,63	9,15
fat	0,12±0,03	14,3	0,03	0,37	0,14±0,05	17,6	0,01	0,71
protein	1,65±0,02	3,72	1,53	1,75	1,42±0,01	4,19	1,30	1,55
lactose	6,87±0,03	1,39	6,74	7,03	6,72±0,09	5,41	5,38	6,97
Dry non-fat milk residue, %	9,27±0,02	0,92	9,15	9,58	8,86±0,09	4,38	7,45	9,15
Fat/protein ratio	0,07±0,02	14,5	0,02	0,15	0,08±0,03	16,6	0,01	0,49
Freezing point, °C	0,66±0,01	1,06	0,649	0,669	0,64±0,01	4,30	0,542	0,663
Somatic cell count, thousand/cm <sup>3</sup>	0,39±0,27	24,8	2	3	2,35±1,50	26,3	3	255

The milk quality indicators of the studied mares in samples from the first and second milking per day are given in Table 2.

Table 2

**Milk quality indicators of the studied mares for the 1st and 2nd milking**

Milk quality indicators	June		August		Reference values
	1 milking	2 milking	1 milking	2 milking	
Content, %: dry matter	9,447±0,087	9,308±0,023	8,928±0,089	8,918±0,078	
fat	0,208±0,079	0,076±0,010	0,105±0,030	0,165±0,060	1,6-1,9
protein	1,613±0,014	1,649±0,020	1,408±0,016	1,345±0,014	2,1-2,2
lactose	6,877±0,029	6,833±0,025	6,701±0,091	6,697±0,085	5,8-6,4
Ratio: fat/protein	0,128±0,049	0,045±0,006	0,074±0,021	0,121±0,044	
Dry non-fat milk residue, %	9,241±0,028	9,233±0,021	8,824±0,098	8,752±0,090	7,0-10,0
Freezing point, °C	0,659±0,002	0,655±0,002	0,642±0,007	0,642±0,006	
Number of somatic cells, thousand/cm <sup>3</sup>	0,308±0,208	0,438±0,302	1,706±0,903	3,353±2,356	

Note: \* $p < 0.05$ ; \*\* $p > 0.1$

It was found that the indicators of daily milk yield and milk quality of experimental mares in samples taken in June and August are significantly correlated with each other (Table 4). The highest probable relationships are between the dry matter content in daily milk yield, the protein and dry matter content in samples obtained from the first milking, the amount of milk, the dry matter, fat and protein content in samples from the second milking.



Table 3

**The relationship between indicators of milk productivity and milk quality of mares in milk samples obtained in June and August (r)**

Milk quality indicators	Correlation coefficient ®		
	1 milking	2 milking	Daily milking
Amount of milk, l	0,769**	0,502*	0,687**
Content, %: dry matter	0,321*	0,528*	0,412*
fat	0,569**	0,708*	0,690**
protein	0,788*	0,342*	0,578*
lactose	0,303**	0,265**	0,281**
Dry non-fat milk residue, %	0,089*	0,067*	0,066*
Fat/protein ratio	0,557**	0,722**	0,704**
Freezing point, °C	0,299***	0,265***	0,280***
Somatic cell count, thousand/cm <sup>3</sup>	-0,096**	-0,126**	-0,121**

Most milk quality indicators are significantly correlated with each other, as proven by the calculations of correlation coefficients (Table 4). The most positively correlated indicators in milk samples taken in June are dry matter and fat content, dry matter and ratio (fat/protein), dry matter and dry non-fat milk residue, fat content and ratio (fat/protein), lactose content and freezing point index, lactose content and dry non-fat milk residue. The somatic cell content was negatively correlated with all studied milk quality indicators.

Table 4

**Correlations between milk quality indicators of mares of the Novoaleksandrovsk heavy-duty breed**

Indicators	Dry matter	Fat	Protein	Fat/protein ratio	Lactose	dry non-fat milk residue, %	Freezing point, °C	Somatic cell content
June								
Dry matter		0,796	0,171	0,808	0,435	0,695	0,588	-0,493
Fat	-0,008		0,258	0,998	-0,067	0,120	0,098	-0,220
Protein	-0,311	0,355		0,221	-0,628	0,003	-0,532	-0,110
Fat/protein ratio	0,032	0,998	0,326		-0,028	0,141	0,136	-0,228
Lactose	0,860	-0,502	-0,548	-0,465		0,776	0,984	-0,329
dry non-fat milk residue, %	0,877	-0,486	-0,435	-0,450	0,992		0,833	-0,537
Freezing point, °C	0,893	-0,444	-0,526	-0,405	0,998	0,992		-0,393
Somatic cell content	-0,830	0,459	0,541	0,422	-0,996	-0,945	-0,954	
August								



In milk samples taken in August, the most positively correlated were the dry matter and lactose content, dry matter and dry non-fat milk residue, dry matter and freezing point index, fat content and ratio (fat/protein). The lactose content index was also highly and positively correlated with the dry non-fat milk residue content and freezing point index, negatively - with the fat, protein content and their ratio.

According to the technology adopted at the Dibrivsky stud farm, mating of mares, and accordingly their foaling, are seasonal, and therefore, the birth of experimental mares occurred in February-March. In this regard, the lactation period index in June fluctuated within the limits: 62-141 days; in August - 114-193 days. Accordingly, mares, based on the results of control milking in June, were distributed according to gradations: 62-90 (3 months), 91-120 (4 months), 121-141 days (5 months); in August: 114-150 (5 months), 151-180 (6 months), 181-193 (7 months).

Considering that the duration of lactation of the share of mares, whose control milking was carried out in June and August, fell on the 5th month, the gradations were adjusted in the summary according to the terms of lactation. In accordance with the established gradations, the value of daily milk yields was determined (Table 5).

Table 5

**Indicators of daily milk yields of mares of the Novooleksandrivka heavy-duty breed depending on the terms of lactation**

Lactation period, days	n	milk yield,kg		
		1 milking	2 milking	Daily milking
June (06/19/2024)				
62-90	2	1,55±0,35	2,65±1,70	4,20±1,70
91-120	6	0,90±0,10	1,63±0,21	2,47±0,24
121-141	8	1,13±0,07	1,48±0,10	2,66±0,17
On average:	16	1,097±0,079	1,684±0,180	2,781±0,240
August (08/10/2024)				
114-150	2	2,15±0,55	1,80±0,35	3,95±0,86
151-180	10	0,82±0,05	1,30±0,13	2,15±0,16
181-193	4	1,11±0,13	1,36±0,14	2,50±0,26
On average:	16	1,078±0,127	1,378±0,098	2,463±0,205

The highest daily milk yield was characterized by mares that began to lactate later. Thus, according to the indicators of control milking in June, the highest milk yield was distinguished by mares that were at the lactation stage of 62-90 days (4.20±1.70 kg, p≤0.01), and in August - at the lactation stage of 114-150 days (3.27±0.86, p≤0.01). The level of the correlation coefficient between daily milk yields and lactation periods was low, negative: lower in June (r=-0.218) and higher in August (r=-0.374).

Correlation analysis proved that the own daily milk yield of the same mares in June and August was significantly interconnected (r=0.695). At the same time, the milk yield from the first milking correlated with a much greater strength (r=0.771) than from the second (r=0.398).

The results of studies on milk quality in connection with the term of lactation are given in Table 6.



Table 6

**Milk quality indicators of experimental mares of the Novoaleksandrivka heavy-duty breed depending on the term of lactation**

Milk quality indicators	Lactation dates			
	June			
n	62-90	91-120	121-141	r
	2	6	8	
Content, %: dry matter	9,435±0,195	9,377±0,051	9,338±0,038	-0,006*
fat	0,230±0,140	0,107±0,034	0,066±0,014	-0,331*
protein	1,675±0,015	1,682±0,023	1,623±0,023	-0,352*
lactose	6,790±0,030	6,838±0,051	6,894±0,023	0,497*
Ratio: fat/protein	0,135±0,085	0,062±0,020	0,041±0,008	-0,297*
Dry non-fat milk residue, %	9,205±0,055	9,273±0,036	9,270±0,027	0,369*
Freezing point, °C	0,653±0,004	0,656±0,004	0,659±0,002	0,457*
Number of somatic cells, thousand/cm <sup>3</sup>	3	0	2	-0,384*
	August			
n	114-150	151-180	181-193	r
	2	10	4	
Content, %: dry matter	9,305±0,305	8,891±0,123	8,995±0,013	-0,206*
fat	0,375±0,335	0,084±0,041	0,048±0,016	-0,251*
protein	1,430±0,010	1,440±0,017	1,390±0,041	-0,153*
lactose	6,775±0,035	6,663±0,149	6,830±0,031	-0,035*
Ratio: fat/protein	0,260±0,230	0,056±0,026	0,035±0,012	-0,259*
Dry non-fat milk residue, %	8,930±0,030	8,813±0,161	8,948±0,020	-0,066*
Freezing point, °C	0,651±0,001	0,639±0,011	0,651±0,002	-0,059*
Number of somatic cells, thousand/cm <sup>3</sup>	0	26,7±25,4	3	0,101*

Note: \* $p < 0.05$

It was found that the highest dry matter content was in milk samples taken in June from mares at the earliest stages of lactation (62-90 days). Accordingly, the fat content in milk was higher in samples taken from mares at the early stages of lactation, and the lactose content, on the contrary, increased in samples from mares at the later stages of lactation. The content of protein, dry non-fat milk residue and freezing point index did not change almost at all.

In August, the highest dry matter and fat content, as well as the fat/protein ratio in milk samples from mares were also at the earliest stages of lactation (114-150 days). The protein content in milk was significantly higher in mares at 151-180 days of lactation, and the lactose and SZZM content was at 191-193 days of lactation. The freezing point index was the same in the milk of mares at the earliest and latest stages of lactation (0.651) and slightly lower at 151-180 days of lactation.

The determination of correlations established a high positive effect of the duration of lactation on the content of lactose in milk ( $r=0.497$ ), dry non-fat milk residue ( $r=0.369$ )



and freezing point ( $r=0.457$ ) in samples taken in June, the remaining relationships are negative. In milk samples taken in August, the duration of lactation is most (negatively) correlated with the fat/protein ratio, fat and dry matter content in milk.

The influence of mares' age on daily milk yield is given in Table 7, the data of which prove the superiority of daily milk yield of mares aged 9-13 years in June, and older ones (14 years and older) in August. The level of the correlation coefficient between daily milk yield and mares' age was positive, of medium strength, almost the same in June ( $r=0.431$ ) and August ( $r=0.352$ ).

Table 7

**Daily milk yield of mares of the Novooleksandrivka heavy-duty breed depending on the age of the mares**

Age of mares, days	n	Milk yield,kg		
		1 milking	2 milking	Daily milking
June				
4-8 years	6	0,883±0,087	1,400±0,100	2,283±0,180
9-13 years	6	1,192±0,156	2,033±0,445	3,225±0,564
14 years and older	4	1,275±0,075	1,588±0,156	2,863±0,221
Average:	16	1,097±0,079	1,684±0,180	2,781±0,240
August				
4-8 years	6	0,842±0,052	1,425±0,070	2,267±0,087
9-13 years	6	1,167±0,313	1,217±0,240	2,400±0,531
14 years and older	4	1,300±0,147	1,550±0,106	2,850±0,205
Average:	16	1,078±0,127	1,378±0,098	2,463±0,205

The influence of mares' age on milk quality indicators is given in Table 8, the data of which prove the superiority of dry matter content in milk of mares aged 10-13 years in June, and older (14 years and older) in August. In groups of mares of the same age category, the fat content in milk was higher. The protein content was highest in milk of mares aged 4-8 years in both June and August, and the lactose content in the oldest mares. The freezing point indicator differed little in different groups of mares, but was higher in older mares (14 years and older). The correlation indicators between the age of mares and the quality indicators of their milk in June and August were low.

The age of mares affects the duration of their foaling, as evidenced by a positive probable correlation coefficient of medium degree ( $r=0.396$ ).



Table 8

**Milk quality indicators of experimental mares of the Novoaleksandrovsk heavy-duty breed depending on the age of the mares**

Milk quality indicators	Age of mares, years			
	June			
n	4-8	9-13	14 and older	r
	6	6	4	
Content, %: dry matter	9,360±0,060	9,425±0,063	9,348±0,073	0,139**
fat	0,063±0,07	0,168±0,022	0,075±0,022	0,153*
protein	1,677±0,038	1,648±0,022	1,638±0,040	-0,005*
lactose	6,870±0,083	6,857±0,033	6,885±0,052	0,046*
Ratio: fat/protein	0,037±0,003	0,100±0,030	0,045±0,013	0,161*
Dry non-fat milk residue, %	9,303±0,058	9,253±0,024	9,275±0,061	0,049**
Freezing point, °C	0,657±0,006	0,657±0,002	0,659±0,004	0,089*
Number of somatic cells, thousand/cm <sup>3</sup>	0	2	3	0,244*
August				
n	4-8	9-13	14 and older	r
	6	6	4	
Content, %: dry matter	8,970±0,082	8,947±0,233	9,000±0,012	0,075***
fat	0,043±0,016	0,227±0,116	0,040±0,011	0,185*
protein	1,442±0,017	1,410±0,035	1,428±0,021	-0,057*
lactose	6,770±0,070	6,608±0,248	6,808±0,012	-0,017*
Ratio: fat/protein	0,030±0,010	0,155±0,078	0,028±0,009	0,186
Dry non-fat milk residue, %	8,938±0,088	8,718±0,257	8,960±0,008	-0,033***
Freezing point, °C	0,647±0,006	0,636±0,019	0,650±0,001	-0,007*
Number of somatic cells, thousand/cm <sup>3</sup>	2,000±1,291	43,000±42,403	0	0,041***

Note: \* $p < 0.05$ ; \*\* $0.1 > p > 0.05$ ; \*\*\* $p > 0.1$

The duration of foaling of experimental mares was on average  $335.6 \pm 2.85$  days (lim=320–358) ( $Cv=3.176$ ). The influence of the duration of pregnancy of mares on the indicators of daily milk yield is given in Table 9. The advantage in daily milk yield of mares with a duration of foaling over 350 days was proven according to the results of control milking in both June and August.

Daily milk yield significantly and positively correlated with high probability ( $p < 0.01$ ) with the duration of pregnancy of experimental mares in June ( $r=0.759$ ), for milk yields in August this correlation was significantly lower ( $r=0.270$ ). The duration of pregnancy was positively and significantly correlated with the age of mares in the average degree ( $r=0.429$ ). It was established that foals were carried by mares for 9.1 days longer than fillies ( $p < 0.05$ ).



Table 9

**Indicators of daily milk yield of mares of the Novooleksandrivka heavy draft breed depending on the duration of pregnancy**

Pregnancy duration, days	n	Milk yield,kg		
		1 milking	2 milking	Daily milking
June (06/19/2024)				
320-330	4	0,867±0,186	1,233±0,088	2,100±0,265
331-340	7	1,000±0,049	1,429±0,071	2,500±0,076
341 and more	5	1,213±0,142	1,988±0,218	3,200±0,117
On average:	16	1,097±0,079	1,684±0,180	2,781±0,240
August (08/10/2024)				
320-330	4	1,015±0,009	1,283±0,017	2,317±0,044
331-340	7	0,857±0,047	1,400±0,072	2,257±0,083
341-350	5	0,988±0,200	1,200±0,331	2,188±0,503
On average:	16	1,078±0,127	1,378±0,098	2,463±0,205

The influence of the duration of pregnancy of mares on milk quality indicators is given in Table 10.

Table 10

**Milk quality indicators of experimental mares of the Novooleksandrivka heavy-duty breed depending on the duration of their pregnancy**

Milk quality indicators	Duration of pregnancy, years			
	June			
n	320-330	331-340	341 and more	r
	4	7	5	
Content, %: dry matter	9,267±0,015	9,338±0,060	9,628±0,204	0,369*
fat	0,067±0,003	0,100±0,033	0,298±0,187	0,242*
protein	1,583±0,032	1,625±0,025	1,620±0,032	0,206*
lactose	6,873±0,039	6,865±0,069	6,950±0,039	0,300*
Ratio: fat/protein	0,043±0,003	0,063±0,021	0,183±0,117	0,230*
Dry non-fat milk residue, %	9,203±0,017	9,240±0,060	9,330±0,028	0,497*
Freezing point, °C	0,657±0,003	0,657±0,005	0,666±0,004	0,364*
Number of somatic cells, thousand/cm <sup>3</sup>	0	2	2	0,024*
August				
n	320-330	331-340	341 and more	r
	4	7	5	
Content, %: dry matter	8,930±0,056	9,001±0,064	8,773±0,298	-0,080*
fat	0,037±0,022	0,046±0,013	0,148±0,098	0,220*
protein	1,393±0,035	1,426±0,024	1,450±0,038	0,203*
lactose	6,787±0,024	6,803±0,062	6,493±0,373	-0,147*
Ratio: fat/protein	0,027±0,017	0,033±0,008	0,098±0,061	0,221*
Dry non-fat milk residue, %	8,903±0,041	8,959±0,073	8,625±0,395	-0,127*
Freezing point, °C	0,648±0,002	0,650±0,005	0,626±0,028	-0,139*
Number of somatic cells, thousand/cm <sup>3</sup>	0	2,143±1,100	225	0,144*

Note: \* $p < 0.05$ ; \*\* $0.1 > p > 0.05$ ; \*\*\* $p > 0.1$



The superiority in dry matter, fat and fat-protein ratio in milk samples of experimental mares with a gestation period of more than 340 days was proven according to the results of control milking in both June and August. This gradation of mares also had the highest lactose content and freezing point index in samples taken in June and protein in samples taken in August. In mares whose gestation period was 331-340 days, the highest protein content and dry non-fat milk residue in milk samples obtained in June and freezing point index in samples obtained in August were found.

It was found that mares that foaled with foals had a high degree of probability ( $p < 0.01$ ) higher daily milk yield, as well as milk yields for the first and second milking (Table 11). This may be due to the fact that foals are born larger and require a larger amount of milk, which is controlled by the hormonal background of the mare (Aoki T., 2013; Robles M., 2018). Milk yield in June in mares that gave birth to foals was significantly higher (by 0.511 l,  $p < 0.01$ ) than in August. In mares that gave birth to fillies, the milk yield level remained unchanged with minor fluctuations in the indicator between the first and second milking.

Table 11

**Daily milk yield indicators of mares of the Novoaleksandrovsk heavy-duty breed depending on the month of milking and the sex of the foals**

The sex of the foal	n	Milk yield, kg		
		1 milking	2 milking	Daily milking
June (19.06.2024)				
Stallions	10	1,094±0,078	1,628±0,148	2,722±0,168
fillies	6	0,910±0,046	1,410±0,105	2,320±0,085
August (10.08.2024)				
Stallions	10	0,933±0,091	1,267±0,137	2,211±0,210
fillies	6	0,920±0,107	1,400±0,114	2,320±0,208

The milk quality indicators of the studied mares depending on the sex of their foals are given in Table 12.

Table 12

**Quality indicators of the daily milk yield of mares depending on the sex of their foals**

Milk quality indicators	June		August	
	stallions	filly	stallions	filly
Content, %: dry matter	9,440±0,043	9,265±0,021	8,992±0,136	8,930±0,071
fat	0,140±0,036	0,060±0,013	0,156±0,073	0,036±0,012
protein	1,644±0,021	1,668±0,031	1,435±0,018	1,412±0,028
lactose	6,900±0,032	6,798±0,025	6,688±0,147	6,770±0,070
Dry non-fat milk residue, %	9,300±0,028	9,208±0,023	8,834±0,156	8,907±0,081
Fat/protein ratio	0,083±0,021	0,035±0,006	0,106±0,049	0,027±0,008
Freezing point, °C	0,660±0,002	0,652±0,002	0,642±0,011	0,647±0,005
Somatic cell count, thousand/cm <sup>3</sup>	0	1,250±0,750	26,00±25,45	1,667±1,174



It was found that in milk samples (selected both in June and August) from mares that gave birth to foals, the content of dry matter was higher (by 0.448 and 0.335 ml, respectively), fat (by 0.08 and 0.12%, respectively), and the ratio of fat to protein. The protein content was higher in samples taken in June from mares that gave birth to fillies, and in August from those that gave birth to foals, however, the difference is small and insignificant. The lactose content, on the contrary, was higher in samples taken in June from mares that gave birth to foals, and in August from mares, the same trend was observed in terms of dry non-fat milk residue and freezing point. A small number of somatic cells was detected in samples taken in June from two mares that gave birth to fillies, in August from four mares that gave birth to foals of both sexes.

**Discussion.** The results of our studies have proven the influence of the month of lactation on the quantity and quality of milk of mares of the Novoaleksandrivka heavy draft breed, with more milk being obtained from the second milking. All milk samples showed low fat and protein content and high lactose content, which is also confirmed by other researchers (Malacarne et al., 2002; Ciesla et al., 2009; Goncharenko, 2012). The dry matter, protein, lactose and SMP content were higher in June, and the protein content (and, accordingly, the fat-protein ratio), freezing point and the number of somatic cells (detected only in a few mares in insignificant quantities) were higher in August. In milk examined in both June and August, a higher content of almost all milk quality indicators was found in samples from the first milking.

High probable positive correlations were established between daily milk yield and dry matter content, fat, and fat-protein ratio. In milk milked in August, these relationships were weaker. In all samples, a negative relationship was established between the amount of milk and the content of somatic cells.

The effect of lactation duration on daily milk yield and milk quality indicators of experimental mares was proven. Thus, the highest daily milk yield was recorded in mares that began lactating later and were in June at 62-90 days, and in August at 114-150 days of lactation. Morresey, (2012) also emphasizes that mares produce the highest amount of milk in the first months of lactation. The highest dry matter, fat and fat/protein ratio were found in milk samples collected in June from mares at the earliest stages of lactation (62-90 days). The protein, dry non-fat milk residue and freezing point values remained almost unchanged. The number of somatic cells was insignificant, and they were not detected at all in milk samples from most mares. In August, the highest dry matter and fat content, as well as the fat/protein ratio, were found in milk samples from mares also at the earliest stages of lactation (114-150 days). The protein content in milk was significantly higher in mares at 151-180 days of lactation, and the lactose content ( $r=0.497$ ) and SZZM ( $r=0.369$ ) at 191-193 days of lactation. The freezing point index was the same in the milk of mares at the earliest and latest stages of lactation (0.651) and slightly lower at 151-180 days of lactation ( $r=0.457$ ).

The influence of the age of mares on the daily milk yield indicators was proven, which is confirmed by the superiority in daily milk yield of mares aged 9-13 years in June ( $r=0.431$ ), and older (14 years and older) ( $r=0.352$ ) in August. In terms of dry matter content in milk, mares aged 10-13 years prevailed in June, and older (14 years and older) in August. In groups of mares of the same age category, the fat content in milk was higher, the protein content was highest in the milk of mares aged 4-8 years in both June and August, and the lactose content was highest in the oldest mares. The freezing point index differed little in different groups of mares, but was higher in older mares (14 years and older).

It was found that the duration of foaling in experimental mares was on average  $335.6 \pm 2.85$  days (lim=320–358) ( $Cv=3.176$ ) and was associated with the age of the



experimental mares ( $r=0.396$ ) and the sex of the fetus - mares carried foals for 9.1 days longer than fillies ( $p<0.05$ ). Aoki T. (2013) provides similar data from the study of heavy-duty mares, the duration of pregnancy of which was on average  $334.9\pm 8.3$  days, and also proves that the sex of the foal is one of the main factors affecting the duration of pregnancy in heavy-duty mares.

The influence of mares' age on daily milk yield indicators was proven, which is confirmed by the superiority of mares aged 9-13 years in June ( $r=0.431$ ), and older mares (14 years and older) ( $r=0.352$ ) in August. In terms of dry matter content in milk, mares aged 10-13 years in June, and older mares (14 years and older) prevailed. In groups of mares of the same age category, the fat content in milk was higher, the protein content was highest in the milk of mares aged 4-8 years in both June and August, and the lactose content was highest in the oldest mares. The freezing point indicator differed little in different groups of mares, but was higher in older mares (14 years and older).

It was found that the duration of foaling in experimental mares was on average  $335.6\pm 2.85$  days (lim=320–358) ( $Cv=3.176$ ) and was related to the age of experimental mares ( $r=0.396$ ) and the sex of the fetus - mares carried foals for 9.1 days longer than mares ( $p<0.05$ ). Aoki T. (2013) provides similar data from the study of heavy-duty mares, the duration of pregnancy of which was on average  $334.9\pm 8.3$  days, and also proves that the sex of the foal is one of the main factors affecting the duration of pregnancy in heavy-duty mares. The advantage in daily milk yield of mares with a duration of foaling over 350 days was proven according to the results of control milking in both June and August. Similar results were reached by Auclair-Ronzaud et al. (2022), who proved that older mares had higher milk yield than young ones. In terms of dry matter, fat, and fat-to-protein ratio in milk samples, experimental mares with a gestation period of over 340 days prevailed according to the results of control milking in both June and August. This gradation of mares also had the highest lactose content and freezing point index in samples taken in June and protein in samples taken in August. In mares whose gestation period was 331-340 days, the highest protein and dry non-fat milk residue content in milk samples obtained in June and the freezing point index in samples obtained in August were found. It was found that mares that foaled with foals had a high degree of probability ( $p<0.01$ ) higher daily milk yield. Aoki T. (2013) and Robles M. (2018) attribute this fact to the fact that foals require a larger amount of milk, which is controlled by the hormonal background of the mare. Milk yield in June in mares that gave birth to foals was significantly higher (by 0.511 l,  $p<0.01$ ) than in August. In mares that gave birth to fillies, the milk yield level remained unchanged with minor fluctuations in the indicator between the first and second milking. At the same time, the milk of mares that gave birth to foals, both in June and August, had a higher content of dry matter (by 0.448 and 0.335 ml, respectively), fat (by 0.08 and 0.12 %, respectively), and the ratio of fat to protein. The lactose content, on the contrary, was higher in samples taken in June from mares that gave birth to foals, and in August - mares, the same trend in terms of dry non-fat milk residue and freezing point. A small number of somatic cells was detected in samples taken in June from two mares that gave birth to fillies, in August - from four mares that gave birth to foals of both sexes.

#### **Conclusions:**

1. It was established that the factors of month, milking number, lactation period, age of mares of the Novoaleksandrivka heavy draft breed, duration of their pregnancy and sex of foals to varying degrees affect the daily milk yield and indicators of its quality.
2. Daily milk yield was higher in June than in August, while in both June and August more milk was obtained from the second milking (by 0.587 l in June and 0.300 l in August). In the milk samples of mares taken in June, a higher content of dry matter,



protein, lactose and SPPW was observed, and the protein content, fat-to-protein ratio, freezing point and the number of somatic cells were higher in the samples taken in August. Almost no somatic cells were detected in the milk of the studied mares, which is explained by its high antibacterial properties. In the milk of the experimental mares taken in both June and August, a higher content of almost all the studied indicators in the milk samples from the first milking was found.

3. A high level of correlation was established between the daily milk yield of milk collected in June and the content of dry matter ( $r=0.856$ ), fat ( $r=0.728$ ), and the ratio of fat to protein ( $r=0.861$ ). In milk collected in August, these correlations were weaker (0.634, 0.461, and 0.489, respectively). In all samples, a negative correlation was established between the amount of milk and the content of somatic cells.

4. It was determined that foaling (birth) of mares is seasonal, and therefore, births of experimental mares occurred in February-March, the lactation period indicator in June fluctuated within the limits: 62-141 days; in August - 114-193 days. The highest daily milk yield was characterized by mares that began to lactate later. By determining the correlation relationships, a high positive effect of the duration of lactation on the content of lactose in milk ( $r = 0.497$ ), dry non-fat milk residue ( $r = 0.369$ ) and freezing point ( $r = 0.457$ ) in samples taken in June was established, the remaining relationships are negative. In milk samples collected in August, the duration of lactation is most (negatively) correlated with the fat/protein ratio, fat and dry matter content in milk.

5. The superiority of mares aged 9-13 years in terms of daily milk yield and dry matter and fat content in milk samples collected in June, and of older mares (14 years and older) in terms of daily milk yield and freezing point in milk samples collected in August, has been proven. The level of the correlation coefficient between daily milk yields and the age of mares was positive, of medium strength, almost the same in June ( $r=0.431$ ) and August ( $r=0.352$ ).

6. It was established that the duration of foaling (pregnancy) of experimental mares on average was  $335.6 \pm 2.85$  days (lim=320–358). Daily milk yield was significantly and positively correlated with high probability ( $p<0.01$ ) with the duration of pregnancy of experimental mares in June ( $r=0.759$ ), for milk yields in August this correlation was significantly lower ( $r=0.270$ ). The duration of pregnancy was positively and significantly correlated with the age of mares in the average degree ( $r=0.429$ ), while mares carried foals for 9.1 days longer than mares ( $p<0.05$ ). The superiority in dry matter content, fat, fat-to-protein ratio, lactose and freezing point index in milk samples of experimental mares with a duration of foaling over 340 days was proven according to the results of control milking in both June and August. In mares whose gestation period was 331-340 days, the highest protein and dry non-fat milk residue content in milk samples obtained in June and the freezing point index in samples obtained in August were found.

7. It was found that mares that foaled with foals had a high degree of probability ( $p<0.01$ ) higher daily milk yield, as well as milk yields for the first and second milking, their daily milk yield in June was significantly higher (by 0.511 l,  $p<0.01$ ) than in August. In mares that gave birth to fillies, the level of milk yield remained unchanged with minor fluctuations in the indicator between the first and second milking. In milk samples (taken both in June and August) from mares that gave birth to foals, the dry matter content, fat (by 0.08 and 0.12%, respectively), and the fat-to-protein ratio were higher. The protein content in all sections differed slightly and insignificantly. The lactose content, on the contrary, was higher in samples taken in June from mares that gave birth to foals, and in August from mares, the same trend was observed in terms of dry non-fat milk residue and freezing point. A small number of somatic cells was detected in samples taken in June



from two mares that gave birth to fillies, and in August from four mares that gave birth to foals of both sexes.

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## **PRODUCTIVITY AND METABOLISM OF SUBSTANCES IN THE BODY OF BULLS BY OPTIMIZING THEIR PROTEIN NUTRITION**

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*The issue of optimizing the protein nutrition of bulls by keeping them in the territory belonging to the III zone of radioactive contamination due to the Chernobyl accident is considered. Experimental studies on young cattle of the Ukrainian black-and-white dairy breed were conducted in the conditions of the physiological yard of the Institute of Agriculture of the Polissya NAAS. For the experiment, 2 groups of experimental animals were formed: Group I (control) - protein deficiency in the diet was provided by feeding 1.02 kg of pea shoots (*Pisum sativum* L.), and in Group II (experimental) - the same amount of field peas (*Pisum sativum*, var *arvense*). Since different types and amounts of feed were used for feeding the bulls, optimization of the nutritional value of the feed rations was carried out monthly. The experimental animals were kept in the same room according to the adopted technology - in stalls on a leash. The feeding and watering regime, microclimate parameters in both groups were the same.*

*Optimization of protein nutrition of fattening bulls due to various high-protein feeds has a positive effect on productivity (875-1023 g) and feed conversion (103.0-120.4 MJ), does not have a negative effect on the slaughter qualities of animals. At the same time, the use of field peas compared to peas to ensure the normalized need of young cattle in digestible protein contributed to an increase in the average daily gain of live weight of animals by 16.9% ( $P>0.95$ ) while reducing the expenditure of metabolic energy per unit of gain by 14.5%. The introduction of field peas into the diet of fattening bulls compared to the use of peas contributed to better digestion of dry matter by 0.2% abs., crude protein - 3.9 ( $P>0.95$ ) and fiber - by 0.9% abs. Experimental animals of group II digested crude fat (by 1.4% abs.) and BER (by 0.3% abs.) somewhat worse than their peers of group I. At the same time, the Nitrogen balance in the body was better in the experimental group bulls compared to the control by 2.9%.*

**Keywords:** *bulls, protein nutrition, peas, field peas, productivity, digestibility, Nitrogen balance.*



## ПРОДУКТИВНІСТЬ І ОБМІН РЕЧОВИН В ОРГАНІЗМІ БУГАЙЦІВ ЗА ОПТИМІЗАЦІЇ ЇХ ПРОТЕЇНОВОГО ЖИВЛЕННЯ

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*Розглянуто питання щодо оптимізації протеїнового живлення бугайців за їх утримання на території, яка належить до III зони радіоактивного забруднення внаслідок аварії на ЧАЕС. Експериментальні дослідження на молодняку великої рогатої худоби української чорно-рябої молочної породи проводили в умовах фізіологічного двору Інституту сільського господарства Полісся НААН. Для проведення дослідів сформовано 2 групи піддослідних тварин: I група (контрольна) – дефіцит протеїну в раціоні забезпечували за рахунок згодовування 1,02 кг дерті гороху (*Pisum sativum* L.), а в II (дослідній) групі - такої ж кількості пелюшки (польовий горох) (*Pisum sativum*, var *arvense*). Оскільки за годівлі бугайців використовували різні види та кількість кормових засобів, оптимізація поживної цінності кормових раціонів проводилися щомісяця. Піддослідних тварин утримували в одному приміщенні згідно з прийнятою технологією – у стійлах на прив'язі. Режим годівлі та напування, параметри мікроклімату в обох групах були однаковими.*

*Оптимізація протеїнового живлення бугайців на відгодівлі за рахунок різних високобілкових кормів позитивно позначається на показниках продуктивності (875-1023 г) та конверсії корму (103,0-120,4 МДж), не має негативного впливу на забійні якості тварин. Водночас використання пелюшки порівняно з горохом для забезпечення нормованої потреби молодняку великої рогатої худоби у перетравному протеїні сприяло підвищенню середньодобових приростів живої маси тварин на 16,9 % ( $P>0,95$ ) за зниження витрат обмінної енергії на одиницю приросту на 14,5 %. Введення пелюшки до раціону бугайців на відгодівлі порівняно з використанням гороху сприяло кращому перетравленню сухої речовини на 0,2 % абс., сирих протеїну – 3,9 ( $P>0,95$ ) і клітковини – на 0,9 % абс. Деяко гірше дослідні тварини II групи відносно ровесників I групи перетравлювали сирий жир (на 1,4 % абс.) та БЕР (на 0,3 % абс.). При цьому баланс Нітрогену в організмі був кращим у бугайців дослідної групи порівняно з контролем на 2,9 %.*

**Ключові слова:** бугайці, протеїнове живлення, горох, пелюшка, продуктивність, перетравність, баланс Нітрогену.

**Introduction.** The problem of feed protein was and remains the main one in organizing full-fledged feeding of farm animals. The deficit of digestible protein in the diets of ruminants is 15-30%, while the production of livestock products decreases by 20-35%, and the cost and consumption of feed increase by 1.5 times (Razumovskyi N.P., Bogdanovych D.M., 2019; Huuskonen A., Joki-Tokola E., 2010). Under such conditions, the search for opportunities to increase the level of protein supply of animals through the use of protein-rich feeds acquires particular importance and is relevant in industrial animal husbandry (Kandyba V. M. et al., 2015; Osmane B. et al., 2017).

According to foreign authors (David L.S. et al., 2024; Volpelli L.A. et al., 2012), grain legumes are a complete source of protein, amino acids and energy, and they can be used as a replacement for soybean meal in animal and poultry feeds, as soybean meal is



becoming scarce and expensive. In addition, the use of legume feeds reduces dependence on fossil-based nitrogen fertilizers and imported protein concentrates, improves carbon sequestration and diversifies crop rotations, thereby improving the economics and sustainability of dairy farming (Räisänen S.E. et al., 2023; Watson C.A. et al., 2018).

An important reserve for replenishing the protein deficiency in animal diets in Polesie can be the use of peas, chickpeas, fodder beans, vetches, and narrow-leaved lupine (alkaloid-free) (Savchuk I.M. et al., 2022; Savchenko Yu.I. et al., 2013). Pea grain is a highly valuable legume that is a source of dry matter, energy, and other nutrients for ruminants (Johnston D.J. et al., 2019). According to reports by domestic researchers (Sloboda O.M., Sloboda L.Ya., 2010; Savchenko Yu.I. et al., 2007), chickpea grain is also a highly nutritious feed for domestic animals, it contains about 18% of digestible protein, which is better than cereal protein in terms of amino acid composition. According to the authors (Halmemies-Beauchet-Filleau A. et al., 2018; Savchenko Y.I. et al., 2014), peas and chickpeas may be attractive alternative substitutes for “high-protein” feed ingredients, as well as cereals in ruminant diets due to their relatively high crude protein content and starch concentration. This is also supported by the findings presented in the review by Rungruangmaitree R. and Jiraungkoorskul W. (2017), which highlighted the health benefits of legume seeds and confirmed the antibacterial, antidiabetic, antifungal, anti-inflammatory, antioxidant and anticarcinogenic effects of pea seeds.

According to the authors (Osmane B. et al., 2017), when dairy cows were fed diets containing beans and peas, the fat and protein content in milk significantly increased, and the total amount of amino acids increased in bulk milk samples. In addition, the addition of field peas to fattening cows that consumed hay from medium-quality grasses increased the overall gain in live weight (Soto-Navarro S.A. et al., 2012). As a protein supplement for feeding beef cattle, the authors (Anderson V. et al., 2007) recommend including field pea grain in an amount of 15 to 30% of the diet (dry matter basis). According to their data, the inclusion of at least 10% of field pea grain in the finishing diet improved the tenderness and juiciness of beef, without affecting the properties of the carcass.

Foreign researchers (Degola L., Jonkus D., 2018; Hanczakowska E. et al., 2019) did not show a negative impact of the use of legume seeds in pig feeding on meat quality. On the contrary, Hanczakowska E. and Świątkiewicz M. (2014) and Sirtori F. et al. (2015) in their studies note a positive impact of feed mixtures containing legume seeds on the quality indicators of pork. Taking into account the results of studies presented by many authors (Prandini A. et al., 2011; Sońta M. et al., 2021) on the use of legume grains in feeding animals for fattening and their various effects on the quality of livestock products, continued research in this regard seems quite justified.

The purpose of the research is to establish the productive and slaughter qualities of bulls, the digestibility of nutrients and the balance of Nitrogen in the body when using various high-protein feeds in animal diets - peas and field peas.

**Materials and methods of research.** For the research, bulls of the Ukrainian black-and-white dairy breed were used, which were kept in the conditions of the physiological yard of the Institute of Agriculture of the Polissya NAAS. For the accuracy of the experiments, two separate groups of animals were formed, which were kept on a leash. The formation of experimental groups was carried out using the balanced group method according to the methodological provisions of Ibatullin I. and Zhukorsky O. (2017). The selection and comparison of animals for testing lasted 31 days. After the end of the comparative period, 10 animals with similar indicators were selected for each group of experimental bulls. The study was conducted in the village of Grozyne, Korosten district, Zhytomyr region, which belongs to the territory with a density of radioactive



contamination up to 185 kBq/m<sup>2</sup> (zone III). The duration of the experimental period is 122 days.

The experimental bulls were fed twice - in the morning and in the evening. The animals had free access to automatic water troughs throughout the day.

According to the main study design, during the experiment, young cattle of the control group were fed with farm ration feed, which included cereal hay, cereal-legume hay, fodder beet, grain mixture, pea husks and table salt. Pea husks were replaced with diaper husks for the animals of the experimental group. The deficiency of trace elements in the diet was ensured by introducing copper and zinc sulfate, cobalt chloride and potassium iodide into its composition.

Diets formed in accordance with modern detailed feeding standards and taking into account the actual composition and nutritional value of feed, fully provide the animal body with energy and make it possible to fully reveal the genetic potential in terms of their productivity (Ibatullin I.I. et al., 2016).

Against the background of scientific and economic research, in accordance with generally accepted methods in zootechnics (Ibatullin I.I., Zhukorsky O.M., 2017), a balance experiment was conducted to determine the influence of the studied factors on the digestibility of nutrients in feed rations and the assimilation of Nitrogen in the body of bulls. For the exchange experiment, three animals were selected from each group, which were kept in individual cages on a leash. The duration of the preparatory period was 10 days, the main (accounting) period was 7 days. During the research period, individual records of feed consumed and excreted feces and urine were kept, with the selection of average samples for analysis.

Before taking the average sample, feces were well mixed and taken in an amount of 10%, placed in glass jars with ground lids, while for its preservation, a 5% solution of hydrochloric acid was added at the rate of 10 ml per 100 g of feces, as well as a few drops of chloroform.

After weighing, urine was filtered and placed in jars with ground lids, taking an average sample in an amount of 5%, and for its preservation, a 10% solution of hydrochloric acid was added at the rate of 5 ml per 100 g of urine and 1-2 ml of thymol. After weighing the feed residues, an average sample (10%) was taken and placed in a glass jar with ground lids, where a few drops of chloroform were added for preservation.

All average samples were stored until laboratory tests at a temperature of 5-70 °C.

The nutritional value of the rations was determined based on the chemical analysis of individual feeds and their residues. Zoochemical analysis of feeds was carried out according to generally accepted methods in the laboratory of agrochemical research, environmental safety of lands and product quality of the Institute of Agriculture of Polissya NAAS (Kostenko V. M. et al., 2008; Vlizlo V. V. et al., 2012). A portion of feeds, their residues, feces, urine was dried in a drying oven to an air-dry state at a temperature of 60-65 °C and the initial moisture was determined. Then, by drying the air-dry portion in a drying oven to a constant mass at a temperature of 100-105 °C, hygroscopic moisture was investigated. Total moisture was determined by calculation.

In addition, the following were determined in the studied material: total Nitrogen – by the classical Kjeldahl method; crude fiber – by Henneberg and Stoman; crude fat – by the method of extraction of an absolutely dry sample in a Soxhlet apparatus; crude ash – by combustion in a muffle furnace at a temperature of 500–600°C; Calcium – by the trilonometric method using fluorexon; Phosphorus – by the photolorimetric method; crude protein, nitrogen-free extractives (BER), metabolic energy – by calculation; digestible protein, sugar, starch, trace elements, vitamins – according to reference data (Bomko V. S. et al., 2010). Nutrient digestibility was calculated by the difference between



nutrients that entered the animal body with feed and their amount excreted with feces. Nitrogen balance in the body of bulls was calculated as the difference between the amount of substances consumed from feed and their excretion with feces and urine.

Live weight of bulls was determined by individual weighing before morning feeding at the beginning and end of each experimental period and monthly, and payment for food feed by live weight gain was calculated (based on the expenditure of metabolic energy per unit of production in animals of the control and experimental groups).

To assess meat qualities, a control slaughter of animals was carried out - 3 heads from each group, the live weight of which corresponded to the average indicators in the groups, using the technology adopted at meat processing enterprises. The weight of a paired carcass and internal fat, slaughter indicators were calculated by dividing the weight of a paired carcass by the live weight after starvation.

The results of the studies were processed by standard methods of variational statistics using POM and computer programs for statistical processing Microsoft Excel were used. The levels of statistical significance (reliability) in the tables were indicated using letter superscripts in the following correspondence: \* $P > 0.95$ ; \*\* $P > 0.99$ ; \*\*\* $P > 0.999$  (Ruban S.Yu. et al., 2020).

During the experimental studies, all biotic requirements for animals were observed, which correspond to the Law of Ukraine "On the Protection of Animals from Cruelty" dated 03/28/2006 and the "European Convention for the Protection of Vertebrate Animals" dated 11/13/1987, the Order of the Minister of Education and Science, Youth and Sports of Ukraine dated 03/01/2012 No. 249, registered in the Ministry of Justice of Ukraine on March 16, 2012 under No. 416/20729 on the "Procedure for Conducting Experiments and Experiments on Animals by Scientific Institutions (European..., 1986; Order of the Ministry of Education..., 2012).

**Research results.** A characteristic feature of young animals is their ability to grow and increase live weight. The increase in body weight of young animals is influenced by the fullness of feeding. The productive effect of the feeding factors studied is usually assessed primarily by the parameters of growth and development of animals, the consumption of feed nutrients and energy per unit of growth.

The results of the studies showed that under the same conditions of feeding and keeping animals, bulls, depending on the type of protein feed in the diet, had different live weights at the end of the experiment (Table 1). At the beginning of the studies, the live weight of the experimental animals ranged from 277.8 kg to 281.7 kg in terms of groups. The intergroup difference in this indicator was insignificant and amounted to 1.0% in favor of bulls of the I (control) group. The use of field peas in the grain mixture for fattening young cattle of the II group led to an increase in their live weight at the end of the experiment to 403.6 kg, which is 18.0 kg more than the indicator of peers of the I group with a significant intergroup difference ( $P > 0.95$ ).

By optimizing the protein nutrition of bulls at the expense of various high-protein feeds, a high intensity of their growth was obtained. Thus, this indicator when using peas for feeding animals was 875 g, field peas - 1023 g. Young cattle of the II (experimental) group in terms of average daily live weight gains exceeded their peers from the I (control) group by 148 g, or by 16.9% with a significant difference ( $P > 0.95$ ). The expenditure of metabolic energy and digestible protein per 1 kg of live weight gain varied within 103.0-120.5 MJ and 818-957 g, respectively. These indicators in bulls of the II group compared to their peers in the I group were lower by 14.5%.



Table 1

**Live weight gain of fattening bulls and metabolic energy expenditure per 1 kg of gain (n=10; M ± m)**

Indicators	Groups	
	control (I)	experimental (II)
Live weight, kg: at the beginning of the experiment	281,7 ± 4,71	278,8 ± 3,32
at the end of the experiment	388,5 ± 3,44	403,6 ± 4,10*
Live weight gain: absolute, kg	106,8 ± 4,51	124,8 ± 4,47*
average daily, g	875 ± 34	1023 ± 33*
+ or – to control: g	-	+148
%	-	+16,9
Nutrient consumption per 1 kg of live weight gain:		
- metabolizable energy, MJ	120,5	103,0
- - digestible protein, g	957	818

Note: \*P > 0.95.

Based on the obtained research results, it can be concluded that balancing the rations for digestible protein at the expense of diaper compared to peas during fattening of bulls in the Polissya zone of Ukraine has a positive effect on their growth rate and feed conversion.

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The meat productivity of young cattle is determined by the genotype, level and quality of feeding, and production technology. The main criteria for its assessment are pre-slaughter live weight, carcass weight and yield, slaughter weight and slaughter yield. The obtained results indicate good slaughter qualities of the experimental animals, while no significant intergroup differences were found in carcass weight and yield and slaughter yield (Table 2). The pre-slaughter live weight of young cattle ranged from 399.3 kg (group I) to 406.3 kg (group II). According to this indicator, the animals of the experimental group exceeded their peers of the control group by 7.0 kg, or by 1.7% with an insignificant difference (P<0.95). Against the background of a greater pre-slaughter live weight of bulls of group II relative to analogues of group I, the carcass yield did not differ significantly - 51.8-52.5%. The slaughter yield varied from 53.6 to 54.3% and is characteristic of fattening young animals of the Ukrainian black-and-white dairy breed. In animals of group II (experimental) the slaughter yield was 0.7% abs. higher than in the control.



Table 2

**Slaughter qualities of experimental bulls (n = 3; M ± m)**

Indicators	Groups	
	control (I)	control (I)
Pre-slaughter live weight, kg	399,3 ± 5,78	406,3 ± 1,81
Weight of paired carcass, kg	206,8 ± 9,32	213,3 ± 4,19
Carcass yield, %	51,8 ± 1,97	52,5 ± 0,78
Weight of internal fat, kg	7,4 ± 0,80	7,4 ± 0,69
Fat yield, %	1,85 ± 0,19	1,82 ± 0,17
Slaughter weight, kg	214,2 ± 10,11	220,7 ± 4,49
Slaughter yield, %	53,6 ± 2,22	54,3 ± 0,93

Analysis of the results of the balance experiment showed that the digestibility of nutrients in the diets of bulls of both groups was at a sufficiently high level (Table 3).

Table 3

**Digestibility of nutrients in the diet of bulls, % (n=3; M ± m) (n=3; M ± m)**

Nutrients	Groups	
	control (I)	control (I)
Dry matter	74,2 ± 1,42	74,4 ± 0,89
Crude protein	59,3 ± 0,38	63,2 ± 0,93*
Crude fat	53,5 ± 2,80	52,1 ± 1,33
Crude fiber	70,2 ± 1,78	71,1 ± 1,72
nitrogen-free extractive substances	83,7 ± 1,82	83,4 ± 1,19

Note. \*P > 0,95.

After analyzing the results of the research, it can be stated that the digestibility coefficients of feed nutrients in the body of the experimental bulls were within the range typical for this live weight of animals, and did not significantly differ between the groups, with the exception of crude protein. The digestibility of crude protein in the body of animals of the experimental group, which were fed diaper rags as part of the diet, was significantly higher compared to the peers of the control group by 3.9% abs. (P > 0.95).

In complex metabolic processes, one of the main places belongs to protein metabolism, the main indicator of which is the balance of Nitrogen in the body of animals. The study of the balance of Nitrogen in animals has both scientific and practical significance, and with the use of new feed - even more so. Balance is one of the factors characterizing the biological value of feed and is the main indicator of the degree of use of nitrogenous substances of feed by animals, allows us to give a fairly objective assessment of metabolic processes associated with the transformation and synthesis of proteins in the body.

The results of the experiment on the use of Nitrogen from feed rations in the body of fattening bulls, under the influence of the studied factors, are presented in Table 4. The analysis of the physiological experiment shows that the balance of Nitrogen in the body of young cattle of both experimental groups was positive and was at a sufficient level to meet physiological needs. At the same time, some intergroup differences in the excretion of Nitrogen with feces and urine were established, which significantly affected the efficiency of the use of digested Nitrogen in the body of animals. It was found that with the feces of bulls of group I, a greater amount of Nitrogen was excreted than in analogues from group II by 3.51% abs.



Table 4

**Average daily balance of Nitrogen in the body of experimental animals, g/head.  
(n=3; M ± m)**

Indicators	Groups	
	control (I)	control (I)
Intaken with feed	240,62	235,22
Excreted with feces	88,21 ± 1,50	77,98 ± 3,96
% of intake	36,66	33,15
Digested	152,41 ± 1,50	157,24 ± 3,96
Excreted with urine	24,59 ± 2,54	25,72 ± 2,53
% of intake	10,22	10,93
Total excretion	112,80 ± 1,90	103,70 ± 1,94*
Deposited in the body	127,82 ± 1,90	131,52 ± 1,94
Absorbed, %: of intake	53,12 ± 1,20	55,91 ± 1,24
of digested	83,87 ± 1,69	83,64 ± 1,36

Note. \*P > 0,95.

The analysis of the physiological experiment shows that the balance of Nitrogen in the body of young cattle of both experimental groups was positive and was at a sufficient level to meet physiological needs. At the same time, some intergroup differences were found in the excretion of Nitrogen with feces and urine, which significantly affected the efficiency of using digested Nitrogen in the animal body. It was found that with the feces of bulls of group I, a larger amount of Nitrogen was excreted than in analogues from group II by 3.51% abs.

According to the results of the research, it was found that in young cattle of group II (experimental), the grain mixture of which included of field peas, the average daily excretion of Nitrogen with urine was the largest - 25.72 g, which is 10.93% of the intake. As a result, the animals of the experimental group excreted 9.10 g/head/day less nitrogen with feces and urine than the same-sex animals of the control group (P > 0.95). Therefore, in the body of bulls of group II, nitrogen deposition was 131.52 g/head/day, or was 2.89% higher than the indicators of analogues from group I (P < 0.95). In addition, the introduction of field peas into the feed rations increased nitrogen absorption in young cattle of the experimental group compared to the control by 2.79% abs. Nitrogen absorption from digested food between animals of both groups did not differ significantly and was 83.64-83.87%.

**Discussion.** *Pisum sativum* L., known as green peas, dry peas or field peas, is an important legume crop that is a source of proteins, vitamins, minerals and biologically active compounds that are beneficial to human health (Fahmi R. et al., 2019). Peas are cultivated in almost all countries of the world and are considered an integral part of the human diet (Han X. et al., 2023). The largest producer of peas in the world is Canada, followed by China, Russia and India (Raghunathan R. et al., 2017).

The energy nutritional value of 1 kg of pea grain is 11.2 MJ of metabolic energy with a content of 192-195 g of digestible protein, 12.7-14.2 - lysine, 4.3-5.5 - methionine with cystine, 2.0 - tryptophan, 2.0-2.5 - calcium and 3.5-4.3 g of phosphorus (Gnoevy V.I. et al., 2009; Ge J. et al., 2020). Higher growth intensity indicators and nitrogen balance in the body of bulls of group II compared to their peers of group I can be explained by a slightly better provision of their diets with protein and its better amino acid composition. The content of essential amino acids in the diet feed can serve as one of the indicators of the overall quality of protein.

At the same time, according to the authors (Shi L. et al., 2018; Hugman J. et al.,



2021), peas contain, albeit in small quantities, anti-nutrients (trypsin inhibitors, tannins, lectins, and phytic acid). Therefore, when pea grains are fed to animals without prior preparation and in significant quantities, protein digestibility decreases and growth slows down (Wu D.-T. et al., 2023), which is consistent with the results of the conducted studies. In addition, according to reports by domestic and foreign authors, in ruminants, an increase in the amount of tannins above a certain level suppresses the rumen microflora, which negatively affects their use of feed nutrients (Savchenko Y.I. et al., 2014; Ge G. Et al., 2021).

### **Conclusions.**

1. The use of field peas in the feed rations of bulls for fattening compared to peas to ensure the normalized need for digestible protein in the conditions of Polissya, Ukraine significantly increases the average daily gain in live weight (by 16.9%) and reduces the expenditure of metabolic energy per unit of gain (by 14.5%), without significant intergroup differences in slaughter indicators.

2. Optimization of protein nutrition of animals by introducing of field peas into the ration had a positive effect on the digestibility of crude protein in the body of bulls of the experimental group, which turned out to be significantly higher compared to the peers of the control group by 3.9% abs. ( $P > 0.95$ ). Nitrogen deposition in the body of young cattle of group II was 131.52 g/head/day, or was higher than the indicator of analogues from group I by 2.89% ( $P < 0.95$ ).

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## **FACTORS OF EFFICIENCY OF THE LIVESTOCK INDUSTRY IN CRISIS CONDITIONS OF WAR**

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*The article examines the current state and dynamics of the development of the livestock industry in Ukraine and considers the impact of systemic and force majeure factors on its development in the pre-war period and in the first two years of the Russian military invasion of Ukraine. It was determined that in Ukraine the rate of reduction of cattle (from 2.6 to 2.1 million heads) and cows (from 1.5 to 1.2 million heads) in the first two years of the war exceeded that in the pre-war years, and the production volumes of milk (from 8.7 to 7.4 million tons) and beef (from 310 to 258 thousand tons) significantly decreased. A steady increase in cow productivity was noted, both in the pre-war years from 4.6 to 5.1 t/head per year and in the two years of the war from 5.1 to 5.4 t/head.*

*The state of the livestock industry in terms of economic entities was considered and it was determined that the share of agricultural enterprises in the structure of milk and meat production is naturally increasing and reached 75.0% and 37.8%, respectively, in 2023.*

*Research has established that there is a significant differentiation in livestock enterprises by the available livestock. Thus, agricultural enterprises with up to 100 heads of livestock occupy 42.5% of the total number of enterprises, medium-sized (from 100 to 1000 heads) - 42.2%, large (more than 1000 heads) - 17.8%. It was determined that a smaller number of enterprises with more than 1000 cows have a larger livestock (63.9%) and produce a larger share of milk compared to small ones (up to 100 cattle).*

*An analysis of the economic activities of the Agro-Novoselivka 2009 private enterprise in Novovodolazha district, Kharkiv region was conducted, which revealed that the enterprise has a meat and grain specialization. Analysis of the income structure by industry revealed the advantages of the livestock industry over crop production, especially in critical war conditions. It was determined that livestock production is less dependent on exports of products, and crop production is more export-dependent.*

**Keywords:** *cattle breeding, cows, milk, meat, efficiency, profit, level of profitability, agricultural enterprises.*



## ФАКТОРИ ЕФЕКТИВНОСТІ ГАЛУЗІ СКОТАРСТВА В КРИЗОВИХ УМОВАХ ВІЙНИ

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*В статті досліджено сучасний стан і динаміку розвитку галузі скотарства в Україні та розглянуто питання щодо впливу факторів системного та форс-мажорного характеру на її розвиток в довоєнний період і за перші два роки російського воєнного вторгнення в Україну. Визначено, що в Україні темпи скорочення поголів'я великої рогатої худоби (з 2,6 до 2,1 млн. голів) та корів (з 1,5 до 1,2 млн. голів) за перші два роки війни випереджали такі у довоєнні роки, а обсяги виробництва молока (з 8,7 до 7,4 млн. тон) та яловичини (з 310 до 258 тис. тон) суттєво скоротилися. Відзначено не впливний ріст продуктивності корів, як у довоєнні роки з 4,6 до 5,1 т/гол за рік так і за два роки війни з 5,1 до 5,4 т/гол.*

*Розглянуто стан галузі скотарства в розрізі суб'єктів господарської діяльності і визначено, що частка сільськогосподарських підприємств в структурі виробництва молока і м'яса закономірно збільшується і досягла у 2023 році відповідно до 75,0% та 37,8%.*

*Дослідженнями встановлено, що в тваринницьких підприємствах існує суттєва диференціація за наявним поголів'ям худоби. Так, сільгосппідприємства, що мають до 100 голів худоби, займають в загальній кількості підприємств 42,5%, середні (від 100 до 1000 голів) – 42,2%, крупні (більше 1000 голів) – 17,8%. Визначено, що менша кількість підприємств з наявністю корів понад 1000 голів налічує більше поголів'я (63,9%) та виробляє більшу частку молока порівняно з дрібними (до 100 голів худоби).*

*Проведено аналіз господарської діяльності ПП «Агро-Новоселівка 2009» Нововодолазького району, Харківської області, який виявив, що підприємство має м'ясо-зернову спеціалізацію. Аналіз структури доходів по галузях, виявив переваги тваринницької галузі відносно рослинництва, особливо у критичних умовах війни. Визначено, що продукція тваринництва є менш залежною від експорту продукції, а продукція рослинництва є більш експортозалежною.*

**Ключові слова.** скотарство, корови, молоко, м'ясо, ефективність, прибуток, рівень рентабельності, сільгосппідприємства.

**Introduction.** The dairy subcomplex is an important component of Ukraine's agro-industrial production, and the livestock sector is its basic raw material foundation and ensures the state's food security in the main types of food - dairy products and meat, and also generates significant amounts of added value due to the significant labor intensity of milk production and processing activities. Even before 2021, structural changes began in the dairy livestock sector, and the conditions of martial law became a catalyst for the corresponding processes. As a result, the livestock sector in Ukraine has undergone



significant structural deformations in recent years, which will only intensify in the near future. In Ukraine, many researchers are studying the effectiveness of the development of the livestock sector. Developments on these issues are presented in the works of such economists as Latynin M. (2000), Lavruk O. (2020), Petrychenko O. (2018), Krasnorutskyi O. (2020), Kernasyuk Yu. (2020), Ryzhkov V. (2007), Shiyan N. (2019), Shust O. (2021), etc.

A number of Ukrainian scientists believe that the functioning of the dairy subcomplex affects the national economy (Lavruk O.V., 2020; Shust O.A., 2021). Kozak O.A. (2020) supports this view and argues that the dairy sector accounts for 36.1% of the value of gross livestock production and 9.5% of all agricultural production in Ukraine. The special importance of dairy farming lies in the fact that its products (milk) are sold daily - this makes it possible to constantly renew the working capital of commodity producers and dairy industry enterprises, to use labor resources, equipment and production facilities more fully and evenly.

Most modern scientists believe that determining ways to improve the economic mechanism in livestock farming lies in the plane of ensuring a level of profitability for commodity producers of the industry's products, which would stimulate them to increase the volume of its production and expanded reproduction (Pidpala T. V., 2018; Shiyan N.I., 2019; Eyfeel A., 2022; Kernasyuk Y. V., 2020).

In her research, Antoshchenkova V. V. (2020) notes that specialized production, as a rule, is highly intensive and efficient. That is, the higher the level of concentration of dairy cattle breeding, the higher the level of specialization of the enterprise on the products of this industry, and narrower specialization allows enterprises to concentrate economic resources on the production of one or several types of products. However, according to L. Melnyk and P. Makarenko (2002), a simple increase in the scale of production by increasing the level of concentration and specialization of production is unlikely to provide an increase in the level of economic effect.

Gladiy M. R., Prosovykh O. P. (2022), studying the current state and trends in the development of the dairy industry of Ukraine, argue that it is systemic rather than situational factors that were the cause of the current crisis in the industry and it is in the conditions of martial law that new opportunities for the development of the dairy business appear. Shiyan N. I. (2021) supports their opinion and proves that profitable production of beef by business entities in the industry is ensured at a level of specialization of more than 60.0%, and milk - above 63.8%.

A number of scientists believe that in households, land, labor and material resources are used more efficiently compared to their use in agricultural enterprises (Tkach G. V., 2004). Therefore, the amount of profit per unit of land area could be higher in them (Makarenko Yu. P., 2013).

However, despite the significant number of studies on the efficiency of the livestock industry, there is a need to identify the factors that most significantly affect the current conditions of its functioning. Important, in our opinion, is the issue of identifying problems that hinder its development and ways to overcome them. No less important is the study of factors and factors that have largely led to the current state of the industry - systemic or force majeure, caused by recent crisis events?

The purpose of this article is to assess the current state of development of livestock farming in Ukraine, to develop theoretical provisions and practical recommendations for increasing the efficiency of production and sale of milk and meat by agricultural enterprises in the current crisis conditions of martial law.

**Materials and methods of research.** The object of the study is the process of functioning of the livestock industry, both in Ukraine as a whole and in a separate



enterprise, in critical conditions of war, factors, processes and force majeure factors that determine its development.

The theoretical and methodological basis of the research is a set of methods, approaches, principles of scientific knowledge, fundamental provisions of economic theory, scientific developments on the formation of a mechanism for the effective development of livestock farming and substantiation of directions for improving the efficiency of livestock farming, especially in crisis conditions of war.

The article uses the method of analysis and synthesis, abstract-logical (formation of theoretical generalizations and conclusions), economic-mathematical, statistical, comparison and generalization. The theoretical and information base of the research is the works of domestic scientists, analytical reviews of Ukrainian and foreign experts, as well as national statistical information on the dynamics of the main indicators of the livestock industry.

Research findings. Russia's full-scale invasion of Ukraine on February 24, 2022, seriously disrupted the country's food production and food security, putting at risk not only its food security but also that of the world. Targeted attacks by the Russian army on Ukrainian agriculture, such as shelling of agricultural facilities and infrastructure across Ukraine, mining and burning of farmland near active combat zones, a five-month blockade of Black Sea ports (and the resumption of the blockade as of July 2023), the Polish border blockade of agricultural exports from Ukraine, and the blowing up of the Kakhovka hydroelectric dam, have made the functioning of the Ukrainian agricultural sector extremely difficult. While export-oriented agribusiness suffered the greatest losses, especially at the beginning of the war, agricultural enterprises and individual peasant farms were able to adapt to extreme conditions and provide food for the Ukrainian army and population.

Dairy and meat cattle breeding is one of the most important food security industries, the purpose of which is to ensure the production of milk and meat in volumes sufficient to load the production capacities of dairy and meat processing enterprises with the subsequent production of dairy and meat products. The state of development of the dairy subcomplex, including its important component - the dairy and meat cattle breeding industry, affects the social and economic stability of the economic system.

The total cattle population in Ukraine from 2015 to 2021 decreased by almost a third and amounted to 2.87 million heads, the number of cows during this period decreased by 493 thousand heads and amounted to 2.64 million heads. In 2022, during martial law, the cattle population decreased by 230 thousand heads or 8%, in 2023 it decreased further by 151 thousand (Table 1).

Before the war, 42% of all milk was produced in the regions that were most heavily shelled and occupied by the Russians. Therefore, as of the end of 2022, the number of cows was 1.35 million heads. This is 191 thousand heads or 12.4% less than in 2021. In 2023, the country continued to trend towards a reduction in the number of cows - it decreased by more than 90 thousand from the beginning of the year. Thus, as of the beginning of 2024, the total number of cattle was 2.15 million heads, cows 1.26 million heads, which is 7.0% and 7.1% less than in the previous year, respectively. At the same time, the number of cows in agricultural enterprises for the period from 2015 to 2021. decreased by only 19.6%. Since 2020, the corresponding process has actually stopped, and in 2021 it amounted to 1.54 million heads. If it were not for the Russian military aggression, the trend towards an increase in the number of cows would have continued in 2022. Instead, the result of the actions of the aggressor country was an 8.3% decrease in the number of cows in 2022 and a 14.2% reduction in 2023 in agricultural enterprises. Thus, the number of cows in agricultural enterprises as of the beginning of



2024 is about 73.3% of the figure at the beginning of 2015 and is 1.26 million heads. The total share of cattle in them as of 2023 is 42.7%, and cows 30.3% (Dezhkomstat of Ukraine, 2023).

*Table 1*

**Main production indicators of livestock farming in Ukraine for 2021-2023  
(farms of all categories)**

<b>Indicators</b>	<b>2021 y.</b>	<b>2022 y.</b>	<b>2023 y.</b>	<b>2023 % to 2021</b>
Cattle, thousand heads	2644,0	2307,1	2156,2	81,6
including agricultural enterprises	1003,4	942,1	919,8	91,7
of households	1640,6	1365,0	1236,4	75,4
Cows, thousand heads	1544,0	1352,8	1262,9	81,8
including agricultural enterprises	424,6	394,2	382,2	90,0
of households	1119,4	958,6	880,7	78,7
Meat production, thousand tons	2438,3	2206,7	2239,5	91,8
including agricultural enterprises	1720,4	1608,1	1679,5	97,6
of households	717,9	598,6	560,0	78,0
Beef production, thousand tons	310,5	268,4	256,9	82,7
Milk production, thousand tons	8713,9	7767,7	7430,4	85,3
including agricultural enterprises	2767,7	2644,2	2809,9	101,5
of households	5946,2	5123,5	4620,5	77,7
Average annual milk yield per cow, kg	5155	5119	5476	106,2
including agricultural enterprises	6863	6611	7568	110,3
of households	4604	4569	4664	101,3
Rate of profitability of operating activities of agricultural enterprises, % 2015 - 43.0%	19,2	41,9	21,0	+1,8

As for households, a similar trend is observed here. The total number of cattle in them in 2021 decreased compared to 2015 by almost 2 times or by 1.11 million heads, cows by 703 thousand heads (73.3%). In 2022, their number decreased by 275 and 161 thousand heads, respectively, or 20.1 and 16.8%, in 2023 by 129 and 79 thousand heads, respectively. heads (10.4% and 8.8%) compared to the previous year. The total share of cattle in the total livestock of the country decreased from 62.0% in 2021 to 57.3% in 2023, and cows to 69.7%.

In general, the share of cows kept by agricultural enterprises in the total livestock has been growing dynamically in recent years - from 23.4% as of the beginning of 2015 to 29.2% at the beginning of 2023., and as of the beginning of 2024, the corresponding figure reached 30.2%. Households, respectively, accounted for almost 70% of the total cattle population in the country.

The reduction in the livestock population is primarily caused by military operations in Ukraine. Direct factors during the war include the physical destruction of livestock and livestock facilities by shelling, bombing, seizure of territories with livestock there, the bombing of the Kakhovka hydroelectric power station, the impossibility of



keeping cows and harvesting feed in front-line zones, mining of territories, etc. Indirect factors influencing the development of the industry include inflation, closed Black Sea ports, the blockade of land exports of Ukrainian agricultural products by the Poles, as a result of which there was a significant decrease in prices for crop products on the domestic market, etc. The difficult macroeconomic situation in the agricultural sector also affected the work of enterprises and households, where animals are increasingly being sent for sale due to the financial inability to maintain them.

In Ukraine, processes of concentration of dairy farms have been taking place for a long time, because keeping a larger number of cows is more economically profitable. Given this, the number of specialized dairy farms and the number of cows they keep is increasing in Ukraine. First of all, it should be noted a significant decrease in the number of enterprises that keep cows: during 2010–2021, their number decreased from 3,741 to 1,686 units (more than 2.2 times). In 2022, their number decreased to 1,440 units (2.6 times less than in 2010). It is especially worth noting that the number of enterprises that suffered losses increased from 11.0% in 2021 to 20.9% in 2022. It is obvious that the trend towards a decrease in the number of agricultural enterprises that keep cows will continue.

Total milk production in Ukraine has been falling rapidly over the last pre-war years (Dezhkomstat of Ukraine, 2023). Yes, they decreased by almost a quarter (by 1.9 million tons) in 2021 compared to 2015, but in the last pre-war year, milk production volumes almost began to level out and in 2021 decreased by only 39 thousand tons compared to 2020. During the years of the war in Ukraine, milk production volumes continued their negative downward trend. With the beginning of Russia's military aggression against Ukraine in 2022, they decreased by 947 thousand tons (12.2%), and in 2023 by another 337 thousand tons (4.5%). However, agricultural enterprises, after reducing milk production volumes in 2022 by 123 thousand tons, increased its production by 165 thousand tons. to the maximum in recent years of 2.8 million tons.

Despite a significant decrease in the number of cows in agricultural enterprises, the volume of milk production by them in 2015–2021 was practically unchanged and fluctuated within the range of 2.67–2.77 million tons (Dezhkomstat of Ukraine, 2023). This became possible due to a significant increase in the productivity of cows kept by agricultural enterprises, which increased by 74% over the period 2010–2021 - from less than 4 thousand kilograms in 2010 to 6.9 thousand kg/head in 2021. In 2022, official statistics recorded a decrease in cow productivity to the level of 6.6 thousand kg/head, which is quite logical for the period of martial law, but in 2023, despite all the hardships (continued war, shelling, power outages, drought, etc.), it increased by as much as 14.4% to 7.5 thousand kg/head, and in 2024, the average milk yield per cow in farms is expected to increase by more than 5-7% and cross the mark of 8 thousand kg/head. The reduction in total milk production is largely due to its decline in households (almost a quarter over 2015–2021). Over the past three years (Statistical Collection, 2022), households have also continued to significantly reduce milk production: in 2022 by 823 thousand tons or 16.1%, in 2023 by another 503 thousand tons (10.9%). This is due to the fact that as a result of hostilities, some agricultural workers and farmers were forced not only to cease economic activity in the agricultural sector, but also to leave their homes.

According to FAO (2022), more than 150,000 farmers / food system workers were directly affected by the war and/or were forced to migrate. Small-scale producers who were engaged in growing seasonal products and played an important role in ensuring employment and income of the rural population found themselves in a difficult situation. Forced population displacement, conscription of men into the Armed Forces of Ukraine lead to a shortage of labor and an increase in the workload of women.



According to FAO (2022), due to the war, rural households in Ukraine suffered about 2.25 billion USD in losses. Of these, about 1.26 billion USD in losses were caused in the field of crop production and 0.98 billion USD in livestock production. In Ukraine, 25% of agricultural households stopped or reduced production volumes due to the war, in front-line regions - 38%. As a result, the share of agricultural enterprises in the total volume of milk production in Ukraine is growing significantly in dynamics from 31.8 in 2021 to 37.8% in 2023, and according to preliminary estimates, in 2024 it will reach almost 40%. Also, in 2024, record milk production volumes by agricultural enterprises are expected to be achieved (about 2.85 million tons, which is 3% more than in 2021). In contrast, milk production volumes by households are forecast to decrease significantly and are expected to be about 4.5 million tons in 2024 (two times lower than in 2015), and their share in the total is 62.2%. Therefore, we can summarize that the dairy cattle breeding sector in the agricultural enterprises segment, despite the unfavorable conditions of martial law, began its growth in 2023. Along with other factors, it is due to the unfavorable situation in the field of crop production, where the cultivation of traditionally high-margin export-oriented crops has ceased to bring surplus profits to agricultural enterprises.

Ukraine has been experiencing a process of concentration of dairy farms for a long time, because keeping a larger number of cows is more economically profitable. Given this, the number of specialized dairy farms and the number of cows they keep is increasing in Ukraine. First of all, it should be noted a significant decrease in the number of enterprises that keep cattle: during 2010–2021, their number decreased from 3741 to 1792 units (more than 2.2 times), including enterprises that kept cows - to 1686. In 2022, their number decreased to 1533 and 1440 units, respectively (2.6 times less than in 2010). Their share in the total number of agricultural enterprises is also decreasing and already reaches less than 10%. It is obvious that the trend towards a decrease in the number of agricultural enterprises that keep cows will continue (Table 2). At the same time, the number of cows in agricultural enterprises decreased by only 19.6% from 2015 to 2021. Since 2020, the corresponding process has actually stopped, and in 2021 it amounted to 1.54 million heads. If it were not for the Russian military aggression, the trend towards an increase in the number of cows would have continued in 2022. Instead, the aggressor country's actions resulted in an 8.3% decrease in the number of cows in 2022 and a 14.2% reduction in 2023 in agricultural enterprises. Thus, the number of cows in agricultural enterprises as of the beginning of 2024 is about 73.3% of the figure at the beginning of 2015 and is 1.26 million heads. The total share of cattle in them as of 2023 is 42.7%, and cows are 30.3% (Dezhkomstat of Ukraine, 2023).

It should be noted that agricultural enterprises engaged in dairy farming are very differentiated in size, given the number of cows in them. Thus, in 2010, the share of enterprises that kept up to 100 cows in the total number of enterprises engaged in dairy farming reached 55.4%. However, in 2021, the share of such enterprises decreased to 43.8%, in 2022 to 42.5%, i.e. decreased by more than a quarter. According to preliminary data for 2023, it decreased to less than 40%.

It should be noted that small enterprises with a cattle population of up to 50 heads at the beginning of 2023 occupy a fifth (20.2%) of their total number, and up to 100 heads - 9.8%, i.e. the total number of small ones is 30%. However, the number of farm animals they have is 17.1%. Enterprises with a livestock of 100 to 500 heads occupy 33.0% in the structure of farms with a specific weight of livestock in its total number at the level of 14.4%. The share of large specialized enterprises from 1000 to 1500 heads is 8.4%, over 1500 heads - 9.4% (Dezhkomstat of Ukraine, 2023). It should be emphasized that the average segment of commodity producers from 100 to 500 cows as of 2022 accounts



for the highest share in the structure of 42.7%. In contrast, the share of enterprises that kept livestock of more than 500 cows in 2010 was only 7.2% in the total number of enterprises engaged in dairy farming. In 2021, it increased to 13.7% and in 2022 to 14.8%, or 1.9 times. The share of enterprises that kept a livestock of more than 1,000 cows increased from 1.2% in 2010 to 4.8% in 2021 and 5.1% in 2022.

Table 2

**Grouping of enterprises by the number of farm animals (as of January 1, 2023)**

Grouping of enterprises by number of animals (head)	Number of enterprises		Number of farm animals	
	units	% of total number	units	% of total number
<b>Cattle</b>				
up to 50	310	20,2	5,9	0,6
50–99	151	9,8	11,2	1,2
100–499	505	33,0	135,8	14,4
500–999	294	19,2	206,2	21,9
1000–1499	129	8,4	156,7	16,6
more than 1500	144	9,4	426,3	45,3
Total enterprises	1533	100,0	942,1	100,0
<b>Cows</b>				
up to 50	403	28,0	8,3	2,1
50–99	209	14,5	15,7	4,0
100–499	614	42,7	157,5	40,0
500–999	140	9,7	93,1	23,6
more than 1000	74	5,1	119,6	30,3
Total enterprises	1440	100,0	394,2	100,0

Farms with an existing livestock of 100 to 500 heads occupy a total of 42.7% with a further increase in the livestock per farm. Accordingly, if in 2010, on average, there were 157 cows per enterprise that kept them, then in 2021 there were already 252, and in 2022 there were 274 cows. It is preliminarily determined that in 2023 the corresponding indicator approached the level of 300 heads.

In contrast, the share of enterprises that kept a livestock of more than 500 cows in 2010 was only 7.2% of their total number. In 2021, it increased to 13.7%, or 1.9 times. The share of farms that kept a livestock of more than 1,000 cows increased from 1.2% in 2010 to 4.8% in 2021, that is, 4 times.

The share of the livestock of cows available in agricultural enterprises that kept up to 100 cows in 2010 was 10% of its total number. In 2021, the share of such livestock decreased to 5.6%, or almost 1.8 times. By the beginning of 2023, this figure had decreased to 1.8% (three times the figure for 2021). However, if in 2010 the share of the cow population in enterprises that kept more than 500 cows was 36.3% of their total population, then in 2021 the corresponding figure increased to 52.5% (1.45 times). For the population in farms that kept more than 1,000 cows, the corresponding figures were 11.3% in 2010 and 29.0% in 2021, that is, it increased almost threefold. It is expected that in 2023 the corresponding trend will continue and the share of cows in farms that keep more than 500 cows will exceed 53% of the total number, and in those that keep more than 1,000 cows, it will reach almost a third of the total number of cows (30.3).

If we analyze the grouping of enterprises by the available number of cattle and cows, the same pattern is determined here, that is, smaller enterprises have a smaller



number of livestock on their balance sheet. That is, small and medium-sized enterprises (up to 500 heads of livestock) make up more than half of all enterprises in Ukraine (63.0%), but keep only 16.2% of the total livestock and at the same time produce approximately 10 percent of livestock products. The share of agricultural enterprises with a livestock of 500 to 1,000 is 19.2%, while holding 21.2% of the livestock. The share of large agricultural holdings and enterprises with a livestock of over 1,000 heads is 17.8%, but they hold more than half (61.9%) of the total livestock.

Despite this, if in 2010 the share of the cattle population available at enterprises that kept more than 500 cows was 36.3% of their total livestock, then in 2022 the corresponding figure increased to 53.9% (1.5 times). For the livestock available at enterprises that kept more than 1,000 cows, the corresponding figures were 11.3% in 2010 and 30.3% in 2022. It is expected that in 2023 the corresponding trend will continue and the share of cows in enterprises that keep more than 500 cows will exceed 55% of the total number, and in enterprises that keep more than 1000 cows, it will reach almost a third of the total number of cows.

All these factors undoubtedly influenced the efficiency of both the livestock and livestock industries and the agricultural sector as a whole. Thus, the level of profitability of the operating activities of agricultural enterprises in 2015 was 43.0%, in 2021 – it decreased by half to 19.2%, in 2022, despite the military actions, it increased again to 41.9%, in 2023 it decreased by half to 21.0%.

General efficiency indicators show the trend of profitability of production, but cannot answer the question - what factors influence the formation of the efficiency of dairy and meat production of each individual enterprise. The representative of the largest middle class of agricultural producers engaged in meat production is the private enterprise "Agro-Novoselivka 2009" of Novovodolazha district, Kharkiv region. It is located only 30 km southeast of the regional center, which provides advantages in terms of proximity to sales markets (the population of the city of Kharkiv is currently about 1.3 million people), especially livestock products. The total land area of the enterprise is about 3 thousand hectares, agricultural land in 2023 was 2.88 thousand hectares (Table 3).

Despite the approaching military operations close to the city of Kharkiv and the almost neighboring Izyum district in 2022, the enterprise is increasing its technical equipment (both in livestock and crop production). At the beginning of 2022, two new tractors, a new feed mixer, and attachments for them were purchased. Therefore, the cost of fixed assets in 2022 increased by 12.6 million UAH. (40.6%), in 2023 by 2.1 million UAH. (4.7%) compared to the previous year. The enterprise has a meat and grain specialization. Meat production is provided by meat cattle breeding and pig breeding. In 2023, the livestock population increased by 26.4% compared to 2021 and amounted to 713 heads, over the same period, the pig population, despite the continuation of the war, increased almost threefold and amounted to 6634 heads at the end of 2023. The main part of meat production on the farm is provided by pig breeding, where gross production is steadily increasing in proportion to the livestock: in 2022, 718.3 tons of pork were produced, which is 28.8% more than the previous year. In 2023, 1.38 thousand tons of pork were produced, which is 2.5 times more than in 2021, and 1.9 times more than in 2022. This was even during the critical years of the war.

The enterprise breeds Aberdeen-Angus cattle, which are kept according to the free-range system, without year-round use of premises. In winter, cows with suckling calves are kept on reconstructed and new free-range feeding grounds, and in summer - on pastures. That is, the conditions for keeping cattle are close to natural conditions (Prudnikov V.G., 2024; Mikhalchenko S.A., 2020). The farm territory is fenced with a reinforced concrete fence made of slabs. Such a fence was also built around the free-range



feeding grounds. It serves as a protective barrier for beef cattle from gusts of wind and drafts. Along the fence on the eastern side (in this natural and climatic zone, the prevailing winds are from the northeast) of the site, canopies are equipped for rest and shelter from the weather. On the walking and feeding sites for cows, heifers of calving age and heifers, the area standards were 15 m<sup>2</sup>/head, for cattle on fattening from 7 to 14 months – 10 m<sup>2</sup>/head. The feed mixture is distributed to the cattle using a feed dispenser 2 times a day.

Table 3

**Resource potential of PE "Agro-Novoselivka 2009"**

Indicators	2021 y.	2022 y.	2023 y.	2023 % to 2021
Total land area, ha.	3013,8	3143,3	3240,4	107,5
including: agricultural land	2661,8	2791,3	2888,4	108,5
including arable land	2553,0	2680,0	2753,0	107,8
hayfields	23,8	26,3	29,9	125,6
pastures	85,0-	85,0	105,5	124,1
Average annual number of employees employed in agricultural production, people	43	42	39	90,7
including: in crop production	32	33	29	90,6
in livestock farming	11	9	10	90,9
Average annual cost of fixed assets, thousand UAH	31072	43672	45730	147,2
Availability, pcs.: tractors	10	12	12	120,0
combines	5	5	5	100,0
forage harvesters	1	1	1	100,0
Average annual livestock, head: cattle	564	634	713	126,4
cows	274	294	304	110,9
pigs	2875	4294	6634	230,7
Gross production, kg:				
live weight gain, cattle	510,8	447,3	580,4	113,6
live weight gain, pigs	5577,5	7183,0	13871,2	248,7

As reported by Kolisnyk O.I. (2018), the formation of the primary layer of long-lasting straw bedding on the walking and feeding sites began in September, with straw being brought in at a layer thickness of 40 cm. At all walking and feeding sites, earthen mounds were arranged with a height of 1–1.5 m, a width of 15–25 m. The mounds were covered with straw for comfortable rest of the livestock. Their area per head was 3–5 m<sup>2</sup>. Due to the thermal processes that occur in the layer of straw, heat is released, as a result of which the livestock rests and warms up in adverse weather. To maintain a dry den, straw was added daily at the rate of 3 kg per head in dry weather and 5-7 kg - in adverse weather conditions (rain, snow, lowering air temperature).

The war left its mark on both production volumes and the efficiency of operational activities. Thus, the volume of sales of agricultural products in 2022 significantly decreased by 21.1 million UAH. or 27.7%, and in 2023 they have already increased by 54.7 million UAH (74.0%) compared to the previous year (Table 4).



*Table 4*

**Industry structure of commodity products of LLC "Agro-Novoselivka 2009"**

Industries and types of products	2021 y.		2022 y.		2023 y.	
	thousand UAH	%	thousand UAH	%	thousand UAH	%
Crop production, total	60933,2	64,5	33628,3	45,5	50601,8	39,3
Including: grains and legumes	47435,5	50,2	23095,2	31,3	30209,0	23,5
Sunflower	13249,7	14,0	10533,1	14,2	20083,2	15,6
Livestock production, total	24169,5	25,6	39623	53,6	77038	59,9
Including: meat: beef	3132,8	3,3	2900,8	3,9	2342,3	1,8
pigs	21036,7	22,3	36722,1	49,7	74696,4	58,1
Sale of other products, works and services	9338,0	9,9	676,7	0,9	1007,6	0,8
<b>Total by household</b>	<b>94440,7</b>	<b>100</b>	<b>73927,9</b>	<b>100</b>	<b>128648,1</b>	<b>100</b>

The structure of commodity products varied significantly over the same period. Thus, in 2021, the share of the crop sector in the total sales of the enterprise was 64.5%, and livestock farming was 25.6%. In 2022, these indicators were 45.5 and 49.7%, respectively, that is, they were approximately equal. And in 2023, livestock farming already formed a larger share of commodity products - 59.9%, and crop farming - 39.3%. The lion's share of commodity crop products has traditionally been grain and leguminous crops and sunflower. Livestock production, respectively, consisted of the sale of pork and beef, where the latter occupied an unfortunately low level - 1.8-3.9%. Of particular note is the following ratio between the crop and livestock sectors in the total volume of commodity production, which was 3 to 1 in 2021, 1:1 in 2022, and 1:1.5 in 2023. This was, of course, significantly influenced by the price situation in the domestic consumer market.

The main obstacle to the efficiency of the crop growing industry was, of course, the full-scale military invasion of our territory launched by Russia, which a priori entailed force majeure circumstances (especially at the beginning of the war, which was March, April, May 2022) a shortage of fuel (and sometimes its complete absence) for the 2022 sowing campaign, power outages, local blackouts, the threat of direct military invasion and shelling, closed ports and blocking of customs on the western border, prices on the domestic market for agricultural products, etc.).

The average purchase prices for wheat in 2021 were 9,650 UAH/t, sunflower seeds 19,650 UAH/t (AgroElita, 2021). In 2022, due to the above-mentioned force majeure factors, wheat prices halved to 4700-4800, and sunflower prices, respectively, 1.5 times to 12560 UAH/t (AgroElita, 2022). In 2023, due to the resumption of grain exports through the Black Sea ports and increased transit through land borders with Poland, Slovakia and Romania, they stabilized to some extent and amounted to 7400 UAH/t for wheat and 15120 UAH/t for sunflower, but still did not reach the level of 2021 (AgroElita, 2023).

Despite all these adversities, the agricultural producer, in particular the Agro-Novoselivka 2009 PE, maintained its production and production capacity and even ended both the first and second years of the war with a positive financial result (Table 5).

A detailed analysis of the financial results of the Agro-Novoselivka 2009 PE (Table 5) showed that the gross income of the enterprise in 2022, for the reasons already



indicated, decreased very significantly - by 22.2 million UAH. or 30.0%. In the same year, the management of the enterprise took drastic measures to stabilize production in both crop production and livestock farming. The result of this work was a sharp increase in gross output in 2023 to 133.3 million UAH., which is 37.1% and 77.3% higher than the results of the previous two years, respectively.

Table 5

**Economic efficiency of Agro-Novoselivka LLC 2009**

Indicators	2021 y.	2022 y.	2023 y.	2023, % to 2021
Gross income – total, thousand UAH	97206,0	74980,5	133317,7	137,1
Profit (loss), total, thousand UAH	11407,8	8973,4	12783,0	112,1
including: in crop production	9183,1	4016,8	5192,1	56,5
in livestock farming	2224,7	4956,6	7590,9	341,2
Profitability level – total, %	13,1	13,6	9,6	73,3
including: in crop production	18,4	5,5	3,8	20,7
in livestock farming	5,3	16,5	12,8	241,5
Gross output per 100 hectares of agricultural land, thousand UAH	3651,9	2686,2	4615,6	126,4
Profit production per 100 hectares of agricultural land, thousand UAH	428,6	321,5	442,6	103,3
including crop production	345,0	143,9	179,8	52,1
livestock farming	83,6	177,6	262,8	314,4

However, gross income characterizes the overall effect of the enterprise's work, and the economic component of the production process reveals the profit received from operating activities, which is the most important structural criterion of production. Thus, the profit of the enterprise as a whole in 2022 decreased by 2.43 million UAH. (27.1%) compared to 2021. However, in 2023 it has already grown almost one and a half times or by 3.81 million UAH. and even exceeded the level of 2021 by 1.37 million UAH or 27.1%.

In the context of individual sectors of the economy, a different picture has emerged. Let's analyze them separately. The crop sector in the first year of the war reduced its profits by more than half or by 5.16 million UAH. In contrast, in 2023, by applying a number of operational measures and adapting the process to critical conditions, profits were increased by 1.17 million UAH. or 29.3%. But due to the negative impact of the price of grain and industrial crops on the domestic market and despite the increase in gross production of crop products in physical terms, this indicator did not reach the level of 2021 and was almost twice as low (5.19 million UAH). Profits in the livestock sector in 2022 exceeded the similar indicator of 2021 by 2.73 million UAH. or 2.2 times. During the second year of the war, the profitability of this sector also tended to grow and exceeded the previous year by 2.63 million UAH. or 53.1%, and compared to 2021 it increased more than threefold - 5.57 million UAH. and amounted to 7.59 million UAH, respectively. Analysis of the structure of income by industry revealed the advantages of the livestock sector over crop production, especially in critical war conditions. Livestock production is less dependent on exports, while crop production is more export-dependent.

This trend is quite logical given the price situation on the domestic meat market. According to AgroPortal (2023), beef prices (in live weight) increased - for bulls with a live weight of more than 400 kg by 8%, and for cows - by 10% and are, depending on the



region, 58.00-62.00 UAH/kg. The first price increase was observed in early autumn 2022, as pre-war export volumes had already been established, which provoked a gradual increase in demand and market activation. In addition, the cost of products is also affected by macro-financial indicators: the level of inflation, subsidies, lending, etc.

A number of economists believe that the most objective and thorough indicator characterizing the economic efficiency of livestock farming is the production of products and profit per 100 hectares of agricultural (agricultural) land. The production of gross products per 100 hectares of agricultural land in 2022 decreased by almost 1 million UAH. or 36.0% compared to the previous year, in 2023 this indicator increased significantly: by almost 1 million UAH. (26.4%) compared to 2021 and by 1.92 million UAH. (71.8%) compared to 2022. As for the profit received per 100 hectares of agricultural land, it should be noted that it also decreased in 2022 by 107.1 thousand UAH. (33.3%), and in 2023 it increased by 14.0 thousand UAH compared to 2021. UAH. against 2022 by 121.1 thousand UAH. or 3.3% and 37.7%, respectively.

In terms of individual industries, it should be noted that the same trend is observed in crop production and livestock production as in gross output. That is, in the crop production sector, profit per 100 hectares of agricultural land in 2022 decreased by half or by 201 thousand UAH., and in 2023 it increased by 36 thousand UAH. (23.6%). In the livestock sector, this indicator in 2022 more than doubled or by 94 thousand UAH., and in 2023 it increased by 85 thousand UAH. (1.5 times) compared to 2022, and four times or 179 thousand UAH compared to 2021. It should be noted separately that in peacetime, crop production brought the farm three times more profit per 100 hectares of agricultural land than livestock production. However, under the influence of negative factors of the war, it was already inferior to the livestock industry in 2022 by 22.7%, and in 2023 - by 43.6%.

Therefore, we must summarize the following. The private enterprise "Agro-Novoselivka 2009" is an existing example of a medium-sized, modern agricultural enterprise of the grain and meat direction. Despite the difficult, and in the spring of 2022, critical situation in our country, the decrease in production and financial indicators in 2022, it withstood all the adversities, applied cardinal, quick decisions regarding production management, continued to produce and produced crop production products so necessary for the population agriculture and livestock farming and at the same time received positive financial results.

**Discussion of research results.** Before the war, economic instability and the economic crisis in the agro-industrial complex of Ukraine negatively affected the development of the livestock industry. From 2015 to 2021, the cattle population in Ukraine decreased by almost a third and amounted to 2.87 million heads, the cow population during this period decreased by 493 thousand heads and amounted to 2.64 million heads. Milk production volumes decreased by 1.9 million tons to 8.7 million tons. However, cow productivity during this period increased from 4.69 to 5.15 t/head.

Force majeure factors caused by the war accelerated the reduction in the cattle and cow populations by 488 thousand heads (22.6%) and 281 thousand heads (22.3%). Milk production during the two years of the war decreased by 1.28 million tons or 17.3%, despite an increase in cow productivity by 321 kg per year. Over the same period, beef production decreased by 53 thousand tons (20.9%).

Due to the war, rural households in Ukraine, according to FAO estimates, suffered about 2.25 billion USD in losses, 25% of agricultural households stopped or reduced production volumes due to the war, in front-line regions - 38%. The total share of cattle in households in the total number of livestock in the country decreased from 62.0% in 2021 to 57.3% in 2023, and cows to 69.7%. Despite the catastrophic impact of direct



factors of the war, households remain an important source of food supply. They produce 75.0% of meat and 37.8% of milk in Ukraine.

In general, the share of cows kept by agricultural enterprises in the total livestock has been growing dynamically in recent years - from 23.4% as of the beginning of 2015 to 29.2% at the beginning of 2023, and their meat and milk production in 2023 amounted to 25.0% and 62.2%, respectively.

In Ukraine, the processes of concentration of dairy farms have been taking place for a long time, because keeping a larger number of cows is considered to be more economically profitable. Small farms with a livestock of up to 100 cows occupy 42.5% of all enterprises (of which 28.0% - up to 50 heads), medium - 42.7%, large - over 500 - 9.7% and 1000 cows - 5.1%. However, the largest share of the cow population is concentrated in large enterprises (over 500 heads) 53.9%, in medium-sized enterprises – 40.0%, and in small ones – 6.1%. Accordingly, the amount of produced products (milk and meat) is distributed in the same proportion.

The reduction in the animal population is primarily caused by military operations in the territory of Ukraine. Also, the difficult macroeconomic situation in the agricultural sector has affected the work of enterprises and households, where animals are increasingly being sent for sale due to the financial inability to maintain them.

Today, 90% of agricultural enterprises in Ukraine are engaged in narrow specialization and concentrate only on the production of crop products, which is considered profitable and win-win. However, the critical force majeure conditions of the war emphasized that in similar conditions, the livestock industry has advantages compared to crop production. This is influenced by both systemic and force majeure factors.

It can be concluded that the livestock sector in the agricultural enterprises segment, despite the adverse conditions of martial law, began its growth in 2023. Along with other factors, it is due to the unfavorable situation in the field of crop production, where the cultivation of traditionally high-margin export-oriented crops has ceased to bring excess profits to agricultural enterprises. In contrast to the arguments of Antoshchenkova V. V. (2020) about the need for narrower specialization, it should be emphasized the need to diversify production and concentrate agricultural enterprises not on one, but on two or three types of products. It is desirable that these be the branches of crop production and livestock farming (most often - livestock farming, where two types of products are formed: milk and meat). And here we completely agree with a number of authors (Pidpala T. V., 2018, Shiyan N.I., 2019, Kernasyuk Y. V., 2020, Eifeel A., 2022), who believe that improving the economic mechanism in livestock farming lies in the plane of ensuring the level of profitability for commodity producers.

An analysis of the production activities of the medium-sized agricultural enterprise PP "Agro-Novoselivka 2009" of Novovodolazha district, Kharkiv region, which has a grain-meat specialization and is engaged in the production of grain, pork and beef, was conducted. Despite the force majeure factors of the war, the enterprise simply maintained production capacity, and ended both the first and second years of the war with a positive financial result. In 2022, 8.9 million profit was received with a profitability of 13.6%. In 2023, having applied a number of measures to overcome critical conditions, the farm increased its profit to UAH 12.7 million with a production profitability of 9.6%. It should be especially noted that the traditionally profitable crop growing sector of the enterprise could not withstand force majeure factors and significantly reduced its profits from UAH 9.1 million in 2021 to UAH 4.0 to 5.1 million in 2022 and 2023. The livestock sector, on the contrary and in two years of war increased its profitability from 2.2 to 7.6 million UAH. It should be noted that the ratio of crop and livestock sectors in the total



profit of the enterprise in 2021 was in favor of the first 3: 1, in 2022, livestock farming already exceeded the first by 25%, in 2023 - the ratio also changed in favor of livestock farming and amounted to 1.5: 1. This became possible due to the specialization of the enterprise not on one type of product, but on three: crop production (wheat and sunflower), pig meat and beef. In this regard, the statement of Antoshchenkova V. V. (2020) that specialized production, as a rule, is highly intensive and efficient, has a place to exist. As reported by Izhdolbina O. O. (2021), research experience shows that large-scale financial support from the state, as a rule, does not guarantee successful production of livestock products, which is more relevant for large-scale producers. Therefore, we believe that in crisis conditions, and especially in war and post-war periods, attention should be paid to the development of small-scale producers (up to 50 and up to 100 cows) with the use of specialization in two or three types of products and resource-saving technologies, including keeping livestock in open pastures, according to the technology adopted in the Agro-Novoselivka 2009 PE.

**Conclusions.** Based on the research conducted to assess the current state of development of Ukrainian livestock farming and the formulation of recommendations for increasing the efficiency of milk and meat production, the following conclusions can be drawn:

1. It has been proven that the state of the livestock industry was negatively affected by both the economic crisis in the agro-industrial complex of Ukraine and force majeure factors caused by the war. The number of cattle and cows in Ukraine decreased sharply from 2015 to 2021 by 1.1 million heads and 493 thousand heads, respectively, and during the two years of the war they decreased by another 488 and 281 thousand heads.

2. It has been determined that the total volume of milk production in Ukraine is declining. Milk production during the two years of the war decreased by 1.28 million tons or 17.3%, despite the increase in cow productivity by 321 kg per year. During the same period, beef production decreased by 53 thousand tons (20.9%). The reduction in total milk production is largely due to its decline in households. According to FAO estimates, in Ukraine, 25% of agricultural households have stopped or reduced production due to the war, in frontline regions - 38%.

3. It was determined that the share of cows kept by agricultural enterprises in the total livestock was growing dynamically and as of the beginning of 2023 reached 30.2%. Households, respectively, accounted for almost 70% of the total number of cows in the country. Meat and milk production by agricultural enterprises in 2023 amounted to 75.0% and 37.8%, respectively. In households, these figures were 25.0 and 62.2%, respectively.

4. It was established that the number of specialized dairy farms is increasing in Ukraine. Over the past ten years, the number of enterprises that keep cattle has halved and amounted to 1,533 units in 2022. Small farms with a livestock of up to 100 cows occupy 42.5% of all enterprises (of which 28.0% - up to 50 heads), medium - 42.7%, large - over 500 heads - 9.7% and 1,000 cows - 5.1%. The largest share of the cow population is concentrated in large enterprises (over 500 heads) 53.9%, in medium - 40.0%, and in small - 6.1%.

5. As of the beginning of 2022, more than 80% of agricultural enterprises in Ukraine were narrowly specialized and focused only on the production of crop products. The level of profitability of the operating activities of agricultural enterprises in 2015 was 43.0%, in 2021 it halved to 19.2%, in 2022, despite the beginning of aggression from the Russian Federation, it increased again to 41.9%, in 2023 it halved to 21.0%.

6. The analysis of the production activities of the medium-sized agricultural enterprise PE "Agro-Novoselivka 2009" of the Novovodolaz district, Kharkiv region showed that despite the force majeure factors of the war, the enterprise maintained



production capacity and received profits of 8.9 million UAH. in 2022 and 12.7 million UAH. 2023 with profitability of 13.6% and 9.6%, respectively. The crop sector could not withstand force majeure and reduced its profits in 2022 by half (by UAH 5.1 million), in 2023 it increased them by UAH 1.1 million, but still almost half as much as in 2021. The livestock industry, on the contrary, increased its profits in 2022 by two times (by 2.7 million UAH), in 2023 by another 1.5 times (by 2.6 million UAH).

7. Agricultural enterprises of Ukraine specializing in grain production are recommended to switch from narrow crop specialization to grain-meat or grain-meat-milk specialization with the ability to use available resources more efficiently and be able to withstand the impact of negative force majeure factors. In livestock farming, it is necessary to apply resource-saving technology with keeping livestock in open grazing areas.

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## **ACTIVITY OF ANTIOXIDANT ENZYMES IN THE BLOOD OF SHETLAND PONY MARES AND STALLIONS INVOLVED IN RECREATIONAL HORSEBACK RIDING: ROLE OF PHOTOPERIOD AND EXERCISE**

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*This study investigated seasonal, sex and exercise-induced variations in the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] in Shetland ponies. region (Pomeranian Voivodeship, northern part of Poland). Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) aged  $6.5 \pm 1.4$  years were used in this study. All horses participated in recreational riding. The training session started at 10:00 am, lasted 1 hour and consisted of a cross-country ride including walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min) and walking (10 min). Blood was collected from the animals' jugular veins in the morning, 90 min after feeding, while the horses were in the stable (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. The research highlights the complex interplay between environmental factors and physiological stressors in modulating antioxidant defence mechanisms. Significant seasonal variations in the activities of SOD, CAT and GPx were observed, with the enzymes showing increased sensitivity to exercise, particularly during the colder months. Mares showed a more pronounced exercise-induced decrease in SOD activity compared to stallions, especially during autumn and winter. Conversely, stable enzyme activity was observed in spring and summer, indicating reduced oxidative stress during milder seasons. Statistical analysis revealed significant seasonal differences in SOD and GPx activities, with higher coefficients of determination for SOD ( $R^2 = 0.45$ ) compared to CAT and GPx. The study suggests that mares have a greater oxidative response to exercise in colder seasons, highlighting sex-specific differences in antioxidant defences. These findings contribute to the understanding of how seasonal and exercise-related stressors influence antioxidant enzyme activity and highlight the role of environmental adaptations in equine health and performance. The results have practical implications for optimising exercise regimes and antioxidant supplementation in equine management. Further research is needed to explore the underlying mechanisms of these differences and their wider implications for animal health.*

**Keywords:** *superoxide dismutase, catalase, glutathione peroxidase, exercise, seasonal alterations, Shetland ponies, mares and stallions*



## АКТИВНІСТЬ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ У КРОВІ КОБИЛ ТА ЖЕРЕБЦІВ ШЕТЛАНДСЬКОГО ПОНІ, ЯКІ БЕРУТЬ УЧАСТЬ У РЕКРЕАЦІЙНІЙ ВЕРХОВІЙ ЇЗДІ: РОЛЬ ФОТОПЕРІОДУ ТА ФІЗИЧНОГО ТРЕНІНГУ

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У цьому дослідженні оцінювали сезонні та статеві зміни активності ключових антиоксидантних ферментів [супероксиддисмутази (SOD), каталази (CAT) і глутатіонпероксидази (GPx)] в крові кобил і жеребців шетландських поні до і після тренування. Ми проаналізували вплив фотоперіоду і фізичних вправ на рівні активності антиоксидантних ферментів 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  $6,5 \pm 1,4$  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). Проби крові відбирали один раз за сезон протягом року. Дослідження підкреслює складну взаємодію між факторами навколишнього середовища та фізіологічними стресовими факторами в модулюванні механізмів антиоксидантного захисту. Спостерігалися значні сезонні коливання активності SOD, CAT і GPx, при цьому ферменти демонстрували підвищену чутливість до фізичних вправ, особливо в холодні місяці року. Кобили показали більш виражене зниження активності SOD, спричинене фізичним навантаженням, порівняно з жеребцями, особливо восени та взимку. Навпаки, стабільна активність ферменту спостерігалася навесні та влітку, що вказує на зниження окиснювального стресу в більш м'які сезони. Статистичний аналіз виявив значні сезонні відмінності в активності SOD і GPx, з вищими коефіцієнтами детермінації для SOD ( $R^2 = 0,45$ ) порівняно з CAT і GPx. Дослідження показує, що кобили мають інтенсивнішу окиснювальну реакцію на фізичні вправи в холодну пору року, підкреслюючи відмінності в антиоксидантному захисті залежно від статі. Ці висновки сприяють розумінню того, як сезонні та пов'язані з фізичними навантаженнями стресори впливають на активність антиоксидантних ферментів, і підкреслюють роль адаптації навколишнього середовища для здоров'я та продуктивності коней. Результати мають практичне значення для оптимізації режимів фізичних вправ і антиоксидантних добавок у догляді за конями. Потрібні подальші дослідження, щоб дослідити основні механізми цих відмінностей та їхній ширший вплив на здоров'я тварин.



**Ключові слова:** супероксиддисмутаза, каталаза, глутатіонпероксидаза, фізичні навантаження, сезонні зміни, шетландські поні, кобили та жеребці.

**Introduction.** Oxidative stress, characterised by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence system, is a critical factor affecting animal health and performance (Durand D. et al., 2022). Antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) play a key role in mitigating oxidative stress by neutralising ROS and maintaining cellular homeostasis (Bhattacharyya A. et al., 2014; Kurutas E. B., 2016). In equine physiology, the activity of these enzymes can be influenced by various intrinsic and extrinsic factors, including exercise, environmental conditions and sex (Kirschvink N. et al., 2006; Andriichuk A. and Tkachenko H., 2017; Bażanów B. A. et al., 2020; Kurhaluk N. et al., 2022).

Recreational riding, a popular activity with Shetland ponies, involves varying degrees of physical exertion which may affect oxidative metabolism (Kurhaluk N. et al., 2022). Exercise-induced oxidative stress is well documented in horses, leading to increased ROS production through increased metabolic activity (Marañón G. et al., 2008; Yavari A. et al., 2015). Seasonal variations, such as changes in photoperiod and temperature, further influence metabolic and physiological processes and may modulate the antioxidant defence system (Chainy G. B. et al., 2016). The interplay between these factors remains an area of active research, particularly in smaller breeds such as Shetland ponies, which are often used for recreational purposes.

The Shetland pony, known for its resilience and adaptability, provides an excellent model for studying the effects of environmental and physiological stressors on antioxidant mechanisms (Brinkmann L. et al., 2012, 2014). Despite their robust nature, little is known about how photoperiod and exercise affect the activity of key antioxidant enzymes in mares and stallions. Understanding these dynamics is essential to optimise management practices and ensure the health and welfare of these animals.

This study aims to investigate the activity of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] in the blood of Shetland pony mares and stallions involved in recreational riding in the central Pomeranian region (Pomeranian Voivodeship, northern part of Poland). Specifically, we are investigating the effects of photoperiod (seasonal variation) and exercise on enzyme activity, providing insights into the adaptive responses of these animals to environmental and physical challenges. By identifying sex- and season-specific patterns, this research contributes to the growing body of knowledge on oxidative stress and its management in equine species.

#### **Materials and methods.**

**Horses.** The study followed the guidelines of the Council of the European Union and current legislation. Twenty-one healthy adult Shetland ponies (11 mares and 10 stallions) from the Central Pomeranian region of Poland (Strzelinko, N54°30'48.0" E16°57'44.9"), aged  $6.5 \pm 1.4$  years, were included. All ponies were involved in recreational riding. They were individually housed in box stalls, fed twice daily (hay and oats) at 8:00 am and 6:00 pm, and had unlimited access to water. Comprehensive clinical examinations and assessments of haematological, biochemical and vital parameters confirmed that all horses were within normal reference ranges. The mares were not in heat and were non-pregnant.

**Training protocol.** Exercise sessions started at 10:00 am, lasted 1 hour, and included the following sequence: walking (5 minutes), trotting (15 minutes), walking



(10 minutes), trotting (10 minutes), walking (5 minutes), galloping (5 minutes), and walking (10 minutes).

**Blood sample collection.** Blood samples were collected from the jugular vein in the morning, 90 minutes after feeding, while the horses were in their stalls (between 8:30 and 10:00) and immediately after the exercise session (between 11:00 and 12:00). Samples were taken once per season over one year: spring, summer, autumn, and winter. Blood was stored in tubes containing K<sub>3</sub>-EDTA or 3.8% sodium citrate and kept on ice until centrifugation at 3,000 rpm for 10 minutes. The plasma was separated and the erythrocyte suspensions were washed three times with five volumes of PBS (pH 7.35) and centrifuged at 3,000 rpm for 5 minutes.

**Assay of superoxide dismutase activity.** The Randox kit method (RANSOD, Cat. N SD 125, Randox Laboratories Limited, UK) was used to measure plasma superoxide dismutase (SOD) activity using xanthine and xanthine oxidase (XOD). The method proposed by Woolliams J. A. et al. (1983) and Suttle N. F. and McMurray C. H. (1983) was used. This method is based on the use of xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The results were calculated and expressed as U per mL.

**Assay of catalase activity.** Catalase (CAT) activity was determined by measuring the reduction of H<sub>2</sub>O<sub>2</sub> in the reaction mixture according to the method developed by Koroliuk M. A. et al. (1988). One unit of CAT activity was defined as the amount of enzyme required to degrade 1 μmol H<sub>2</sub>O<sub>2</sub> per minute per mL.

**Assay of glutathione peroxidase activity.** Glutathione peroxidase (GPx) activity was measured in blood by the standard method using a Ransel Glutathione Peroxidase Assay Kit (RX Monza, RS 504, Randox Laboratories Limited, UK). This method is based on GPx catalysis of the oxidation of glutathione by cumene hydroperoxide. All procedures followed the methodology proposed by Paglia D. E. and Valentine W. N. (1967) and Kraus R. J. and Ganther H. E. (1980) with our modification. The decrease in absorbance at 340 nm was measured on an Rx Monza analyser in a 1 cm cuvette light path at +37°C and expressed as U per mL.

**Statistical analysis.** Results are presented as mean ± S.D. Significant differences between means were determined using a multiple range test with a threshold of at least  $p < 0.05$ . Data that did not follow a normal distribution were log-transformed. Statistical tests with 95% confidence intervals ( $\alpha = 0.05$ ) were used to assess the significance of differences between the studied parameters (Stanisz A., 2006, 2007). Homogeneity of variance was assessed using Levene's test and normality was assessed using the Kolmogorov-Smirnov test.

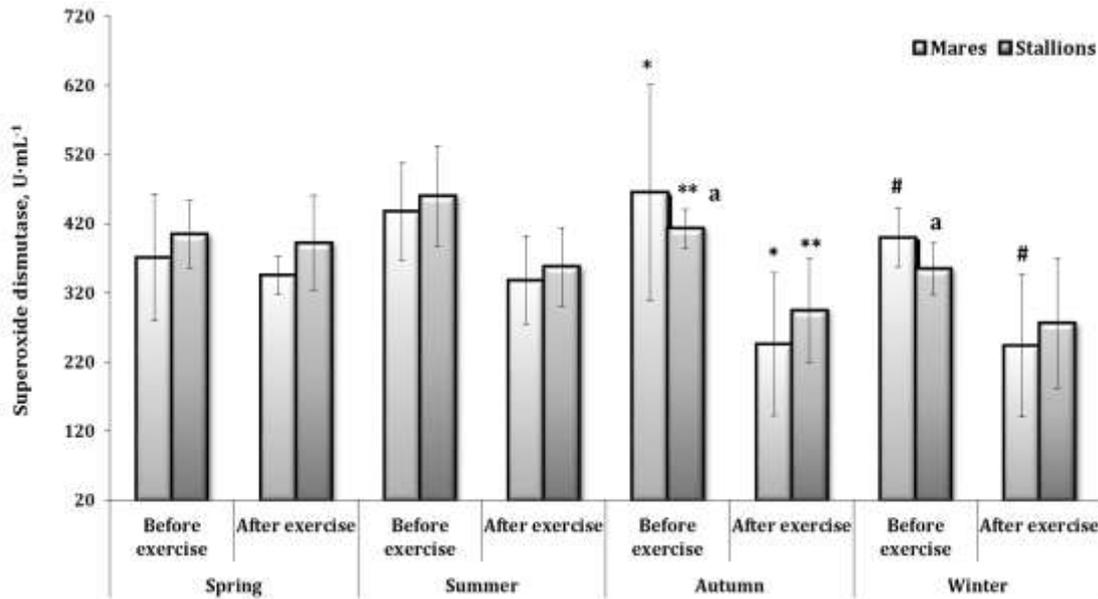
Parametric correlations were analysed using Pearson's regression analysis within the multiple regression module. The results of the MANOVA analysis were further supported by the sum of squares test (total SS model) versus residual SS, taking into account the multiple correlation analysis (R), the coefficient of determination (R<sup>2</sup>) and the adjusted coefficient of determination (R<sup>2</sup> adjusted), which takes into account random errors.

Basic statistical analyses, including significance of regression slopes and analysis of variance, were performed using the STATISTICA 13.3 software package (TIBCO Software Inc., USA). The SS test was used to quantify the contributions of all analysed biomarkers of oxidative stress and biochemical parameters in the assessment of antioxidant defences, using the F test and its significance (Stanisz A., 2006, 2007).

**Results.** We examined the activity of antioxidant enzymes in the blood of Shetland ponies under the influence of three factors: photoperiod, sex, and exercise. The



enzyme activity in the blood of Shetland pony mares and stallions, both before and after exercise, during spring, summer, autumn, and winter, is illustrated in Figures 1–3.



**Fig. 1. Superoxide dismutase activity in the blood of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in spring, summer, autumn, 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 autumn;

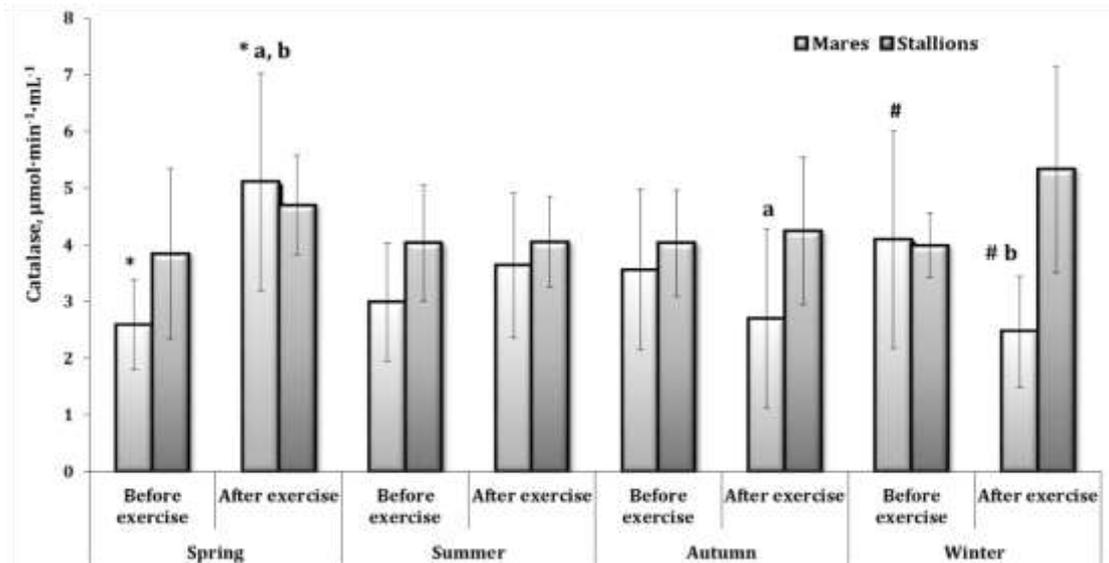
\*\* – between values obtained before and after exercise in stallions in autumn;

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

a – between values obtained in stallions before exercise in winter and autumn.

The results of our study showed that the blood SOD activity of Shetland pony mares in autumn was statistically significantly decreased after exercise to ( $245.7 \pm 102.89 \text{ U} \cdot \text{mL}^{-1}$ ) compared to the state before exercise ( $465.01 \pm 156.10 \text{ U} \cdot \text{mL}^{-1}$ ). On the other hand, a statistically significant decrease in blood SOD activity was observed in stallions after exercise ( $294.38 \pm 75.52 \text{ U} \cdot \text{mL}^{-1}$ ) compared to the state before exercise ( $413.06 \pm 27.95 \text{ U} \cdot \text{mL}^{-1}$ ). The percentage decrease was 47.2% ( $p < 0.05$ ) for mares and 28.7% ( $p < 0.05$ ) for stallions. Similarly, in winter, SOD activity in the blood of mares of Shetland ponies was statistically significantly decreased after exercise to ( $243.60 \pm 103.10 \text{ U} \cdot \text{mL}^{-1}$ ) compared to the state before exercise ( $399.89 \pm 42.80 \text{ U} \cdot \text{mL}^{-1}$ ). The percentage decrease was 39.1% ( $p < 0.05$ ). Blood SOD activity in Shetland pony mares was statistically significantly lower before exercise in autumn ( $399.89 \pm 42.80 \text{ U} \cdot \text{mL}^{-1}$ ) compared to before exercise in winter ( $465.01 \pm 156.10 \text{ U} \cdot \text{mL}^{-1}$ ). The percentage decrease was 14% ( $p < 0.05$ ). After exercise, SOD activity in the blood of both mares and stallions of summer and spring was statistically non-significantly lower than before exercise. Higher levels of SOD activity were observed in the blood of mares compared to stallions in autumn and winter (Fig. 1).

Catalase activity in the blood of Shetland pony mares and stallions before and after exercise in spring, summer, autumn, and winter were illustrated in Figure 2.



**Fig. 2. Catalase activity in the blood of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in spring, summer, autumn, 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 spring;

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

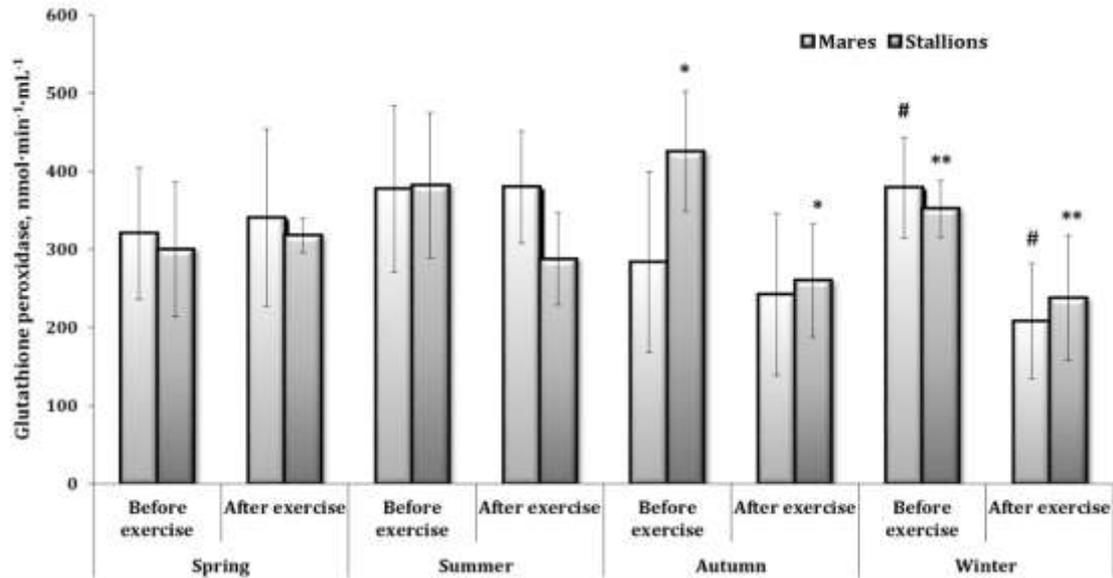
a – between values obtained in mares after exercise in spring and autumn;

b – between values obtained in mares after exercise in spring and winter.

The results of our study showed that catalase activity in the blood of Shetland pony mares in autumn was statistically significantly increased after exercise to ( $5.11 \pm 1.92 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) compared with the pre-exercise state ( $2.59 \pm 0.79 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ). The percentage of increase was 97.3% ( $p < 0.05$ ). Conversely, in winter, catalase activity in the blood of mares of Shetland ponies was statistically significantly decreased after exercise to ( $2.47 \pm 0.98 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) compared with the pre-exercise state ( $4.09 \pm 1.92 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ). The percentage reduction was 39.6% ( $p < 0.05$ ). Blood catalase activity in Shetland pony mares was statistically significantly lower after exercise in autumn ( $2.70 \pm 1.58 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) and winter ( $2.47 \pm 0.98 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) than before exercise in spring ( $5.11 \pm 1.92 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ). The percentage decreases were 47.2% ( $p < 0.05$ ) and 51.7% ( $p < 0.05$ ) respectively. In summer and spring, catalase activity in the blood of both mares and stallions after exercise was not statistically significantly higher than before exercise. Higher levels of catalase activity were observed in the blood of stallions compared to mares in all seasons (Fig. 2).

GPx activity in the blood of Shetland pony mares and stallions before and after exercise in spring, summer, autumn, and winter were illustrated in Figure 3.

The results of our study showed that GPx activity in the blood of Shetland pony stallions in autumn was statistically significantly decreased after exercise to ( $260.13 \pm 72.71 \text{ nmol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) compared with the pre-exercise state ( $425.54 \pm 77.07 \text{ nmol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ). The percentage reduction was 38.9% ( $p < 0.05$ ). Similarly, in winter, GPx activity in the blood of mares of Shetland ponies was statistically significantly decreased after exercise to ( $208.31 \pm 73.82 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) compared with the pre-exercise state ( $379.12 \pm 64.39 \text{ nmol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ). The percentage reduction was 45.1% ( $p < 0.05$ ). Blood GPx activity in Shetland pony mares and stallions was at the same level during exercise in both spring and summer (Fig. 3).



**Fig. 3. Glutathione peroxidase activity in the blood of Shetland pony mares (n = 11) and stallions (n = 10) before and after exercise in spring, summer, autumn, 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 stallions in autumn;

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

# – between values obtained before and after exercise in mares in winter.

**Discussion.** The results of this study demonstrate distinct seasonal, sex and exercise-induced variations in the activity of key antioxidant enzymes (SOD, catalase and GPx) in the blood of Shetland ponies. These variations highlight the complex interplay of environmental and physiological factors in modulating antioxidant defence mechanisms in horses.

This study identifies GPx, CAT and SOD as key markers of enzymatic antioxidant defence. These enzymes are considered part of the first-line antioxidant defense system as they rapidly neutralize free radicals or molecules with the potential to form free radicals (Jomova K. et al., 2024). SOD activity showed a marked decrease in both mares and stallions following exercise in autumn and winter, with mares showing a more pronounced reduction than stallions (Fig. 1). This suggests that mares may have an increased oxidative response to exercise during these colder seasons. The seasonal comparison showed that SOD activity was significantly lower in autumn than in winter before exercise, suggesting possible seasonal adaptations in oxidative stress management. Conversely, no significant changes in SOD activity were observed in spring and summer, probably reflecting a more stable oxidative state during these milder seasons (Fig. 1). In our study, the activity of this enzyme showed higher statistically significant variability ( $F_{15,152} = 8.34$ ;  $p = 0.000$ ) compared to the activities of CAT ( $F_{15,152} = 4.18$ ;  $p = 0.000$ ) or GPx ( $F_{15,152} = 5.95$ ;  $p = 0.000$ ). This finding was further supported by the correlation (R) and determination coefficients, as well as the corrected form of the latter used in the statistical analysis. In our study, SOD activity was more strongly influenced by the sex and exercise variables, but not by the photoperiodic factor. However, the differences in SOD activity were statistically significant in the total SS model versus the residual SS, with correlation coefficient values ( $R = 0.67$ ), coefficient of determination ( $R^2 = 0.45$ ), and the adjusted form ( $R^2_{adj} = 0.40$ ) at  $F = 8.34$  ( $p = 0.000$ ).



In the study of Bażanów B. A. et al. (2020), these researchers observed significantly higher activities of total SOD, MnSOD, and CuZnSOD in blood samples from Hucul horses compared to Arabian horses. The superoxide dismutase (SOD) family serves as the first line of defense against ROS (Wang Y. et al., 2018). Changes in SOD activity are known to protect hosts against microorganisms, bacteria, and parasites, with such variations influenced by physical activity, diet, and chemical factors (Rahal A. et al., 2014).

SOD activity was positively associated with long-term improvements in maximal oxygen uptake in horses following a 12-week conditioning programme in the study by de Moffarts B. et al. (2004). However, during a single exercise session, SOD activity does not appear to change significantly during short duration, high intensity work. In Standardbreds undergoing a 14-minute high-intensity treadmill test, no changes in resting, peak or recovery SOD activity were observed, although a reduction in reduced glutathione levels was noted (de Moffarts B. et al., 2004). Similarly, Ji L. L. et al. (2001) found no significant changes in SOD, catalase or glutathione peroxidase activity in response to acute exercise when measured at rest, 2 minutes and 30 minutes after a 12-minute high-intensity treadmill session.

The response of SOD activity to prolonged, lower intensity exercise is less well understood. Balogh N. et al (2001) reported no significant differences in SOD activity when measured 24 hours before, immediately after and 24 hours after exercise in horses competing in a pentathlon. This event involved two consecutive rounds of 20-minute warm-up followed by 1-minute jumping sessions for each horse, although no measurements were taken during the recovery period after the second round. The response of SOD activity to low-intensity standardised exercise tests remains unclear. In addition, the nutritional status of the horses used in these studies was not reported, which could influence the functionality of their antioxidant systems.

A longitudinal cohort study was conducted by Bollinger L. et al. (2023) with 49 healthy horses participating in the 160 km endurance ride at the 2016 World Endurance Riding Championships in Samorin, Slovakia. The study of Bollinger L. et al. (2023) found that lower pre-ride SOD levels were associated with better race results. It is possible that SOD levels are altered primarily after prolonged exercise, such as endurance rides over 120 km (Ono K. et al., 1990). This is consistent with the findings of Kinnunen S. et al. (2005) who reported that general resting markers of oxidative stress – such as oxygen radical scavenging capacity, vitamin E levels, lipid hydroperoxide (LPO) concentrations and glutathione-related enzyme activities - were higher in endurance horses than in trotters. However, even prolonged exercise, such as an 80 km ride, did not induce oxidative stress in endurance horses (Kinnunen S. et al., 2005).

Although it was initially expected that higher pre-ride SOD levels would reduce the likelihood of elimination, the opposite was observed. SOD levels are known to increase after prolonged exercise, suggesting that horses with higher pre-ride SOD levels may have experienced sustained oxidative stress prior to sampling. This stress could be due to overtraining or inadequate recovery periods (Balogh N. et al., 2001; Ferrarresso R. L. et al., 2012). Blood SOD levels reflect current SOD activity and oxidative stress defence rather than stored SOD reserves (Bollinger L. et al., 2023).

After a race, lower SOD availability in failed horses compared to successful horses may make them more susceptible to oxidative damage (Bollinger L. et al., 2023). Further studies, possibly including muscle biopsies to assess muscle SOD levels prior to competition, may provide more detailed insights. Currently, there is limited data on pre-ride or resting SOD levels in endurance horses, apart from a small sample studied by Fraipoint A. et al. (2011). As a result, it remains unclear which levels are indicative of a



healthy resting state and which are indicative of increased SOD activity due to ongoing oxidative stress.

CAT is a cytoplasmic protein that plays a crucial role in reducing hydrogen peroxide by catalyzing its conversion into water and oxygen. The efficiency of CAT in metabolizing hydrogen peroxide is dependent on the level of hydrogen peroxide accumulation (Nandi A. et al., 2019). Catalase activity showed contrasting seasonal patterns (Fig. 2). In autumn, exercise induced a significant increase in catalase activity in mares, suggesting an upregulated response to counter oxidative stress. In winter, however, catalase activity decreased significantly after exercise, possibly indicating a reduced capacity for antioxidant defence during the colder months. Stallions had consistently higher catalase activity in all seasons, which may reflect sex differences in enzymatic antioxidant defences (Fig. 2). Plasma CAT activity showed statistically significant differences only in the group of mares after exercise during the spring-summer and spring-winter photoperiods. The following coefficients were calculated for CAT activity: correlation coefficient ( $R = 0.54$ ), coefficient of determination ( $R^2 = 0.29$ ), and its adjusted form ( $R^2_{adj} = 0.22$ ) at  $F = 4.18$  ( $p = 0.000$ ). For GPx activity, the statistical analysis revealed the following values:  $R = 0.61$ ,  $R^2 = 0.37$ , and  $R^2_{adj} = 0.31$  at  $F = 5.94$  ( $p = 0.000$ ). Therefore, the activities of the selected antioxidant enzymes showed the following relationships in the statistical model:  $SOD > GPx > CAT$ . Siqueira R. F. et al. (2014) reported that prolonged endurance exercise had no effect on CAT activity in racehorses.

Another enzyme involved in maintaining redox balance is GPx, which removes  $H_2O_2$  by converting it to water and reducing lipid peroxides to their corresponding alcohols. This function is essential for the prevention of lipid peroxidation (Pei J. et al., 2023). GPx activity followed similar trends, with significant decreases observed in stallions during autumn and in mares during winter after exercise (Fig. 3). These findings highlight the sensitivity of GPx to both seasonal and exercise-induced oxidative challenges, particularly during the colder months when environmental stressors may exacerbate oxidative demands. The consistent GPx levels observed in spring and summer further support the notion of seasonal stability of antioxidant enzyme activity during these periods (Fig. 3). The observed seasonal and gender differences in enzyme activity may be due to variations in metabolic demands, hormonal influences and environmental conditions, including photoperiod and temperature. Exercise-induced oxidative stress appears to be modulated differently by season and sex, reflecting the dynamic adaptability of Shetland ponies to their environment.

This finding is consistent with previous research suggesting that antioxidant enzyme activity may decrease during intense exercise due to increased ROS production, but may return to higher levels during recovery (Fisher-Wellman K. and Bloomer R. J., 2009). Conversely, Balogh N. et al. (2001) reported no significant changes in SOD or GPx activities in pentathlon horses between blood samples taken before, immediately after and 24 hours after exercise. Ott E. C. et al. (2022) observed unchanged GPx activity after prolonged exercise in horses. However, their study reported an increase in SOD activity, another key marker of enzymatic antioxidant defence.

In our previous study (Kurhaluk N. et al., 2022), we aimed to determine the photoperiod-induced variations and the effects of exercise on oxidative stress biomarkers (2-thiobarbituric acid reactive substances [TBARS], aldehyde [AD] and ketone [KD] derivatives of oxidatively modified proteins [OMP], total antioxidant capacity [TAC], and biomarkers of metabolic changes (glucose, urea and uric acid, and lactate dehydrogenase [LDH] activity) in the blood of Shetland pony mares and stallions involved in recreational riding. A MANOVA analysis showed that photoperiod played a



predominant role in the changes in these biomarkers, while exercise and sex had a lesser effect. Lipid peroxidation biomarkers, such as plasma TBARS levels, showed the highest adjusted coefficient of determination ( $R^2_{adj} = 0.77$ ). Pre-exercise (resting) plasma TBARS levels in stallions and mares were lowest during the spring and summer photoperiods and highest during the autumn and winter photoperiods. A statistically significant reduction in the levels of both aldehyde and ketone derivatives of OMP was observed in the blood of ponies during the autumn and winter periods; in addition, the level of ketone derivatives of OMP decreased after exercise in the spring. TAC was statistically significant in the spring and winter photoperiods, both before and after exercise. Photoperiod and exercise-induced changes in markers of oxidative stress and antioxidant defences may play a role in the adaptation of animals to exercise, with sex differences observed. Seasonal variations in antioxidant defences and energy metabolism substrates in the blood of mares and stallions as a function of exercise capacity may be crucial in understanding how animals' endogenous adaptive mechanisms prepare for environmental changes associated with different seasons (Kurhaluk N. et al., 2022).

**Conclusions.** This study highlights the significant influence of photoperiod, sex and exercise on antioxidant enzyme activity in Shetland ponies. Antioxidant enzyme activity was generally more affected during autumn and winter, indicating increased oxidative stress during these seasons. Stable enzyme activity in spring and summer suggests reduced oxidative stress during these periods. Mares were more sensitive to exercise-induced oxidative stress in colder seasons, while stallions had higher overall catalase activity. Exercise consistently modulated antioxidant enzyme activity, with the magnitude and direction of change influenced by both season and sex.

These results provide valuable insights into the physiological adaptability of Shetland ponies to environmental and physical stressors. The findings may inform strategies for optimising equine management practices, including seasonally adapted exercise regimes and dietary supplementation to support antioxidant defences. Future research should explore the underlying mechanisms driving these variations and assess their implications for equine health and performance.

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## ASSESSMENT OF GENETIC DIVERSITY IN POPULATIONS OF DAIRY CATTLE BREEDS OF UKRAINIAN SELECTION USING MICROSATELETT MARKERS

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*The article presents the results of a study of the genetic and population structure in herds of Ukrainian Black-and-White and Red-and-White dairy breeds kept in the Kharkiv region (DPDH "Hontarivka"). The analysis of genetic variability in the experimental groups of animals was carried out using 10 microsatellite loci recommended by FAO-ISAG: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA12, ETH10. The amplification products were separated in native polyacrylamide gels of different concentrations (5 – 8 %). All studied loci were found to be polymorphic. The number of detected alleles per locus ranged from 4 to 8 (on average 5 alleles per locus) the size of which ranged from 115 bp (ETH3) to 307 bp (ILSTS006). The vast majority of studied loci belong to informatively valuable markers ( $PIC > 0.5$ ). The most polymorphic loci for both breeds were TGLA053 (8 alleles), BM2113 (6) and ETH3(6). The main population genetic parameters were calculated for the studied loci. The highest values of heterozygosity indices ( $H_e$ ) and effective number of alleles ( $n_e$ ) were characteristic of the BM2113 locus ( $H_e=0.80-0.81$ ,  $n_e=5.1-5.3$ ). The minimum values of expected heterozygosity were established for the ETH3 loci (0.53-0.55; Ukrainian Black-and-White and Red-and-White dairy breeds) and BM1818 (0.59, Ukrainian Black-and-White dairy breed).*

*For most microsatellite loci, an equilibrium state between the actual and expected genotype frequencies is characteristic. A probable deviation in the form of a deficit of heterozygotes was established only for the BM1818 locus in both experimental populations ( $F_{is} = 0.37$ ;  $p < 0.05$ ).*

*Changes in the genetic structure of the experimental cattle population (Kharkiv region) were analyzed in comparison with data from previous years of research, other regions, and with data from the initial forms involved in the creation of these breeds. Analysis of genetic changes that occurred during the reproduction of experimental cattle populations indicates a narrowing of genetic variability and the need to control genetic processes in breeding work.*

**Keywords:** microsatellites, polymorphism, population, cattle, allele, genotype, heterozygosity.



## **ОЦІНКА ГЕНЕТИЧНОГО РІЗНОМАНІТТЯ В ПОПУЛЯЦІЯХ МОЛОЧНИХ ПОРІД УКРАЇНСЬКОЇ СЕЛЕКЦІЇ ЗА ВИКОРИСТАННЯ МІКРОСАТЕЛІТНИХ МАРКЕРІВ**

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*У статті наведені результати дослідження генетико-популяційної структури в стадах корів українських чорно-рябої та червоно-рябої молочних порід, які утримуються в Харківській області (ДПДГ «Гонтарівка»). Аналіз генетичної мінливості в дослідних групах тварин проводили з використанням 10 мікросателітних локусів рекомендованих FAO-ISAG: ETH225, VM2113, ETH3, VM1818, VM1824, ILSTS006, INRA023, TAGLA053, TAGLA12, ETH10. Продукти ампліфікації розділяли в нативних поліакриламідних гелях різних концентрацій (5 – 8 %). Всі досліджені локуси виявились поліморфними. Кількість виявлених алелів на локус коливалась від 4 до 8 (у середньому 5 алелів на локус) розмір яких знаходився в межах від 115 п.н. (ETH3) – до 307 п.н. (ILSTS006), Переважна більшість досліджених локусів належить до інформативно цінних маркерів ( $PI_C > 0,5$ ). Найбільш поліморфними для обох порід виявились локуси TGLA053 (8 алелів), VM2113 (6) та ETH3(6). Розраховано основні популяційно-генетичні параметри за досліджуваними локусами, Найвищі значення показників гетерозиготності ( $H_e$ ) і ефективної кількості алелів ( $n_e$ ) були властиві локусу VM2113 ( $H_e=0,80-0,81$ ,  $n_e=5,1-5,3$ ). Мінімальні значення очікуваної гетерозиготності встановлені для локусів ETH3 (0,53-0,55; УЧР, УЧер) і VM1818 (0,59, УЧР).*

*Для більшості мікросателітних локусів властивим є рівноважний стан між фактичними і очікуваними показниками частот генотипів, Вірогідне відхилення у вигляді дефіциту гетерозигот встановлено лише для локусу VM1818 в обох дослідних популяціях ( $F_{is} = 0,37$ ;  $p < 0,05$ ).*

*Проаналізовано зміни в генетичній структурі дослідних популяцій корів (Харківська обл.) порівняно з даними попередніх років досліджень, інших регіонів та з даними вихідних форм, задіяних у створенні цих порід. Аналіз генетичних змін, які відбувалися в процесі відтворення дослідних популяцій ВРХ свідчить про звуження генетичної мінливості та необхідність контролю генетичних процесів в селекційно-племінній роботі.*

**Ключові слова:** мікросателіти, поліморфізм, популяція, корови, алель, генотип, гетерозиготність

**Introduction.** To solve a number of tasks related to the scientific support of breeding work, in particular regarding the certification of animal breeds, determining the level of consolidation of created groups and the degree of genetic differentiation of populations, a separate class of molecular genetic markers - microsatellites - is widely used (Debrauwere H. et al., 1997, Senan S. et al., 2014). Due to the high level of polymorphism of microsatellite markers (SSR, Simple Sequence Repeat), which is



reflected in a larger number of alleles per locus compared to classical biallelic systems, microsatellites can be used as a rather subtle and effective tool for studying genetic variability, which allows successfully solving the entire range of these issues (Shelyov, 2015; Mishra S. et al., 2017, Zhao J. et al., 2017). SSR markers are considered to be selection-neutral and, due to their wide localization in the genome, are of interest for controlling genetic processes that occur in artificially reproduced animal populations (Shelyov, 2017, Al-Jubori & Senkal, 2023). Given the importance of this issue in the context of preserving the biodiversity of animal breeds, the international organizations FAO and ISAG have proposed microsatellite panels for the main species of farm animals and recommendations for their use in scientific research (ISAG/FAO, 2004, FAO, 2011). According to the FAO recommendations for cattle, 30 microsatellite loci have been identified with localization in each chromosome. ISAG proposes the use of the 12 most polymorphic of them. The first publications on the polymorphism of microsatellite loci in cattle (*Bos taurus*, Bovidae) appeared in the early 90s of the 20th century, and to date, a considerable amount of information has been accumulated on the genetic variability of individual breeds within this type of farm animals.

In Ukraine, studies of microsatellite variability in cattle have mainly concerned local herds of indigenous breeds of domestic selection, which are valuable as carriers of specific biological and economic characteristics for specific geoclimatic breeding conditions. These are breeds such as Ukrainian gray (Shkavro N. et al., 2010), Lebedynskaya (Shkavro et al., 2018, Ladyka et al., 2019), red steppe (Kramarenko et al., 2018), southern meat (Kramarenko, 2019), buffaloes (Dzitsiuk et al., 2020).

**Purpose of the study.** Changes occurring in the genetic structure of populations of factory-type breeds used in intensive milk and meat production are also of great interest from the point of view of optimizing the selection and breeding process in the direction of maintaining biological diversity.

In Ukraine, the vast majority of dairy cattle are represented by two breeds - ukrainian black-and-white and ukrainian red-and-white dairy breeds (glady m.v. et al., 2015, vyshnevskiy et al., 2019). more than 28 years have passed since the official registration of these breeds (black-mottled (1996), red-mottled (1993)). there are data on the number of allelic variants of microsatellite loci of cattle of 24 breeds bred in Ukraine, including the ukrainian black-and-white and red-and-white dairy breeds (podoba b.e. et al., 2013). among the publications over the past 5-7 years, one can note the work of shelyov a.v. et al. (2017) on the study of microsatellite variability in herds of ukrainian black-and-white and red-and-white dairy breeds of the kyiv region. another joint publication concerned microsatellite variability in cattle populations of ukrainian and russian origin (snegin et al., 2019).

Given the wide distribution area of Ukrainian black-and-white and red-and-white dairy breeds, our goal was to analyze changes in the genetic structure of the experimental cow population in the Eastern region of Ukraine (Kharkiv region) compared with data from previous years of research, other regions, and data from the initial forms involved in the creation of these breeds.

**Materials and methods of research.** The population of cows of Ukrainian black-and-white and red-and-white dairy breeds (DPDH "Hontarivka" of Vovchansky district of Kharkiv region) was used as the object of research. The sample consisted of 30 individuals for each experimental population. DNA isolation was performed from hair follicles using the "NeoPrep DNA" reagent set (Lab Neogene P.C., Ukraine).

According to the recommendations of FAO-ISAG, 10 microsatellite loci were selected for research: ETH225, BM2113, ETH3, BM1818, BM1824, ILSTS006, INRA023, TAGLA053, TAGLA122, ETH10 (Table 1).



Table 1

**Nucleotide sequences of primers for microsatellite loci**

№	Microsatellite	Primers	Annealing, °C	Amplicon, bp
1	ETH225 (Chromosome 9)	gatcaccttgccactatttct; acatgacagccagctgctact	58	131-159
2	BM2113 (Chromosome 2)	gctgccttctaccaaataccc; cttctgagagaagcaacacc	58	122-156
3	ETH3 (Chromosome 19)	gaacctgcctctcctgcattgg; actctgcctgtggccaagtagg	60	103-133
4	BM1818 (Chromosome 23)	agctgggaatataaccaaagg; agtgccttcaaggctcatgc	58	248-278
5	BM1824 (Chromosome 1)	gagcaaggtgttttccaate; cattctccaactgcttctctg	56	176-197
6	ILSTS006 (Chromosome 7)	tgtctgtatttctgctgtgg; acacggaagcgatctaaacg	56	277-309
7	INRA023 (Chromosome 3)	gagtagagctacaagataaacttc; taactacaggggtgtagatgaactc	58	195-225
8	TGLA53 (Chromosome 16)	gcttcagaaatagtttcattca; atcttcacatgatattacagcaga	58	143-191
9	TGLA122 (Chromosome 21)	ccctcctccaggtaaatcagc; aatcacatggcaataagtacatac	58	136-184
10	ETH10 (Chromosome 5)	gttcaggactggccctgctaaca; cctccagcccactttctctctc	62	207-231

Amplification of fragments of the studied loci was carried out using a thermocycler "Amply-4" (Biocom, Russia) using the appropriate program: 1 cycle - denaturation 94°C 3 min; 35 cycles - denaturation 94°C 30 s, annealing 30 s (56-62 °C depending on the locus), elongation 72°C 50 s; 1 cycle - final elongation 72 °C 10 min. The volume of the reaction mixture was 10 µL, which included 5 µL of Mastermix (2×buffer with 4 mM MgCl<sub>2</sub>, 0.4 mM dNTP mixture and 0.5 units of DreamTaq DNA polymerase (Thermo Scientific), 2.5 µL of 1 mM primer and 2.5 µL of DNA template.

The amplification products were separated in polyacrylamide gels of different concentrations (5-8%), both native and denaturing. Gels were stained using ethidium bromide (visualization was performed in the ultraviolet spectrum) or silver nitrate. The size of the fragments was determined using molecular mass markers pUC19 and O'RangeRuler 20 bp («Thermoscientific», USA). Calculation of molecular masses of amplification products was performed using the GelAnalyzer program (Version 2010a freeware).

Genotyping of individuals by a set of microsatellite markers in native polyacrylamide gels was performed according to the authors' method (Kulibaba & Liashenko, 2016).

Based on the obtained data, genotype and allele frequencies, actual ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, effective number of alleles ( $n_e$ ), Wright fixation index ( $F_{is}$ ) was calculated, and the Hardy-Weinberg genotype distribution was checked using the GenAIEx 6.503 add-in integrated into Excel (Peakall & Smouse, 2012.) (<https://biology-assets.anu.edu.au/GenAIEx/Download.html>).

**Research results.** According to the results of the studies, it was found that all microsatellite loci used in the experimental animal populations are polymorphic (the proportion of polymorphic loci was 100%).



The number of detected alleles per locus ranged from 4 (ETH10, etc.) to 8 (TGLA53). The analysis of the obtained results of genotyping of individuals allowed to detect a total of 51 alleles for 10 microsatellite loci, the size of which was in the range from 115 bp (ETH) to 307 bp (ILSTS006) (Table 2).

Table 2

**Allele frequencies of the studied breeds for 10 loci**

Locus	Allele, bp	Black-and-White	Red-and-White	Red-and-White	Red-and-White	Red-and-White	Red-and-White
<i>ETH</i> <i>225</i>	140	0.41	0.37	<i>TGLA</i> <i>053</i>	160	0.03	0.03
	146	0.15	0.18		168	0.06	0.06
	150	0.05	0.04		170	0.54	0.46
	152	0.19	0.16		174	0.07	0.1
	154	0.2	0.25		178	0.06	0.07
<i>TGLA</i> <i>122</i>	148	0.14	0.11	<i>ETH</i> <i>3</i>	115	0.61	0.26
	152	0.35	0.28		117	0.02	0.02
	156	0.02	0.06		119	0.03	0.02
	160	0.40	0.45		121	0.03	0.04
	172	0.09	0.10		125	0.03	0.03
<i>BM</i> <i>2113</i>	125	0.16	0.23	<i>ILSTS</i> <i>006</i>	127	0.28	0.63
	127	0.25	0.15		291	0.26	0.07
	135	0.06	0.13		295	0.17	0.22
	137	0.12	0.07		301	0.22	0.31
	139	0.26	0.17		307	0.35	0.40
	141	0.15	0.25		<i>BM</i> <i>1818</i>	266	0.30
<i>BM</i> <i>1824</i>	190	0.35	0.42	268		0.13	0.13
	192	0.05	0.07	276		0.55	0.35
	194	0.24	0.29	278		0.02	0.04
	196	0.36	0.22	<i>ETH</i> <i>10</i>	216	0.14	0.17
<i>INRA</i> <i>023</i>	199	0.02	0.03		218	0.23	0.25
	203	0.07	0.17		222	0.17	0.43
	211	0.52	0.33		224	0.46	0.15
	215	0.05	0.06				
	219	0.34	0.41				

The most polymorphic loci in terms of the number of alleles were the TGLA53 loci (8 alleles) and 2 loci that had 6 alleles (BM2113 and ETH3). The studied microsatellite loci differed significantly in terms of the number of detected genotypes. Thus, for the most polymorphic locus TGLA53, out of 32 possible genotypes for the black and red-and-pigmented breeds, only 15-16 were detected. At the same time, the frequency of occurrence of two genotypes out of all detected was about 50%, and for 11 genotypes the frequency of occurrence was less than 5%. This distribution of genotypes affected the value of the effective number of alleles ( $n_e$ ), which was 3.0-3.9 (37-48%) (Table 3)



Table 3

**Main genetic and population indicators of the studied breeds by microsatellite loci**

Locus	Parameter											
	N <sub>a</sub>		n <sub>e</sub>		H <sub>o</sub>		H <sub>e</sub>		F <sub>is</sub>		PIC	
	I	II	I	II	I	II	I	II	I	II	I	II
ETH225	5	5	3,71	3,86	0,69	0,71	0,73	0,74	0,05	0,04	0,70	0,72
BM2113	6	6	5,10	5,30	0,82	0,77	0,80	0,81	-0,02	0,05	0,79	0,80
ETH3	6	6	2,20	2,14	0,59	0,57	0,55	0,53	-0,07	-0,08	0,50	0,49
BM1818	4	4	2,44	2,69	0,37	0,40	0,59	0,63	0,37	0,37	0,55	0,59
BM1824	4	4	3,20	3,19	0,66	0,68	0,69	0,69	0,04	0,01	0,65	0,66
ILSTS006	4	4	3,74	3,23	0,70	0,66	0,73	0,69	0,04	0,04	0,70	0,65
INRA023	5	5	2,54	3,22	0,63	0,65	0,61	0,69	-0,03	0,06	0,57	0,67
TGLA53	8	8	3,02	3,88	0,64	0,72	0,67	0,74	0,04	0,03	0,68	0,72
TGLA122	5	5	3,22	3,26	0,67	0,65	0,69	0,69	0,03	0,06	0,66	0,65
ETH10	4	4	3,19	3,35	0,70	0,74	0,69	0,70	-0,01	-0,06	0,67	0,68
Mean	5,1± 0,41	5,1± 0,41	3,24 ± 0,26	3,41 ± 0,26	0,65 ± 0,04	0,66 ± 0,03	0,67 ± 0,02	0,69 ± 0,02	0,04 ± 0,04	0,05 ± 0,04	0,65 ± 0,03	0,66 ± 0,03

Notes: I – Ukrainian Black-and-White dairy breed; II – Ukrainian Red-and-White dairy breed; N<sub>a</sub> – number of alleles; n<sub>e</sub> – effective number of alleles; H<sub>o</sub> – observed heterozygosity; H<sub>e</sub> – expected heterozygosity; F<sub>is</sub> – Wright’s fixation index; PIC – Polymorphic Information Content.

Among the 6-allelic loci for dairy breeds, the most balanced in terms of allele frequencies (0.17 ± 0.03) and the maximum value of their effective number (n<sub>e</sub> = 5.2; 87%, Table 3) was BM2113. Among the loci with 5 alleles, the most uniform distribution of allele frequencies was observed for the ETH225 locus (0.2 ± 0.05), of which 74-77% can be considered effective.

Analysis of the distribution of expected heterozygosity (H<sub>e</sub>) in the studied cattle breeds by the set of loci revealed an average level of genetic variability in dairy cattle populations (H<sub>e</sub> = 0.67 ± 0.023 and 0.69 ± 0.023. The highest expected heterozygosity indices were characteristic of the studied populations at the BM2113 locus (0.80 and 0.81, respectively). For the remaining loci, the minimum estimated number of heterozygous individuals was at the level of 53 (ETH3, Ukrainian Red-and-White dairy breed) -59 (BM1818, Ukrainian Black-and-White dairy breed) %.

For most microsatellite loci within the studied cattle populations, an equilibrium state between the actual and expected indices is characteristic (Table 4).

Checking the nature of the distribution of genotype frequencies according to Hardy-Weinberg revealed a probable deviation in the form of a deficit of heterozygotes for the VM1818 locus in only 2 cases (F<sub>is</sub> = 0.37; χ<sup>2</sup> = 8.0-8.3; p < 0.05). The average value of the Wright fixation index gives grounds to believe that in the study populations there is an independent state of genotype distribution with a certain tendency to increase the number of homozygous individuals (F<sub>is</sub> = -0.04 ÷ 0.13 (black-pigmented) and F<sub>is</sub> = -0.03 ÷ 0.14 (red-pigmented).

The obtained analysis results prove the possibility of using 9 out of 10 SSR markers for passporting, identification and confirmation of the origin of individual individuals within the studied cattle populations.



Table 4

**Results of the assessment of the reliability of deviations in the distribution of genotype frequencies according to Hardy-Weinberg**

Locus	$\chi^2$	
	Ukrainian Black-and-White dairy breed	Ukrainian Red-and-White dairy breed
<i>ETH225</i>	0,150	0,099
<i>BM2113</i>	0,037	0,146
<i>ETH3</i>	0,317	0,342
<i>BM1818</i>	8,342*	7,997*
<i>BM1824</i>	0,113	0,013
<i>ILSTS006</i>	0,101	0,113
<i>INRA023</i>	0,064	0,202
<i>TGLA53</i>	0,120	0,044
<i>TGLA122</i>	0,050	0,202
<i>ETH10</i>	0,013	0,196

Note. \* –  $\chi^2 > \chi^2_{cr}$ . level of significance  $p < 0,05$

According to the results of the conducted studies, it was found that the vast majority of the studied loci belong to informatively valuable markers ( $PIC > 0.5$ ). The exception is the *ETH3* locus ( $PIC = 0.50$ ) for both breeds.

**Discussion.** The obtained data on the genetic structure of the studied cattle population according to the complex of microsatellite loci are a valuable source of information in terms of both the preservation of the gene pool of breeds and for the control of genetic processes in artificially reproduced animal populations. Given the potential value of the studied dairy breeds as carriers of specific biological and economic characteristics for specific geoclimatic breeding conditions, it would be advisable to analyze changes in their genetic structure according to microsatellite markers compared with data from previous years of research, with data from the initial forms involved in the creation of the breed. Unfortunately, in the available literary sources there is little information on the subject of the study, which could be correctly used for analysis.

Similar studies on the determination of allelic polymorphism of microsatellite loci of biological objects, starting from the end of the 90s of the last century, are carried out on appropriate equipment. We are talking about DNA analyzers (sequencers), which allow to unify the fragmentary analysis of amplified fragments and, thereby, minimize the influence of the human factor on the decision-making process regarding the number and size of microsatellite alleles. This allows, taking into account the recommended FAO-ISAG list of SSR markers, to correctly conduct a comparative analysis of genetic polymorphism both within one and several animal populations.

However, most domestic scientists are deprived of the opportunity to use such equipment and, at best, send samples for analysis abroad. We have accumulated considerable experience in studying microsatellite variability in various animal species and have developed a method for assessing the conformational structure of DNA under native PAGE electrophoresis conditions based on the use of available equipment (Kulibaba & Liashenko, 2016). This allows us to accurately determine the allelic spectrum (number of alleles) of the studied SSR loci. The disadvantage of this approach is certain inaccuracies in determining the sizes of amplified fragments. In the case of a DNA analyzer, a molecular mass marker for each individual sample and software data processing based on a given mathematical regression equation are used to determine the



size of the allele. We use one molecular marker for several samples and unlicensed GelAnalyzer software (Version 2010a freeware) with manual selection of the appropriate approximation equation for electrophoresis analysis and a regular millimeter ruler for control. This may give certain inaccuracies and deviations from the data obtained on the DNA analyzer. Considering the above, we will conduct a comparative analysis obtained by other authors based on the assessment of the total number of alleles per locus, their effective number, heterozygosity indicators, Wright's F-statistics and deviation from the Hardy-Weinberg equilibrium. We found three options for analysis. The first source that provides information on microsatellite variability of breeding bulls of different breeds of cattle from the Genetic Resources Bank is the work of scientists of the Institute of Genetic Resources of the National Academy of Sciences of Ukraine (Podoba et al., 2013). The studies were conducted using a microsatellite panel (10 loci) on an ABI Prism 3130 DNA analyzer. This work presents only data on the total number of allelic variants detected. It should also be noted that the comparative analysis was performed for 6 of the 10 microsatellite loci that are common to both studies, namely the loci: ETH3, ETH10, BM2113, BM1824, INRA023, TAGLA122 (Table 5).

*Table 5*

**Total number of alleles detected in the studied cattle breeds**

Cattle breed	Locus					
	BM1824	BM2113	ETH10	ETH3	INRA023	TGLA122
Holstein	13	9	9	9	12	18
Simmental	7	8	4	6	9	9
Ukrainian Black-and-White (genbank)	5	5	5	6	7	7
Ukrainian Red-and-White (genbank)	10	10	8	6	11	10
Ukrainian Black-and-White	4	6	4	6	5	5
Ukrainian Red-and-White	4	6	4	6	5	5

The largest number of alleles was observed in Holstein cattle, which is a component breed in the creation of Ukrainian dairy breeds. The maximum number of allelic variants in this breed was recorded at the TGLA122 locus (n=18). The magnitude of allelic polymorphism in breeding herds of black and red-and-white breeds of the experimental farm of the NAAS network "Hontarivka" is significantly lower (5 alleles per locus) compared to Holstein (11.7), Simmental (7.2), Ukrainian Red-and-White (9.2) and Ukrainian Black-and-White (5.8) breeding herds.

Another study of microsatellite variability in herds of cows of Ukrainian Black-and-White and red-and-white dairy breeds was dated 2017 (Shelyov et al.). The authors of the article analyze population genetic processes in experimental groups of cows from the Boryspil district of the Kyiv region (Voronkiv village). Unfortunately, this work presents data only on the level of heterozygosity (No and No). Of the 10 microsatellite loci used, we present values for 7 (ETH3, , ETH10, ETH225, BM2113, BM1824, INRA023, TAGLA122, Table 6).



Table 6

**Level of genetic variability in experimental herds of dairy cows**

Locus	Kyiv region				Kharkiv region			
	I		II		I		II	
	H <sub>o</sub>	H <sub>e</sub>						
TGLA122	0,76	0,86	0,74	0,86	0,67	0,65	0,69	0,69
INRA23	0,73	0,86	0,88	0,88	0,63	0,65	0,61	0,69
ETH3	0,84	0,77	0,86	0,77	0,59	0,57	0,55	0,53
ETH225	0,78	0,80	0,91	0,80	0,69	0,71	0,73	0,74
BM1824	0,80	0,84	0,81	0,86	0,66	0,68	0,69	0,69
BM2113	0,87	0,86	0,77	0,83	0,82	0,77	0,80	0,81
ETH10	0,82	0,77	0,81	0,77	0,70	0,74	0,69	0,70
Середнє	0,80± 0,018	0,82± 0,016	0,83± 0,023	0,82± 0,017	0,68± 0,027	0,68± 0,025	0,68± 0,030	0,69± 0,032

Notes: I – Ukrainian Black-and-White dairy breed; II – Ukrainian Red-and-White dairy breed; H<sub>o</sub> – observed heterozygosity; H<sub>e</sub> – expected heterozygosity.

Analysis of actual and expected heterozygosity in dairy herds of Kyiv region indicates a relatively high level of genetic variability. (H<sub>e</sub>=0.82). Some cases of consanguineous inheritance (excess homozygotes) were noted at loci TGLA122 and INRA23 for the black and white breed (F<sub>is</sub> =0.12 and 0.15, respectively) and for TGLA122 of the red and white breed (F<sub>is</sub> =0.14). A significantly lower level of heterozygosity occurred in the experimental groups of animals of Kharkiv region (H<sub>e</sub>=0.68-0.69), although without significant deviations from the equilibrium state in the distribution of genotypes.

Another option for analysis was the article by Snegin, Kramarenko et al., (2019) in which the authors assess the genetic diversity and relationships among eight Russian and Ukrainian breeds of cattle using 10 microsatellite markers. The object of the study was two Ukrainian (Southern Beef and Red Steppe, Kherson, Mykolaiv regions) and four breeds kept in the Belgorod region (Ayrshire, Simmental, Black and Piebald Holstein, Swiss, Russian Black and Piebald and Red and Piebald). First of all, we were interested in data on the genetic structure of the populations of the Ayrshire, Simmental, Holstein breeds, which were the initial forms in the creation of the Ukrainian Black and Piebald and Red and Piebald dairy breeds. It was also possible to compare our data with the results obtained in herds of black-and-white and red-and-white dairy breeds from a neighboring region (Bilgorod region).

It should be noted that the comparative analysis was carried out for 8 out of 10 microsatellite loci that are common to both studies, namely for the loci: ETH3, , ETH10, BM2113, BM1818, BM1824, INRA023, TAGLA053, TAGLA122. Analysis of the total allele pool for these loci indicates a narrower interval of variation of allele sizes for our experimental populations of dairy breeds. This applies to both the upper and lower limits of variation in the lengths of amplification fragments for most microsatellite loci (Table 7).

Among the studied breeds, the smallest number of alleles per locus was observed in the herds of Ukrainian Black and Red-motley cows. In particular, it is almost half as low as in the Ayrshire, Holstein, Simmental breeds of foreign selection and the Black and Red-motley breeds from the neighboring Belgorod region (Table 8).



Table 7

**Allele sizes of microsatellite loci in the compared cattle breeds**

Locus	Alleles, bp	
	BPua-RPua	Ayr, BPH, Sim, Sw, RPru, BPru
BM2113	125-141	122-156
ETH3	115-127	103-133
BM1818	266-278	248-278
BM1824	190-196	176-197
INRA023	199-219	195-225
TGLA53	160-190	142-191
TGLA122	148-172	129-184
ETH10	216-224	206-231

Notes: Ayr – Ayrshire; BPH – Holstein black-and-white; Sim – Simmental; Sw – Swiss Brown; RPru – Russian red-and-white; BPru – Russian black-and-white; BPua – Ukrainian black-and-white (Hontarivka); RPua – Ukrainian red-and-white (Hontarivka).

Table 8

**Indicators of genetic variability in the studied cattle populations**

Cattle breed	Parameter				
	N <sub>a</sub>	n <sub>e</sub>	H <sub>o</sub>	H <sub>e</sub>	F <sub>is</sub>
Ayr	10,3±1,2	5,2±0,6	0,870±0,046	0,775±0,034	-0,120±0,030
BPH	13,6±1,3	5,6±0,6	0,823±0,037	0,800±0,024	-0,033±0,044
Sim	10,2±1,3	4,0±0,5	0,808±0,059	0,698±0,051	-0,161±0,033
Sw	6,0±1,0	3,3±0,7	0,619±0,074	0,597±0,067	-0,042±0,062
RPru	9,4±0,9	4,5±0,5	0,824±0,047	0,745±0,035	-0,104±0,024
BPru	9,6±1,0	4,8±0,6	0,794±0,044	0,735±0,035	-0,064±0,056
SMua	10,4±0,8	4,7±0,2	0,673±0,047	0,780±0,012	0,138±0,056
RSua	6,4±0,6	3,8±0,4	0,603±0,094	0,700±0,037	0,185±0,116
BPua	5,1±0,4	3,24±0,3	0,647±0,036	0,675±0,023	0,044±0,038
RPua	5,1±0,4	3,41±0,3	0,655±0,033	0,691±0,023	0,052±0,039

Notes: Ayr – Ayrshire; BPH – Holstein black-and-white; Sim – Simmental; Sw – Swiss Brown; RPru – Russian red-and-white; BPru – Russian black-and-white; SMua – Ukrainian Southern Meat cattle; RSua – Ukrainian red steppe; BPua – Ukrainian black-and-white (Hontarivka); RPua – Ukrainian red-and-white (Hontarivka).

In terms of the number of alleles per locus (N<sub>a</sub>), effective alleles (n<sub>e</sub>) and the level of heterozygosity (H<sub>e</sub>), the populations of dairy breeds studied by us are similar to the indicators obtained in the population of the Ukrainian Red Steppe breed (N<sub>a</sub>=6.4; n<sub>e</sub>=3.8; H<sub>e</sub>=0.7). Among the breeds of foreign selection, the lowest level of genetic variability was found in the herd of cows of the Swiss breed (N<sub>a</sub>=6.0; n<sub>e</sub>=3.3; H<sub>e</sub>=0.6). The maximum values of the indicators of genetic diversity were characteristic of the populations of the Ayrshire and Holstein breeds (N<sub>a</sub>=10.3-13.6; n<sub>e</sub>=5.2-5.6; H<sub>e</sub>=0.78-0.8).

In relation to the Hardy–Weinberg Equilibrium (HWE), a characteristic feature of Ukrainian breeding breeds is the deficit of heterozygous individuals (4.4-13.8%), which is reflected in the magnitude and sign of Wright's fixation index (F<sub>is</sub> (SMua) = 0.138; F<sub>is</sub> (RSua) = 0.185; F<sub>is</sub> (BPua) = 0.185; F<sub>is</sub> (RPua) = 0.185; Table 8). A significant deviation from HWE towards the excess of homozygotes was established at the BM1818 locus for



Ukrainian black and red-motley breeds, ETH3, INRA023, TGLA053 for southern meat breeds and INRA023 and TAGLA053 for red steppe breeds (Table 9).

Table 9

**Results of Hardy-Weinberg Equilibrium (HWE) testing in cattle populations for 8 microsatellite loci**

Locus	Cattle breed									
	Ayr	BPH	Sim	Sw	RPrU	BPrU	SMua	RSua	BPua	RPua
BM1818	NS	E	NS	NS	NS	E	NS	NS	D	D
BM1824	E	NS	E	D	E	NS	NS	NS	NS	NS
BM2113	NS	NS	E	NS	E	NS	NS	NS	NS	NS
ETH10	E	NS	NS	NS	NS	E	NS	NS	NS	NS
ETH3	E	D	NS	NS	E	D	D	NS	NS	NS
INRA023	E	E	NS	NS	NS	NS	D	D	NS	NS
TGLA122	NS	NS	NS	NS	E	NS	NS	NS	NS	NS
TGLA53	NS	NS	NS	NS	NS	E	D	D	NS	NS

For breeds of foreign origin, out of 18 cases of deviation from HWE, only 3 showed a deficit of heterozygotes.

Thus, the results of the comparative analysis of microsatellite variability give reason to believe that in the herds of Ukrainian Black and Red-motley dairy breeds kept in the DPDG "Hontarivka" of the Kharkiv region, a significant narrowing of genetic variability is observed. First of all, this is manifested in a significant decrease in the number of allelic variants for all studied loci (an average of 5 alleles per locus), as well as the number of heterozygous individuals ( $H_o=0.65-0.67$ ). The reason for this may be several factors. First of all, this is the selection of individuals for crossing in artificially reproduced animal populations and, as a consequence, gene drift. On the example of artificial populations, we have an example of the action of the main factors of the evolutionary process, which arise in nature by chance. A person himself isolates individuals, preventing free crossing, purposeful selection of desired genotypes, as a result of which changes in allele frequencies occur primarily in small populations. According to reported data as of 01.01.2022, there were 714 dairy cows in the experimental farm (370 Red-mottled and 344 Black-mottled dairy breeds). Is this a lot or a little for the normal functioning of the genetic potential of an artificial population of animals. If we draw an analogy with natural populations, it is believed that the threshold values of the effective population size ( $N_e$ ) are 50 individuals, which is an insufficient number to prevent inbreeding depression. Even 500 individuals are too few to fully preserve the evolutionary potential of a given species of animals (the best approximation is  $N_e \geq 1000$ , Frankham et al., 2014). In our case, when there are objective reasons for limiting the number of animals, the first priority should be the control of genetic processes in artificially reproduced animal populations, which must be taken into account in selection and breeding work.

**Conclusions**

1. Analysis of polymorphism of 10 microsatellite loci in experimental populations of Black-and-White and Red-and-White dairy breeds allowed to detect a total of 51 alleles for each of the populations. The number of detected alleles per locus ranged from 4 (ETH10 and others) to 8 (TGLA53) (average 5.1).

1. The average level of genetic variability was established for the set of microsatellite loci in dairy cattle populations ( $H_e = 0.68 \pm 0.025$ ).



2. For most microsatellite loci, an equilibrium state between the actual and expected genotype frequencies is characteristic. A probable deviation in the form of heterozygote deficiency was established only for the BM1818 locus in both experimental populations ( $F_{is} = 0.37$ ;  $\chi^2 = 8-8.3$ ;  $p < 0.05$ ).

3. The vast majority of the studied loci belong to informatively valuable markers ( $PIC > 0.5$ ), The exception is the ETH3 locus ( $PIC = 0.50$ ) for both breeds.

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## **BIOTECHNOLOGICAL METHODS OF STIMULATION OF REPRODUCTIVE FUNCTION OF MAIN SOWS**

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*Development of new and improvement of existing biotechnological methods for activating the reproductive function of sows remains one of the current areas of scientific research, which is a necessary condition for the current revival of industrial complexes with a full cycle of pig production.*

*The results of experiments on studying the influence of vitamin-hormonal stimulation of older sows to extend their productive longevity are presented. The experiments were carried out in the conditions of industrial pig farming separately in the spring and summer seasons. The work was aimed at increasing the efficiency of pig farming in conditions of intensified production.*

*According to the results of the research, it was found that in the spring period of the year, when using vitamin and hormonal treatment, an increase in the fertility of older sows (3 years and older) is observed to 86.6 %, compared to 76.9 % in the control group. In the experimental group, 15 sows came into heat within 4–5 days, of which 13 were fertilized; in the control group, out of 15 animals, 13 animals came into heat within 8 days, of which 10 or 76.9 % of the sows were fertilized. Comparing the obtained data, it should be noted that the sows of the experimental group came into heat 3 days earlier than the control animals.*

*It has been proven that when vitamin and hormonal preparations are administered to the main sows older than 3 years and using natural mating, an increase in the fertility rate is observed by 9.7 %.*

*Such an important indicator as the multiparity of sows was higher by 4.0 % (from 13 experimental sows 34 piglets were obtained more compared to the control group of animals).*

*Studying the main reproductive indicators of sows in the summer season: it was found that when using vitamin-hormonal treatment, fertilization was at the level of 80 %, which is 6.7 % higher than in the control. Within 6 days, all 15 animals of the experimental group came into sexual heat, while 12 of them (80 %) became fertile after mating. The sows of the control group, which were not treated with vitamin and hormonal agents, came into sexual heat within 8 days; out of 15 animals, only 11 animals or 73.3 % were fertilized. The multiparity rate of sows in the experimental group was 8.4% higher than in the control group (10 piglets were born from 12 sows).*

**Keywords:** *vitamin-hormonal stimulation, pigs, fertilization, multiparity*



## БІОТЕХНОЛОГІЧНІ МЕТОДИ СТИМУЛЯЦІЇ РЕПРОДУКТИВНОЇ ФУНКЦІЇ ОСНОВНИХ СВИНОМАТОК

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*Розробка нових та удосконалення існуючих біотехнологічних методів активізації відтворної функції свиноматок залишається одним із актуальних напрямків наукових досліджень, що є необхідною умовою при теперішньому відродженні промислових комплексів з повним циклом виробництва продукції свинарства.*

*Представлено результати експериментів з вивчення впливу вітамінно-гормональної стимуляції свиноматок старшого віку для подовження їх продуктивного довголіття. Досліди було здійснено в умовах промислового свинарського господарства окремо у весняну та літню пору року. Робота була спрямована на підвищення ефективності ведення свинарської галузі в умовах інтенсифікованого виробництва.*

*За результатами досліджень встановлено, що у весняний період року при застосуванні вітамінно-гормональної обробки спостерігається збільшення заплідненості свиноматок старшого віку (3-х років і більше) до 86,6 %, проти 76,9 % у контрольній групі. У дослідній групі прийшли в охоту 15 свиноматок протягом 4–5 діб, з яких запліднилось 13 голів; у контрольній групі з 15 голів в охоту протягом 8 діб прийшло 13 голів, із них запліднились 10 або 76,9 % свиноматок. Порівнюючи отримані дані, слід відмітити, що в охоту свиноматки дослідної групи прийшли на 3 доби раніше ніж контрольні тварини.*

*Доведено, що при введенні вітамінного та гормонального препаратів основним свиноматкам старше 3-х років та використанні природного парування спостерігається підвищення показника заплідненості на 9,7 %.*

*Такий важливий показник, як багатоплідність свиноматок, була вищою на 4,0 %, (від 13 дослідних свиноматок отримано на 34 поросят більше порівняно із контрольною групою тварин).*

*Вивчаючи основні репродуктивних показники свиноматок у літню пору року: встановили, що при застосування вітамінно-гормональної обробки заплідненість була на рівні 80 %, що 6,7 % вище ніж у контролі. Протягом 6 діб у статеву охоту прийшли всі 15 тварин дослідної групи, при цьому 12 із них (80 %) стали поросними після парування. Свиноматки контрольної групи, які не оброблялися вітамінними та гормональними засобами у статеву охоту прийшли протягом 8 діб; із 15 тварин запліднилось лише 11 голів або 73,3 %. Показник багатоплідності свиноматок у дослідної групи був вище на 8,4 % ніж у контролі, (від 12 свиноматок народилося на 10 поросят більше).*

**Ключові слова:** вітаміно-гормональна стимуляція, свині, заплідненість, багатоплідність

Introduction. In solving the meat problem, an important place is given to pig breeding as one of the most effective branches of animal husbandry, which is due to the



biological characteristics of pigs, which are manifested in their high multiparity, early maturity and significant growth intensity. (Usenko, S. O., et al., 2020).

Intensification of the pig breeding industry should provide for the maximum use of the biological characteristics of pigs not only through the provision of progressive breeding and feeding methods, but also through the application of new biotechnological methods of stimulating the reproductive capacity of animals. (Bezverkha, L. et al., 2021; Paliy, A. P. et al., 2024).

Reproductive ability is largely determined by the morphofunctional state of the reproductive system of sows, which depends on environmental factors, the level of feeding and conditions of detention (Ulyanchenko, O. V. et al., 2015; Mykhalko, O. H., 2021; Pankeyev, S. P., 2022).

In most pig farms, the main problem is the lack of gilts available to replace old and unproductive sows. The experience of specialists indicates that the number of gilts in the herd should be regulated, depending on the goals of the strategy for repairing the breeding stock (it is advisable to replace old sows only when you have the best young gilts) (Radchenko, V. I., 2017).

One of the best indicators for herd repair is the number of farrowings. The indicators of animal productivity, in particular the number of live-born piglets in the litter, depend on them. Usually it is highest in sows of the 2nd–4th farrowing, and starting from the 5th it gradually decreases. The age of the animals and their productivity determine the terms of economic use of the breeding stock, which in turn necessitates the study of the reproductive qualities of sows. As a rule, in most pig farms, animals are culled after 3 years of intensive use, but since sows of this age still retain good indicators of multiparity and good maternal qualities, the animal is left for further use (Kharenko, M. I., Khomyn, S. P., 2010; Ivanov, V. O., Voloshchuk, V. M., 2013).

The reproductive qualities of sows depend on the state of the reproductive system and are determined by the following indicators - sexual behavior, intensity of estrus, and depend on negative factors of maintenance - stress, monotonous feeding, imbalance of nutrients according to the physiological state of sows, lack of active exercise and pastures with green fodder (Baban, O. A. et al., 2018).

The sow's body is physiologically weak after weaning piglets. To restore its reproductive function, preparations containing vitamins, amino acids and necessary trace elements are required. One of the veterinary preparations that has proven itself well is "Introvit". This is a complex preparation that contains all the necessary components for the treatment and prevention of metabolic disorders in the animal body (Veterinaryye preparaty, 2024; Pylypchuk, O. S., Sheremet, V. I., 2015).

In the technology of modern pig farming, the issue of maintaining maximum productivity and reproduction of sows over a long period of time is important. This, in turn, requires full-fledged balanced feeding, stable operation of the neurohumoral system of regulation of reproductive function and endocrine glands (Baban, O. A., 2018; Pylypchuk, O. S., Sheremet, V. I., 2014).

Scientists' studies have experimentally proven the possibility of stimulating sexual activity for planned regulation of reproduction of a herd of pigs. management of reproductive functions of females, synchronous induction of heat and ovulation in them by using various hormones and their synthetic analogues (Robert, V Knox, 2015; Drahan, P. O., Ivasenko, B. P., 2023).

To intensify the reproductive function of sows by normalizing, restoring, stimulating and synchronizing the reproductive function, a variety of methods and techniques are currently successfully used in the practice of veterinary obstetrics and reproductive biotechnology, including the use of combined hormonal preparations, which



contain two gonadotropins: mare serum (stimulates the development of follicles) and human chorionic (stimulates the onset of ovulation and the formation of the corpus luteum) (Bodnar, O. O. et al., 2010).

The relevance of vitamin-hormonal regulation of the reproductive system of pigs lies in the requirements of modern intensive technologies and economic efficiency (Humennyi O. H., Shpilevska V. V. 2015; Sukhin, V. M., et al., 2012; Andrushko, O. B., Sharan, M. M., 2010). The efficiency of reproduction can be increased by using methods of stimulating the heat of the main sows older than 3 years, to extend their productive longevity.

**The purpose of the research** is to study the effectiveness of individual biotechnological methods for a more complete realization of the reproductive potential of pigs.

**Materials and methods of research.** In previous years, vitamin-hormonal stimulation was studied in studies on replacement pigs and main sows. At the same time, several variants of stimulation schemes were tested and the most effective one was selected in terms of improving reproductive indicators (coming to heat after weaning of piglets, fertilization and multiparity). This made it possible to reduce the number of experimental groups in the current work and use the most effective variant of vitamin-hormonal treatment.

For this purpose, 2 groups of Landrace sows were formed, each with 15 heads in the group, aged 3 years and older, weighing 200–250 kg. The research was conducted in the private company "Saenko L. V." Krasnogradsky district of Kharkiv region.

In the experimental group of animals, the complex vitamin preparation "Introvit" was used in a dose of 8 ml intramuscularly to each animal 3 days before weaning the piglets and on the day of weaning the piglets, the hormonal preparation "Gestavet" was used in a dose of 5 ml intramuscularly. The control group of animals was not administered vitamin and hormonal preparations.

The farm keeps 12 Landrace boars and natural mating is used. The selection of sows in the hunt is carried out twice a day. After establishing the immobility reflex in the sows by the boar - the probe, the uterus is allowed to the boar-breeder assigned to it.

**Research results.** The effect of stimulation of sows older than 3 years was studied to extend their productive longevity. The reproductive indicators of sows were determined: coming into heat after weaning piglets, fertility, multiparity in the spring and summer seasons.

According to the results of research in the spring season, it was proven that when using vitamin-hormonal treatment, an increase in the fertility of sows older than 3 years is observed to 86.6 %, compared to 76.9 % in the control. 15 sows came into heat within 4–5 days, of which 13 were fertilized (experiment). Of the 15 heads, 13 heads came into heat within 8 days, of which 10 or 76.9 % of sows were fertilized (control). Comparing the data obtained, it should be noted that the sows of the experimental group came into heat 3 days earlier than the control group of animals.

According to the results of the conducted studies, it was found that when vitamin and hormonal preparations were administered to main sows older than 3 years and natural mating was used, the fertility rate increased by 9.7 % compared to the control group.

The multiplicity of sows in the experimental group was higher by 4.0 % compared to the control group of animals, sows that were administered complex preparations gave birth to 34 piglets more than in the control group.

It was also found that per inseminated sow (those that became pregnant and those that were not inseminated), the difference between the groups is 14.9 % in favor of the



experimental one, so 1.29 piglets more than in the control were obtained ( $P > 0.999$ ). The results of the studies are presented in the table.

*Table*

**Reproductive ability of main sows of older age when using vitamin and hormonal treatment**

Group	Animal treatment	Fertilized, head	Farrowing		Number of piglets born, head.	Fertility, (per farrowing uterus), head	Average number of piglets (per inseminated uterus) head.
			гол.	%			
<b>Spring time</b>							
Experiment	Vitamin-hormonal	15	13	86,6	130	10,00 ±0,20	8,67 ±0,13 ***
Control	No treatment performed	13	10	76,9	96	9,60 ±0,27	7,38 ±0,21
<b>Summer time</b>							
Experiment	Vitamin-hormonal	15	12	80	117	9,75 ±0,25	7,80 ±0,26 *
Control	No treatment performed	15	11	73,3	107	9,72 ±0,24	7,13 ±0,19

*Note. \*  $P > 0.95$ ; \*\*\*  $P > 0.999$  - compared to the control.*

The study on sows in the summer season was carried out according to the same scheme as in the spring, using the same number of animals, 15 animals in the experimental and control groups. Natural mating of sows with boars attached to them was used.

Determining the reproductive indicators of sows: coming into heat after weaning piglets, fertility and multiparity of animals in the experimental and control groups, the following was established: when using vitamin-hormonal treatment in the experimental group, the fertility indicators were at the level of 80 % and 73.3 % in the control group. The studies revealed that in the experimental group, the fertility indicator was 6.7% higher compared to the control group. 15 animals in the experimental group came into heat within 6 days, 12 of which 80 % became fertile.

The sows of the control group, which did not receive any drugs, came into heat within 8 days, and out of 15 animals, only 11 or 73.3 % were fertilized.

In the summer, according to the results of the studies, 10 more piglets were born in the experimental group than in the control, which is 8.4 %. The multiparity index of the sows of the experimental group was 9.75 piglets, in the animals of the control group the multiparity was 9.72, that is, the index was 0.4 % lower. In terms of overall efficiency per inseminated sow (fertilized and unfertilized), a small difference of 8.6% was obtained and 0.67 piglets were obtained, with the advantage of the experimental group over the control ( $P > 0.95$ ).



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Determining the influence of the season on the reproductive performance of sows, it was found that the difference in the fertilization rate between the experimental groups was 6.6 % in favor of sows that became pregnant in the spring. Between the control groups, the difference in this indicator was 3.6 %, that is, many times smaller. Determining the difference in the multiparity rate between the experimental groups, it was found that it was 2.5 % superior to the group of animals that were pregnant in the summer. In the control groups, the difference was only 1.2 %. Comparing the number of piglets born in the experimental groups, a difference of 13 heads or 10% in favor of sows that farrowed in the summer was found. The advantage of 11 piglets or 1.3 % was between the animals of the control group in favor of sows that farrowed in the summer.

Thus, it was established that the indicators of reproductive ability were better when using the vitamin-hormonal treatment scheme for sows, both in the spring and summer seasons.

**Discussion.** In the scientific literature, there are a number of reports on the relationship between the level of reproductive traits of sows and their age. In particular, researchers (Pokhodnya, H. S., 2011) claim that with increasing age of sows, their multiparity increases. Studies conducted by scientists (Nekrasova M. A., 2020) revealed a significant influence of the age of sows on the manifestation of their reproductive qualities. In particular, it was established that the multiparity of sows increased with increasing age. Thus, at the first farrowing, the value of this indicator was 9.9 goals. Gradually increasing with each farrowing, it reached its maximum of 11.3 goals in sows with the sixth farrowing, the difference between the first and sixth farrowings was 1.4 goals ( $p < 0.001$ ).

According to our data, it was found that when using the vitamin preparation "Introvit" and the hormonal preparation "Gestavet" in the sows of the experimental group, an increase in the fertility rate was observed compared to the control group by 9.7 % in the spring season and by 6.7 % in the summer. The indicators obtained by us are confirmed by the data of other researchers. In particular (Pylypchuk, O. S., Sheremet, V. I., 2015) feeding sows the biologically active preparation "Glutame 1M" at a dose of 20 ml for three days immediately after weaning the piglets in combination with an injection



of "Introvit" at a dose of 10 ml on the day of weaning increases their fertility by 21.4 %, multiparity by 1.6 heads and halves the number of stillborn piglets.

Many studies have proven (Drahan P. O., Ivashenko B. P., 2023, Bodnar, O. O. et al., 2010, Humenny O. H., Shpilevska V. V., 2015) that the administration of combined hormonal preparations to sows, which contain two gonadotropins: mare serum (stimulates the development of follicles) and human chorionic (stimulates the onset of ovulation and the formation of the corpus luteum), is an effective means of restoring the sexual function of animals (Andrushko, O. B., Sharan, M. M. (2010). Scientists (Pylypchuk, O. S., Sheremet, V. I., 2014) found that the administration of a preparation containing gonadotropic hormones to stimulate sexual function in sows after weaning piglets helps to reduce the idle period and increase the fertility of animals. Our studies confirm this conclusion. Thus, in the spring season, 15 sows came into heat within 4–5 days, of which 13 animals were fertilized (experiment). In the control group, 13 animals came into heat within 8 days, of which 10 or 76.9 % of the animals were fertilized. In the summer season, 15 animals of the experimental group came into heat within 6 days, of which 12 or 80 % became pregnant. The sows of the control group, which did not receive any drugs, came into heat within 8 days, and of the 15 animals, only 11 or 73.3 % were fertilized.

The same authors (Pylypchuk, O. S., Sheremet, V. I., 2014) noted that with a sows' idle period of 4–5 days, a higher percentage of fertilization is observed, compared to its duration of 7–8 days. The same pattern was observed in our experiments.

According to (Kramarenko, S.S. et al., 2008), in most sows, the number of piglets in the litter increases to the fifth - sixth farrowing. Piglets obtained from sows in the second and sixth farrowings increase their live weight faster.

According to the studies of scientists (Leontev V.V., 2008; Topchiy L. I., 2009). Topchiy L. I., 2009) one of the problems of industrial pig farming is the influence of the season on the reproductive function of animals. This is probably due to the fact that environmental factors also change with the season of the year, among which the most important are ambient temperature, air humidity, etc.

The issue of the influence of individual factors on the indicators of reproductive characteristics of sows is widely discussed in the scientific literature. It is no coincidence that we divided the studies into spring and summer in our experiments. It was reported (Javier Piñán et al., 2021) in particular about a significant difference in fertility and multiparity indicators depending on the season of the year. It was also reported about the influence of age on the reproductive characteristics of sows. Therefore, in our studies, sows after 6 or more farrowings were taken to obtain correct results.

Studies (Mykhalko, O. H., Povod M. H., 2019) showed that the multiparity of sows in the experimental group in spring was significantly higher by 2.21 heads or 15.13% ( $p < 0.001$ ) in the experimental group compared to the control group. Scientists noted that in the summer months, despite the increasing influence of external seasonal factors, which should theoretically reduce the total number of piglets at birth, this indicator had some increase in the experimental group, in the summer the total number of piglets born was 16.07 heads, which is 3.01 heads (18.73 %) more than in the control group ( $p < 0.001$ ). The presence of a significant effect of the season of the year on the reproductive qualities of sows is confirmed by the findings (J. Hagan, 2018, Y.H. Huang, 2003).

Our studies on a group of sows have shown that the multiparity index of sows in the experimental group was 4.0% higher than that of the control group in the spring. The difference in the multiparity index of sows in the summer between the groups was 0.4 %. The effect of stimulation of sexual function in sows with a combination of gonadotropins



and vitamins on multiparity indices has also been noted by other scientists (Sukhin, V. M., Chumak, V. O., Kryva, O. A., 2012).

Our experiments have shown a positive effect on sows over 3 years of age not only by the injection of “Introvit” and “Gestavet”, but also by the use of natural mating with boars with proven reproductive function. In our opinion, this additionally increased the fertility and multiple birth rates in the older age group of animals.

**Conclusions.** Based on the results obtained, a scheme for vitamin and hormonal stimulation of the reproductive function of sows is proposed.

It was found that when vitamin and hormonal preparations were administered to sows in the experimental group and natural mating was used, the fertility rate increased compared to the control group by 9.7 % in the spring and by 6.7 % in the summer.

It was studied that the multiple birth rate in sows in the experimental group in the spring period was higher when using the complex preparation “Introvit” and the hormonal preparation “Gestavet” by 4.0 % compared to the control. The difference in the multiparity index of sows in the summer season between the groups was 0.4 %.

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## **BIOLOGICAL NOMENCLATURE (TAXONOMY) AND CLASSIFICATION OF HONEY BEE. CURRENT STATE AND PROBLEMS**

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*The chronology of scientific research achievements in the process of determining the biological nomenclature and classification of honey bees and the implementation of the results obtained for use in the practice of beekeeping in the selection and preservation of aboriginal breeds (races) of bees in modern environmental conditions is highlighted. An attempt is made to focus this study in the field of analysis of the chronology of methods used in the historical aspect of determining the taxonomy of insects, in particular bees. At the same time, the problems and prospects of scientific research in modern economic and natural and climatic conditions of the development of the industry are reflected. It is shown that initially only morphometry was used worldwide to identify bee breeds. However, morphometric features are not always informative in identifying subspecies, since they are subject to variability under the influence of environmental conditions. Later, biochemical methods for identifying bee subspecies based on polymorphism of allozyme loci were developed. It is shown that at the same time, methods for identifying bee subspecies based on polymorphism of mitochondrial DNA (mtDNA) loci were developed. This polymorphism was successfully used in phylogenetic and phylogeographic studies of honey bees. The disadvantage of mtDNA markers is the exclusively maternal type of inheritance. At the same time, methods for identifying bee subspecies were developed taking into account polymorphism of nuclear DNA (nDNA) loci. Recently, methods for identifying bee subspecies based on SNP analysis have been developed. These markers have become widely used in population, evolutionary and phylogenetic studies of bees due to the development of next-generation sequencing methods NGS (Next Generation Sequencing) Illumina. SNP markers are characterized by high resolution due to their number and stable inheritance over several generations, which can be successfully used in genetic mapping, population and evolutionary studies, selection of lines for economically useful traits and disease resistance, identification of taxonomic affiliation of bee families.*

**Keywords:** honey bee, taxonomy, morphometric, molecular genetic research methods.



## **БІОЛОГІЧНА НОМЕНКЛАТУРА (ТАКСОНОМІЯ) ТА КЛАСИФІКАЦІЯ МЕДОНОСНИХ БДЖІЛ. СУЧАСНИЙ СТАН ТА ПРОБЛЕМАТИКА**

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*Висвітлено хронологію здобутків наукових досліджень в процесі визначення біологічної номенклатури і класифікації медоносних бджіл та впровадження отриманих результатів для використання у практиці ведення галузі бджільництва щодо селекції та збереження аборигенних порід (рас) бджіл у сучасних умовах довкілля. Зроблено спробу зосередити дане дослідження в полі аналізу хронології методів, що застосовувались в історичному аспекті визначення таксономії комах, зокрема бджіл. Разом з цим відображено проблеми і перспективи наукових досліджень в сучасних економічних та природно-кліматичних умовах розвитку галузі. Показано, що для ідентифікації порід бджіл спочатку у всьому світі використовували лише морфометрію. Однак морфометричні ознаки не завжди інформативні при ідентифікації підвидів, оскільки під впливом умов довкілля схильні до мінливості. Надалі набули розвитку біохімічні методи ідентифікації підвидів бджіл на основі поліморфізму алозимних локусів. Відображено, що одночасно розроблялися методи ідентифікації підвидів бджіл на основі поліморфізму локусів мітохондріальної ДНК (мтДНК). Цей поліморфізм успішно застосовувався у філогенетичних та філогеографічних дослідженнях медоносної бджоли. Недоліком маркерів мтДНК є виключно материнський тип успадкування. У той самий час розвивалися методи ідентифікації підвидів бджіл з урахуванням поліморфізму локусів ядерної ДНК (ядДНК). Останнім часом набули розвитку методи ідентифікації підвидів бджіл на основі аналізу SNP. Дані маркери набули широкого поширення у популяційних, еволюційних та філогенетичних дослідженнях бджіл завдяки розвитку методів секвенування наступного покоління NGS (Next Generation Sequencing) Illumina. SNP-маркери характеризуються високою роздільною здатністю в силу їхньої кількості та стабільної успадкованості у ряді поколінь, які можуть бути успішно використані в генетичному картуванні, популяційних та еволюційних дослідженнях, селекції ліній за господарсько-корисними ознаками та стійкістю до захворювань, ідентифікації таксономічної приналежності сімей бджіл.*

**Ключові слова:** медоносна бджола, таксономія, морфо метричні, молекулярно-генетичні методи дослідження.

Beekeeping has existed for millennia. Pollination of crops by honey bees is even more important than its products from both a commercial and ecological point of view. Knowledge about the main pollinators of agricultural plants, in particular, bee breeds, is one of the modern sections that expand the understanding of the most valuable species of insects and encourage a deeper understanding of their role in the direct and indirect impact of both pollination activity on the reproduction of entomophilous plants and the production of food products for humans. *Apis mellifera* (honey bee) is one of the economically valuable species represented in Ukraine. It is characteristic that bees show the greatest adaptive efficiency precisely to those climatic conditions, species composition of honey plants, parasites and bee diseases, where their breed (race) was historically formed. (Metlytska O. I., 2016).



As in other branches of animal husbandry, in beekeeping, significant attention is paid to the breed resources of bees, the rational use of which in appropriate natural and climatic conditions ensures an increase in the received products and balances the multifaceted connections in wildlife. (Metlytska O.I, 2010).

The achievements of any science are the result of historical development, which is manifested in the concepts of leading representatives, through whose efforts it moves forward.

The goal was to generalize the issue of taxonomy and classification of honey bees, distributed throughout the territory of Ukraine through the analysis of population and molecular genetics methods.

**Research methods.** The methodological tools included both general scientific methods - logical analysis and synthesis, classification and systematization, generalization, and special ones - problem-chronological and source and terminological analyses, as well as analysis of literary sources.

**Research results.** According to the main taxonomic categories (ranks), bees – *Apis* – belong (Zlotin O. Z., 1988., Cherkasova A. I., 1991, Kotthoff U. et al., 2013) to:

- Phylum: Arthropoda / *Arthropoda*
- Class: Insecta / *Insecta*
- Order: Hymenoptera / *Hymenoptera*
- Family: Apidae / *Apidae*
- Genus: Bee / *Apis*

Hymenoptera is a large order of insects, consisting of bees, sawflies, ants and wasps. Currently, about 150,000 living and about 2,000 extinct species of hymenoptera have been described.

The only genus *Apis* of honey bees is included in the family *Apidae*. All species of honey bees of the genus *Apis* are very similar in behavior, social biology, morphology, nest architecture and nutrition. However, despite the great similarity between species of honey bees, differences in behavior and ecology are observed.

According to some authors, species of honey bees of the genus *Apis* are grouped into three lineages:

- dwarf - *Apis andreniformis* and *Apis florea*;
- giant - *Apis dorsata*;
- with closed nests - *Apis koschevnikovi*, *Apis cerana* and *A. Mellifera*.

Some scientists divide bees into four species:

- *A. mellifera* – western cryptic bee;
- *A. cerana* – eastern cryptic bee;
- *A. dorsata* – giant bee;
- *A. florea* – dwarf bee.

Each of these species occurs in a wide range of climatic conditions and habitats. Each lineage includes many ecologically and morphologically differentiated allopatric subspecies and related species that live in the same territory but occupy different ecological niche (Meixner et al., 2013). In particular, *A. mellifera* L. bees were distributed throughout Europe, Africa, and were divided into many subspecies, and are currently distributed on all continents except Antarctica.

*Apis mellifera* (honey bee) is one of the most well-studied species of honey bees, although many questions about its biology, taxonomy and evolution remain unanswered.

At the initial stage of the classification of honey bee subspecies, there were several identification methods, in particular, morphometric: morphometry of body parts; classical morphometry of the wing. It was used to establish the breed composition of bee colonies, starting from the first half of the 20th century. (Ruttner, 1973).



Some of the most important exterior indicators are listed below (Yarovets, 2022):

1. Length of the proboscis. This indicator is taken into account when determining the breed of bees. It also has independent breeding, biological and economic significance - bees with a long proboscis are able to extract nectar from deeply located nectaries.

2. Length and width of the right front wing. Some researchers associate wing size with the potential foraging capabilities of bees.

3. Number of hooks on the hind wing. This indicator is rarely used, but it is of interest because it is not associated with seasonal variability.

4. Cubital index. This indicator does not change during the season, weakly correlates with other exterior indicators.

5. Length and width of the third tergite. The dimensions of the tergite correlate well with the overall dimensions and body weight of the bee and can be a reliable criterion for determining the breed of bees.

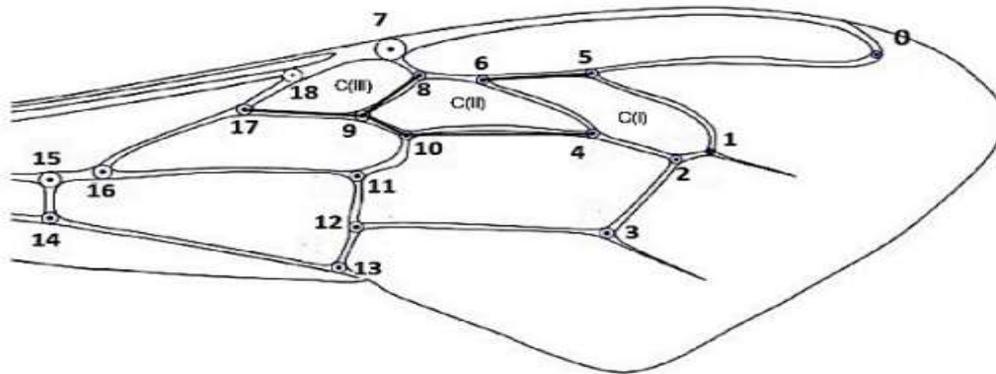
6. Length and width of the third sternite.

7. Length and width of the wax mirror.

8. Tarsal index. The indicator is practically independent of seasonal influence, and it can be successfully used to determine the breed of bees.

9. Discoidal displacement.

Classical wing morphometry is an important and inexpensive method for determining the breed composition of bee colonies by phenotypic characteristics. The latter include the so-called indices - the values of the ratios of the lengths of certain segments on the wings. Over time, classical morphometry has developed, and the DAWINO discriminant analysis (Fig. 1) has appeared; geometric analysis of the wing shape.



**Fig. 1. Scheme of drawing and numbering points on the wing according to the DAWINO method**

Morphometric methods are based on numerous measurements of many individuals. From a large number of characters for the analysis of honey bees from a wide range of geographical locations, researchers selected 42. This set of characters was called the “standard morphometric” indicators. They have stood the test of time and continue to remain relevant and used in a wide range of studies of geographical variations (Diniz-Filho et al, 2000, Bouga M., 2011, Vinutha R Bhatta et al, 2022, ).

The biodiversity of the honey bee *Apis mellifera* L was first assessed using morphometry. After the development of multivariate statistical methods, they were used by Ruttner (1988) to systematize the taxonomy of honey bees of the genus *Apis*.



Initially, the biodiversity of honey bees was determined using the morphometric method, which was entirely based on a large collection of samples with subsequent multivariate analysis of morphometric characters. This identification method was long considered definitive among honey bees.

The systematics and nomenclature of honey bees of the genus *Apis* are quite complex (Sheppard, Meixner, 2003; Chen., 2016.). Maa T.C., (1953) distinguished 24 species of honey bees and three genera. Kanderir, Fuchs et al., 2011, provide tabular data obtained from the Oberurse Database and used in their works for 24 names of subspecies of honey bees (Fig. 2).

Subspecies	Sample size	
	Colony	Bee
<b>Tropical African</b>		
<i>A. m. adansonii</i>	11	108
<i>A. m. capensis</i>	3	30
<i>A. m. lamarckii</i>	7	69
<i>A. m. litorea</i>	6	53
<i>A. m. monticola</i>	8	77
<i>A. m. scutellata</i>	14	136
<i>A. m. unicolor</i>	7	67
<i>A. m. yemenitica</i>	14	126
<b>Western Mediterranean–Northern African</b>		
<i>A. m. iberica</i>	2	18
<i>A. m. intermissa</i>	8	78
<i>A. m. mellifera</i>	14	136
<i>A. m. sahariensis</i>	2	19
<b>Central Mediterranean–Southeastern European</b>		
<i>A. m. carnica</i>	15	146
<i>A. m. cecropia</i>	9	87
<i>A. m. ligustica</i>	11	107
<i>A. m. macedonica</i>	2	20
<i>A. m. sicula</i>	4	39
<b>Middle Eastern</b>		
<i>A. m. adami</i>	5	50
<i>A. m. anatoliaca</i>	5	50
<i>A. m. armeniaca</i>	6	54
<i>A. m. caucasica</i>	12	119
<i>A. m. cypria</i>	4	39
<i>A. m. meda</i>	8	79
<i>A. m. syriaca</i>	9	85

Fig. 2. List of honey bee subspecies



Based on a large volume of samples and multivariate analysis, a classification was proposed, the basis of which is the division of bee subspecies into branches. Initially, three branches were distinguished - A (from South and Central Africa), M (North Africa and Northern Europe), C (Eastern Europe and the Mediterranean). The classification was further refined by adding a fourth evolutionary branch - O (Near and Middle East).

Recently, two more branches have been described as lineage Y from Ethiopia and lineage Z from Syria (table 1) (Franck et al., 2001; Ruttner, 1988.; Alburaki et al., 2013, Whitfield, 2006, Wallberg, 2014, Chen C. et al., 2016).

According to other literature data, currently about 30 subspecies of the species *A. Mellifera* are recognized by most scientists. In particular: Species: *Apis mellifera*. Subspecies: *A. m. adami*; *A. m. adansonii*; *A. m. anatoliaca*; *A. m. artemisia*; *A. m. capensis*; *A. m. carnica*; *A. m. caucasia*; *A. m. cecropia*; *A. m. cypria*; *A. m. iberica*; *A. m. intermissa*; *A. m. jemenitica*; *A. m. lamarckii*; *A. m. ligustica*; *A. m. litorea*; *A. m. macedonica*; *A. m. meda*; *A. m. mellifera*; *A. m. monticola*; *A. m. remipes*; *A. m. ruttneri*; *A. m. sahariensis*; *A. m. scutellata*; *A. m. siciliana*; *A. m. sicula*; *A. m. simensis*; *A. m. sinixinyuan*; *A. m. sossimai*; *A. m. syriaca*; *A. m. taurica*; *A. m. unicolor* (Linnaeus, C. 1758, Engel, M.S. 1999,).

Table 1

**Classification of *Apis* subspecies by morphological characteristics and distribution area**

№	Name	
	Branch, distribution area	Subspecies
1	A, Africa	<i>A. m. adansonii</i>
2		<i>A. m. capensis</i>
3		<i>A. m. lamarckii</i>
4		<i>A. m. litorea</i>
5		<i>A. m. monticola</i>
6		<i>A. m. scutellata</i>
7		<i>A. m. unicolor</i>
7		<i>A. m. yemenitica</i>
9	M, Western and Northern Europe	<i>A. m. ibericensis</i>
10		<i>A. m. intermissa</i>
11		<b><i>A. m. mellifera</i></b>
12		<i>A. m. sahariensis</i>
13	C, Mediterranean. Eastern Europe	<i>A. m. carnica</i>
14		<i>A. m. cecropia</i>
15		<i>A. m. ligustica</i>
16		<i>A. m. macedonica</i>
17		<i>A. m. caucasia</i>
18		<i>A. m. sicula</i>
19	O, Near and Middle East	<i>A. m. adami</i>
20		<i>A. m. anatoliaca</i>
21		<i>A. m. armeniaca</i>
22		<i>A. m. cypria</i>
23		<i>A. m. meda</i>
24		<i>A. m. syriaca</i>
25	Z, Syria	<i>A. m. syriaca</i>
26	Y, Yemen	<i>A. m. jemenitica</i>



Ilyasov R. et al. (2017) provide a list of subspecies of honey bees, the names of which differ somewhat from the data of previous authors (Table 2).

Table 2

**Division of *A. Mellifera* subspecies into branches**

№	Branch	Subspecies	№	Branch	Subspecies
1	A	<i>A. m. sahariensis</i>	16	C	<i>A. m. ligustica</i>
2		<i>A. m. intermissa</i>	17		<i>A. m. carnica</i>
3		<i>A. m. lamarckii</i>	18		<i>A.m. carpathica</i>
4		<i>A. m. litorea</i>	19		<i>A. m. macedonica</i>
5		<i>A. m. scutellata</i>	20		<i>A. m. cecropia</i>
6		<i>A. m. monticola</i>	21		<i>A.m.sicula</i>
7		<i>A. m. adansonii</i>	22		<i>A.m. pomonella</i>
8		<i>A. m. unicolor</i>	23		<i>A. m. ruttneri</i>
9		<i>A. m. capensis</i>	24		<i>A. m. caucasia</i>
10		<i>A. m. simensis</i>	25		<i>A. m. remipes</i>
11		<i>A.m. nubica</i>	26	<i>A.m.armeniaca</i>	
12	M	<b><i>A. m. mellifera</i></b>	27	O	<i>A. m. adami</i>
13		<i>A. m. iberiensis</i>	28		<i>A. m. meda</i>
14	Z	<i>A. m. syriaca</i>	29		<i>A. m. cypria</i>
15	Y	<i>A. m. jemenitica</i>	30		<i>A. m. anatoliaca</i>

They differ in many features, both behavioral and morphological. Initially, the recognition of subspecies was based on a descriptive basis, but they turned out to be insufficient.

Important remarks regarding the use of classical, standard wing morphometry were expressed by A. Tofilski (2008), emphasizing the possibility of using different options in terms of the number of wing features, which, as a rule, lead to classification results that differ little from each other.

In addition, the use of stepwise discriminant analytics allows you to reduce the number of features and leave only those that make a significant contribution to the discrimination of wing phenotypes.

However, there are several circumstances that prevent the effective use of this method to its full extent. The use of segment lengths and segment areas requires studying only bees of the same age, which is extremely problematic, especially if a significant number of colonies from different regions and apiaries are being studied, and the use of segment areas requires the presence of specific technological and software, to which most Ukrainian beekeepers do not have access (Cherevatov V.F. et al., 2023).

The difficulty also lies in the further use of the obtained classification data for the purpose of correct comparison of wing phenotypes from different apiaries or regions. From this point of view, the use of a set of 8 indices has the advantage that it allows creating sufficiently compact banks of reference index data. According to the authors, such banks must meet certain requirements - contain the minimum number of signs that would be sufficient for reliable classification of bee wings; signs must be unified; signs must be established without technological difficulties. The use of such reference data banks is the key to reliable identification of the breed composition of bees by wing phenotype. Thus, morphometric research methods, despite their low accuracy, allow for a quick and inexpensive preliminary assessment of the taxonomic affiliation of bee



colonies. Additional assessments of biological and ethological signs of bee colonies allow for increasing the accuracy of identification of their taxonomic affiliation. These facts make morphometric methods of studying bees widespread in the practical activities of beekeepers today.

Determining the breed of bees by studying exterior features using the classical method is complicated by the fact that the indicators of some measurements of body parts in breeds are within the same or close limits. In addition, some authors indicate that the results of morphometric measurements carried out using the standard method are influenced by subjective factors.

However, phylogeographic studies have shown that variations in the morphological features of these subspecies as a result of adaptation to local conditions often complicate their correct identification. Therefore, molecular methods based on DNA markers, such as mitochondrial DNA (mtDNA), microsatellites and single nucleotide polymorphisms (SNP, Single Nucleotide Polymorphism), have been developed. Molecular genetic studies have generally confirmed the previously obtained morphometric data for branches A, M, C; O. More recently, the Y branches, which include the subspecies *A. m. yemenitica* from Ethiopia (Franck et al., 2000, 2001) and the Z branches, which include the subspecies *A. m. syriaca* from Syria (Alburaki et al., 2013, Meixner et al., 2013, Babu K. N. et al., 2020), have been isolated.

Among molecular markers, non-recombinant and maternally inherited mtDNA is one of the most widely used objects of study. PCR-based assays for bee typing were first used in connection with the Africanization of honey bees in the Americas. The introduction of the African subspecies *A.m.scutellata* in Brazil, and its subsequent hybridization with previously imported European subspecies (Michener, 2000), led to the rapid spread of wild populations of the new hybrid strain (Africanized honey bee) over a large area covering most of South America, Central America, Mexico, and the southwestern United States (Texas, New Mexico, Arizona, Nevada, and California) (Pinto et al., 2012).

A feature of mtDNA as a molecular marker is that it is inherited from only one parent: it is transmitted through the maternal line (from mother to offspring) during fertilization. Due to maternal inheritance, there are no genetic recombinations in the mitochondrial genome, thus a single haplotype is inherited - a set of closely linked loci. The mutational process acts as the only factor in the formation of mtDNA polymorphism, and mutations that are stored and accumulate in the mitochondrial genome can be a trace of the evolutionary history of a species or an individual (Metlitskaya et al. 2012).

One of the first molecular genetic studies to identify Africanized bees in California was based on polymorphisms of three mtDNA loci and required the use of three restriction enzymes (Hall Smith, 1991. Smith, 1995). Later, the analysis methodology was improved, and today the most informative for typing is the intergenic locus located between the genes of cytochrome oxidase subunits I and II (COI-COII) of the mitochondrial genome of *Apis mellifera*.

Proposed by Garnery et al. (1993) PCR protocol involves the use of one pair of primers and restriction endonuclease Dra I. Classification of mitotypes of the COI-COII locus is carried out on the basis of a complex analysis, which includes the length of the amplification fragment and polymorphism of the Dra I restriction endonuclease sites. Based on the Dra I restriction of the COI-COII locus of bee mtDNA, 91 mitotypes of the evolutionary branch M, 30 of the branch A, 5 of the branch C and 7 of the branch O mitotypes have been identified to date (Ilyasov et al., 2016). As an alternative approach to determining the mitochondrial genome of African bees, an analysis method based on the polymorphism of the cytochrome b gene of mtDNA can be considered. Crozier et al.



(1992) developed primers within cytochrome b and proposed to diagnose Africanized bees by the presence/absence of the Bgl II restriction site. The advantage of this method is its simplicity and ease of interpretation of the results (convenient for analysis of amplification 485 nucleotide pairs (bp) and restriction 291/194 bp fragments, use of agarose gel). However, this method does not allow to distinguish the mitotypes of bees from the southern part of Africa *A. m. scutellata* from *A. m. intermissa* (Northeast Africa) and the genetically related *A. m. iberica* (Southern Spain).

In addition, the intergenic region of cytochrome oxidase subunits I and II (COI-COII) of the *Apis mellifera* mitochondrial genome is also widely used as a simpler, but less informative method for classifying mitotypes of bee subspecies, which is based on the analysis of insertions/deletions in this region. At least 7 variants of the lengths of the amplified fragments PoQ, PoQQ, PoQQQ, PQ, PQQ, PQQQ and Q are known, which can be explained by a combination of 3 sequences: Po (67 bp), P (54 bp) and Q (192-196 bp). For their analysis, primers 5' CAKATTTAΓAAATCCATAT 3' and 5' ATAAATATAAATCATGTGGA 3' are used, which flank the 3'- and 5'-terminal sequences of the COI and COII genes, respectively. The size of the amplified fragment using the specified pair of primers is 350 bp for the case of the simplest combination Q (*A. m. sausasica* and other southern subspecies of *A. mellifera*) and 600 bp for the case of a more complex combination PQQ in *A.m.mellifera*. Mitotype classification is carried out using agarose gel electrophoresis.

In addition to the mitochondrial genome, to confirm breed affiliation, analysis of allelic polymorphism of microsatellite loci is used according to the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the International Foundation for Animal Genetics (ISAG). The use of a set of microsatellites allows you to successfully determine the main genetic and population parameters of the study groups and carry out breed differentiation, assess the level of inbreeding/outbreeding, the level of population cross-breeding, etc. (Grygorchuk D.I. et al., 2020).

One of the promising areas of work using molecular genetic markers is the possibility of a comprehensive assessment of the level of hybridization of bees (using the types of molecular genetic markers listed above) to address the issue of their adaptive abilities. As a result of all this, continuous hybridization leads to greater mortality and corresponding economic costs.

Timely assessment of bee colonies using different types of molecular genetic markers (primarily using microsatellites and analysis of mitochondrial genome variability) allows you to determine with a high level of probability the level of genetic variability and, accordingly, the degree of hybridization. All this allows you to effectively control the selection process (Shaibi, 2008).

Regarding the study of the genetic foundations of the adaptive qualities of bees at this stage of the development of molecular genetics, researchers' attention is focused on finding a set of allelic variants of genes, the functioning of which is directly related to the regulation of the adaptive properties of the organism. The ability to identify a molecular genetic marker associated with an economically useful trait, in particular, hygienic behavior, etc., provides the broadest prospects for the application of marker-associated selection (MAS) methods in beekeeping. However, at this stage of genetics development, there are no effective DNA tests for assessing the polymorphism of quantitative trait loci (QTL), which significantly complicates the possibility of using MAS. In this direction (search for potential QTL for the needs of MAS), a lot of work is being done to study the expression features of various candidate genes (associated with any economically useful trait); a search for single-nucleotide polymorphisms in the nuclear and mitochondrial



genomes is being conducted; DNA markers are being searched / developed that could be used in breeding programs (primarily in the areas of bee resistance), etc.

Today, uncontrolled hybridization of bees belonging to different races (breeds) of *Apis mellifera mellifera* has reached an alarming pace in Ukraine. Preservation of the gene pool of local (aboriginal) bees well adapted to local natural conditions is an urgent area of scientific research, which cannot be solved without the use of molecular genetic methods for monitoring local populations (Van der Zee, R. et al, 2009, Brodschneider, 2016, Cherevatov et al., 2019).

Correct identification of these subspecies is important for sustainable beekeeping and the preservation of honey bee biodiversity, since the artificial movement of subspecies/breeds between different regions leads to the loss of purity of the aboriginal gene pool as a result of hybridization. Until recently, beekeeping used mainly morpho-physiological criteria to assess the degree of genetic characteristics of subspecies (purebredness): body color, length of the proboscis, features of wing venation, cubital index, egg production of queens, etc. However, they vary greatly in hybridized colonies, which significantly reduces the reliability and even makes it impossible to determine breed affiliation using traditional morphometric analysis. Phylogeographic studies have shown that variations in morphological features as a result of adaptation to local conditions often complicate their correct identification.

In solving important issues of establishing the breed affiliation of bees, preserving its unique gene pool within natural ranges, there is an urgent need to apply molecular genetic methods (Magnus, 2014).

Preservation of the gene pool of local forms of *Apis mellifera* seems to be particularly relevant for Ukraine due to the significant diversity of natural conditions and the prevalence of several races (breeds) of the honey bee. During the last years of the late 20th and early 11th centuries, queens of different breeds were massively imported to Ukraine from abroad, then a hybrid of several breeds began to be imported, in particular *Backfast*. Uncontrolled crossing of different species led to the loss of useful traits by bees and a decrease in their adaptability to the environmental conditions of the territory in which this species is widespread. It also creates obstacles to the selection of bees and the preservation of their purebredness. To prevent this, it is necessary to establish and monitor the genetic structure of bee populations kept in Ukraine today.

**Conclusions.** The material presented above argues that each individual indicator does not allow for a full assessment of the state of the population. Only by taking into account the results of applying a set of indicators can one draw the right conclusions. The prospects of using different types of molecular genetic markers for solving a number of practical problems in selection and breeding work in Ukrainian beekeeping are undeniable. Summarizing all of the above, we can conclude that the prospects of using different types of molecular genetic markers for solving a number of practical problems in selection and breeding work in Ukrainian beekeeping are undeniable.

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## **PRODUCTION OF COMPOUND FEEDS IN THE CONDITIONS OF FARMS WITH LOADED RESERVATION OF DOSING AND MIXING**

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*Two options for resource-saving technology of mechanized production of compound feeds and PVMS with flexible connections were developed and implemented in farm conditions with loaded backup of weighing batchers and mixers and with loaded backup of mixers.*

*The studies have established that the dosing accuracy of 0.1 kg., the mixer shaft revolutions have an impact on the mixing quality indicator, so at 30 rpm. mixing quality is 95.18%, and at 46 rpm. – 96.1% and 1 min of mixing. Based on a multifactor experiment, polynomial linear and quadratic models were obtained for the mixing quality of compound feed ingredients due to the factors: rotation speed, number of blades and operating time with a mixer volume of 0.54 m<sup>3</sup>. The time spent on performing operations for the production of compound feed was determined and two versions of the cycle diagrams of the process of compound feed production of technological lines were constructed: with loaded redundancy of weighing batchers and mixers and with loaded redundancy of mixers.*

*The time of the initial cycle of the process of compound feed production of technological lines was established:*

*- with the redundancy of weighing batchers and mixers, the time of the initial cycle was 9.7 min. and the established cycle was 6.9 min.*

*- with the redundancy of mixers, the time of the initial cycle of the process of compound feed production of technological lines with a capacity of 2 tons per hour was 9.7 min. and the established cycle was 5.9 min.*

*It was established that the productivity of technological lines for the production of compound feed was:*

*- using technology with flexible connections and loaded redundancy of two weighing batchers and two mixers - 1.974 t.*

*– using technology with flexible connections and a weighing dispenser, which provides unloading of raw material components in two opposite directions and loaded redundancy of two mixers – 2.307 t.*

**Keywords:** *technologies, compound feed, dosing, displacement, destruction, components*



## ВИРОБНИЦТВО КОМБІКОРМІВ В УМОВАХ ГОСПОДАРСТВ З НАВАНТАЖЕНИМ РЕЗЕРВУВАННЯМ ДОЗУВАННЯ ТА ЗМІШУВАННЯ

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*Проведено розробку та реалізацію двох варіантів ресурсозберігаючої технології механізованого виробництва комбікормів та БВМД з гнучкими зв'язками в умовах господарства з навантаженим резервуванням вагових дозаторів і змішувачів та з навантаженим резервуванням змішувачів.*

*Дослідженнями встановлено, що точність дозування 0,1 кг., обороти валу змішувача мають вплив на показник якості змішування, так при 30 об/хв. якість змішування – 95,18 %, а при 46 об/хв. – 96,1 % та 1 хв змішування. На основі багатофакторного експерименту отримані поліномні лінійна та квадратична моделі для якості змішування інгредієнтів комбікормів за рахунок факторів: швидкості обертання, кількості лопаток та часу роботи з об'ємом змішувача 0,54 м<sup>3</sup>. Визначено витрати часу на виконання операцій по виробництву комбікормів та побудовані два варіанта циклограм процесу виробництва комбікормів технологічних ліній: з навантаженим резервуванням вагових дозаторів та змішувачів і з навантаженим резервуванням змішувачів.*

*Установлено час початкового циклу процесу виробництва комбікормів технологічних ліній:*

*- з резервуванням вагових дозаторів та змішувачів час початкового циклу склав 9,7 хв. а циклу, що установився – 6,9 хв.*

*- з резервуванням змішувачів час початкового циклу процесу виробництва комбікормів технологічних ліній продуктивністю 2 тони на годину склав 9,7 хв. а циклу, що установився – 5,9 хв.*

*Установлено, що продуктивність технологічних ліній виробництва комбікормів склала:*

*– по технології з гнучкими зв'язками та навантаженим резервуванням двох вагових дозаторів та двох змішувачів – 1,974 т.*

*– по технології з гнучкими зв'язками та ваговим дозатором, який забезпечує вивантаження компонентів сировини в двох протилежних напрямках і навантаженим резервуванням двох змішувачів – 2,307 т.*

**Ключові слова:** технології, комбікорми, дозування, змішування, деструкція, компоненти

Creating a comprehensively developed and highly profitable agro-industrial production, including in animal husbandry, is possible only on the basis of accelerated development of achievements of science and technology and is one of the conditions for stable socio-economic development of the country. The main conceptual approaches of the strategy for the development of modern feed production are the introduction of science-intensive, innovative technologies for the use of feed, which will become the



basis for the revival of competitive animal husbandry and guarantee the food security of the state. Innovative feed production, and in particular compound feed, should ensure the sustainable functioning of the agricultural sector. The provision of high-quality compound feed largely determines the level of development and economy of this direction, since in the cost structure of livestock production the cost of feed reaches 65-75%. Therefore, the compound feed industry is an important link in the development of the country's agro-industrial complex. The compound feed industry is characterized by large-scale production and produces significant products that play a leading role in the development of industrial livestock farming in Ukraine.

The most important factors of effective livestock farming and providing it with high-quality feed are taking into account the peculiarities of the territorial distribution of feed raw material production, the availability of resources, and the procurement of feed using domestically produced protein-vitamin-mineral supplement (PVMS) and based on new resource-saving technologies.

Only under such conditions will the implementation of the strategy make it possible to create organizational and economic foundations for the effective, socially directed development of the agricultural sector of the economy. Therefore, the development of compound feed production is very relevant for the stable provision of the industry with agricultural raw materials, and the population with high-quality and safe domestic livestock products (Petrychenko V. et. al, 2022, Voronetska I. et. al, 2021, Fihurska, L., 2024 et. al )

The feed industry in Ukraine requires significant attention, as this industry is one of the main factors in the development of livestock and poultry farming.

In modern conditions, feed is produced at large specialized feed plants, regional and district plants, and directly on farms. The production of compound feed, both at large specialized compound feed plants, regional and district plants and directly on farms, is associated with the use of processes: various types of destruction of raw materials (Hulavskiy V. 2014, Yehorov B. 2019, Postnikova M., et. al., 2019, Soldatenko, L 2020 et. al., Aleksashin, A 2021, Rybalchenko V., et. al. 2023), dosing (Milko D. 2015, Buryma M. A. et. al. 2017, Yehorov B. 2018 , 2019) and mixing (Yeremenko O. et. al.. 2015, Yehorov B. 2019, 2019, Malakov O. et. al. 2019).

Manufacturers of large specialized feed mills declare the completeness of the composition of feed and the maximum productive effect from their use, but it is not always possible to obtain the declared level of quality from them. A study was conducted on the effectiveness of using feed from different manufacturers in feeding hybrid pigs. The effect of using pre-starter and starter feed from the manufacturers of TM "TrauNutryshin Ukraine" and "Yedynstvo" in feeding hybrid piglets was studied. (Chudak R. et al. 2017). Based on these studies, we conducted a multifactorial analysis of the use of feed from these manufacturers. The results of the comparative analysis of the use of pre-starter and starter feeds of different manufacturers in feeding hybrid piglets by the complex efficiency index of each of the alternative options N(Ck) compared to the idealized one allow us to note a significant advantage of using pre-starter and starter feeds of manufacturers of TM "TrauNutryshin Ukraine" in feeding hybrid piglets, for which the objective function according to the considered criteria is the smallest and is 0.02103, while the objective function for "Yedynstvo" feeds is 5.5342 times worse. That is, feeds manufactured by different powerful specialized feed mills differ in quality for the same age groups of pigs. Improved nutrient absorption is necessary to increase productivity, profitability and environmental protection. Incomplete digestion of nutrients reduces the profitability of livestock farming due to limited consumption, reduced animal productivity and increased production of by-products (organic waste), which leads to



increased environmental pollution, in particular greenhouse gases (Novakovska V., 2023; Zolotaryov, et. al., 2021, 2023).

Low-component compound feeds with guaranteed productive effect should be prepared directly on farms from grain of own production and purchased balancing additives. In this case, any one type of grain or grain mixture can be used. Production of low-component compound feeds directly on farms makes it possible to:

- expand the availability of the use of high-quality compound feeds in animal feeding and implement it according to scientifically based standards;
- significantly increase animal productivity and increase the volume of livestock production;
- significantly reduce the cost of compound feeds by reducing transportation costs and energy consumption;
- reduce losses of raw materials and finished products during transportation and storage;
- reduce the cost of production by not including VAT on compound feed (since compound feed is prepared for own consumption).

Research objective. To develop and test the technology of compound feed production with loaded backup of weighing batchers and mixers and with loaded backup of mixers under production conditions to determine the main technical and economic indicators.

**Materials and methods.** The study was conducted on the basis of GSTU 46.007 - 2000 "Machines and equipment for feed preparation"; "DSTU 3218-95 Crushers Test methods"; DSTU OIML R 61 - 1 : 2008 Discrete action weighing batchers.

The following parameters were determined: unit productivity, loading and unloading time, grinding module, dosing accuracy, quality of mixing of compound feed ingredients, operation time.

The mixer productivity ( $W$ ) was determined by the formula (1):

$$W = \frac{m_n}{t}, \quad (1)$$

where  $m_n$  - mass of feed obtained after the mixer, kg;  $t$  - mixing time, h.

The experiment was repeated three times. The mixing duration was determined by a stopwatch SOS-pr-26-2 TU 25.1819.0021.

The quality of mixing of compound feeds was determined by the distribution of the control component in 15-20 samples, which were taken at equal intervals during the unloading of the finished compound feed. The repetition of the experiment was three times.

The weight of the selected samples for compound feed mixtures was 100 g each.

As a control component for compound feed mixtures, table salt was used (for mixtures of crushed concentrated feeds).

The uniformity of mixing of components ( $\rho_m$ ) in percent was determined by the formula (2):

$$\rho_m = 100 - Va, \quad (2)$$

Where  $V_a$  – the coefficient of variation of the actual distribution of the control component in the samples.

$$V_a = \frac{100}{m_{av}} \sqrt{\frac{\sum_{i=1}^n (m_n - m_{av})^2}{n-1}}, \quad (3)$$



Where  $m_n$  is the value of the content of the key component in the samples;  $n$  is the number of selected samples;  $m_{av}$  is the average value of the content of the key component in the grain mixture samples.

In the case of deviation of the actual dose of the control component from 1%, the indicator  $V_a$  was multiplied by  $\sqrt{n_a}$ , where  $n_a$  is the actual and percentage content of the control component in the mixture (obtained in experiments).

The revolutions of the mixer working shaft were determined using the "Time tachometer" brand T410-R, GYU 2.780.001PS, TU 25-07-1051.79.

The problem of obtaining the dependences of the mixing quality by changing three factors:

- rotation speed (rpm);
- number of blades;
- operating time (min).

To solve this problem, experimental studies were conducted - mixing quality at the following values of variable factors:

- rotation speed: 30, 37 and 46 rpm;
- number of blades: 0, 2 or 3;
- operating time: from 1 to 6 minutes with a step of 1 minute.

In total, a full factorial experiment was conducted, containing  $3 \times 3 \times 9 = 54$  studies.

Based on the cycle diagrams of the feed production process on technological lines and a lunch break and two technological breaks of 20 minutes each, the productivity of the mechanized feed production technology was determined, taking into account the dependencies (Chernyaev A.I.):

$$q_1 = \left\{ 1 + \frac{[60 - (T_{IC} + T_{TB})]}{T_{EC}} \right\} \cdot G_{PFI}, \quad (4)$$

where  $q_1$  - productivity of the feed production technological line in the first hour of operation and technological breaks, kg/h;  $T_{IC}$  - initial cycle time, min;  $T_{TB}$  - technological break time, min;  $T_{EC}$  - established cycle time, min;  $G_{PFI}$  - weight of the portion of feed ingredients prepared for mixing, kg.

$$q_2 = \left( \frac{60}{T_{EC}} \right) \cdot G_{PFI}, \quad (5)$$

where  $q_2$  - productivity of the feed production line at the established cycle, kg/h.

Multicriteria analysis was performed using the method of evaluating the integral criterion of the distance to the target using the approach of collapsing all criteria to one  $N$  using. For comparative assessment by a complex indicator based on the multicriteria analysis method, the relative distance  $N(C_k)$  was found for each alternative solution from the expression (Piskun V.I. et. al., 2020).

$$N(C_k) = \frac{\sum_{i=1}^n u_{ij}^N - \sum_{i=1}^n u_{i_0}^N}{\sum_{i=1}^n u_{i_0}^N}, \quad (6)$$

where  $N(C_k)$  - the efficiency of each of the options under study compared to the idealized one;

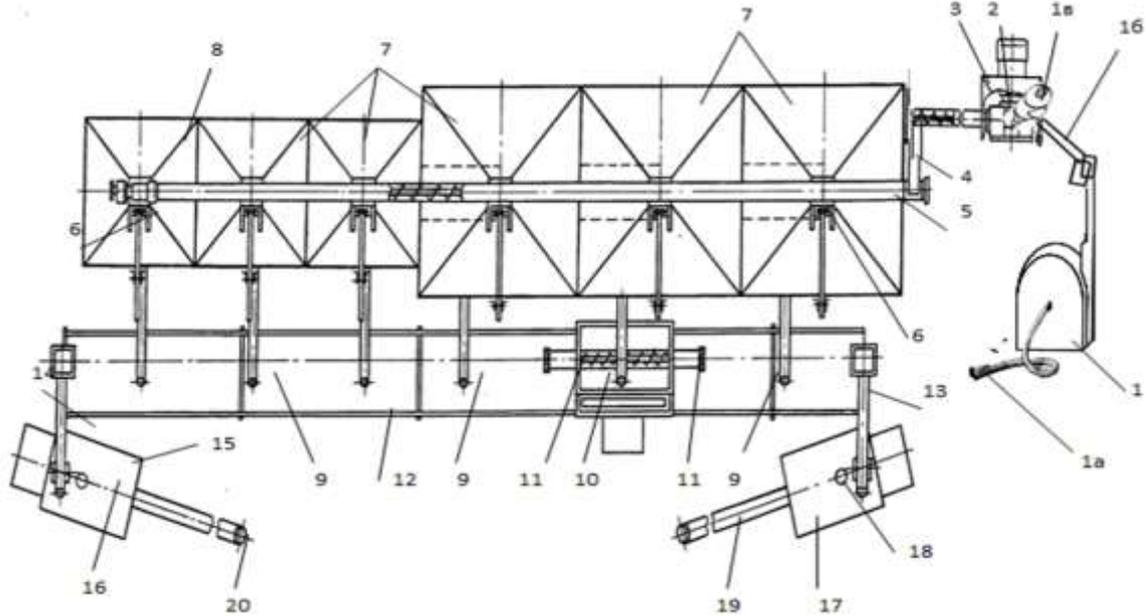
- $u_{ij}^N$  - the normalized  $j$ -th indicator of the option under study;
- $u_{i_0}^N$  - the normalized 0-th indicator of the idealized option;
- $n$  - the number of evaluated criteria.



**Research results.** Two original variants of resource-saving technology for mechanized production of compound feed and PVMS with flexible connections in farm conditions have been developed and implemented.

The first variant includes the following main equipment: a grain loader, a crusher hopper, a crusher, a conveyor, a distribution auger, a gate valve and feed ingredient hoppers, each of which has unloading augers that ensure the unloading of raw material components in two opposite directions, two mobile weighing batchers that are installed with the ability to move along the feed hoppers, loading augers and mixers of compound feed ingredients, unloading augers.

The second variant includes the following main equipment: a pneumatic grain loader that has corrugated hoses at the inlet and outlet and a cyclone; a crusher hopper; feed ingredient hoppers, each of which has unloading augers, a mobile weighing batcher, which provides unloading of raw material components in two opposite directions, which is installed with the possibility of moving along the consumable hoppers; loading augers and mixers of feed ingredients for two or more weighing batchers, unloading augers. The scheme of the resource-saving technology of mechanized production of feed and PVMS in the conditions of the farm with loaded backup of mixers is presented in (Fig. 1).



**Fig. 1. Scheme of resource-saving technology of mechanized production of compound feed and BVMD in farm conditions**

Scheme of resource-saving technology of mechanized production of compound feed and PVMS in farm conditions with loaded backup of compound feed and PVMS production line functions as follows: the initial components of the pneumatic grain loader 1 through the input and output corrugated hose 1a, 1b and cyclone 1c are fed to the crusher 3, in which they are crushed, then using the elevator 4 and the distribution screw conveyor 5 the components are distributed to the corresponding bunkers 7, by overlapping the valves 6. Components that do not require crushing, bypassing the crusher, go directly to the storage bunker. Further, through the unloading screws from the component hoppers 9, according to the requirements of the recipe, the components are loaded one by one as they move to the weighing batcher 10. Mineral components are also loaded to the weighing batcher. The mass of finished components is fed to the mixer 17 through the reverse unloading screw 11 of the weighing batcher and the loading screw 13. After mixing, the compound feed is unloaded from the mixer 17 through the screw 19. When



the weighing batcher 10 moves in reverse, again, according to the requirements of the recipe, the components are loaded one by one to the weighing batcher 10 as it moves. Mineral components are also loaded to the weighing batcher. The mass of finished components is loaded into the mixer 15 through the reverse unloading screw 11 of the weighing batcher 10 and the loading screw 14. Subsequently, the ingredients of the compound feed are mixed in the mixer 15. The finished compound feed is unloaded from the mixer 15 by the screw 20. Then the cycle is repeated.

The general view and fragment of the resource-saving technology of mechanized production of compound feed with loaded backup of mixers with a capacity of 2 t/h are presented in Fig. 2 and 3, respectively.

In this variant, one mobile weighing batcher is used, which provides unloading of raw material components in two opposite directions, and is installed with the possibility of moving along the consumable bunkers and mixers of compound feed ingredients, two more than weighing batchers.



**Fig. 2. General view of the resource-saving technology of mechanized production of compound feed**

*1 - auger for loading compound feed ingredients to the weighing batcher; 2 - hopper of compound feed ingredients; 3 - weighing batcher; 4 - auger for loading compound feed ingredients to mixer No. 1; 5 - control panel No. 1; 6 - mixer No. 1; 7 - control panel No. 2; 8 - screw for loading feed ingredients into mixer No. 2; 9 - unloading screw of mixer No. 2; 10 - mixer No. 2*

The results of the study of the technological line show that grinding feed ingredients on a “Kharkiv’yanka” type crusher on a sieve with mesh diameter of 3 mm provides a grinding module: for grain mixture in the range from 2.6 to 2.9 mm., for meal – 1.6-1.8 mm, for corn – 2.3-2.4 mm.

The average value of the grinding module and bulk density of feed ingredients when using a sieve with a diameter of 3 mm and 2.5 mm for grinding and the moisture content of the ingredients are given in Table 1.

By studying the influence of the mixer shaft speed on the mixing quality index, it was found that at 30 rpm. the mixing quality level is 95.18%, and at 46 rpm. – 96.1% and

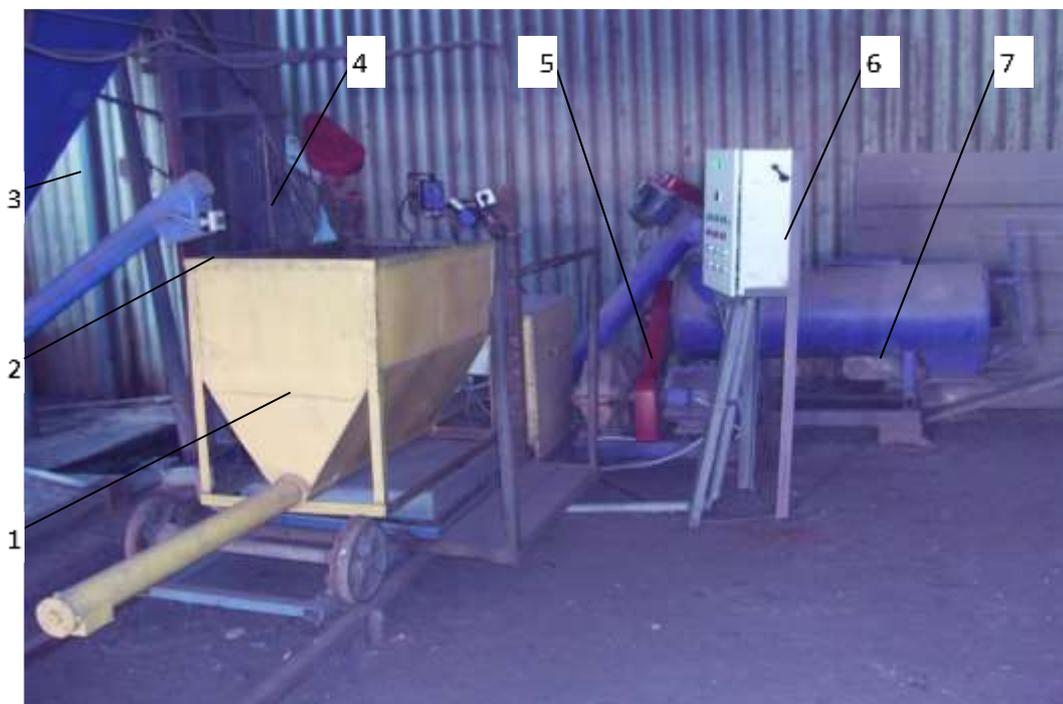


the mixing duration is 1 min. The conducted studies revealed the influence of the presence of blades in the mixer on the mixing quality.

Table 1

**Crusher performance indicators**

Type of ingredient in the feed mill	Sieve with 3 mm mesh diameter						Sieve with 2,5 mm mesh diameter					
	Grinding module, mm		Bulk density, g/l		Humidity, %		Grinding module, mm		Bulk density, g/l		Humidity, %	
	d	d	V	V	W	W	d	d	V	V	W	W
Corn	2,45	0,09	569,67	1,86	16,35	0,25	1,48	0,07	591,0	0,58	16,68	0,55
Grainmix	2,63	0,15	536,33	0,33	11,46	0,49	1,68	0,07	547,67	0,33	14,05	0,45



**Fig. 3. Fragment of resource-saving technology of mechanized production of compound feed**

1 – weighing batcher hopper; 2 – screw for loading compound feed ingredients to the weighing batcher; 3 – compound feed ingredient hopper; 4 – elevator for loading crushed compound feed ingredients; 5 – screw for loading compound feed ingredients to the mixer; 6 – control panel; 7 – mixer

It was established that when mixing feed ingredients for 1 min in the absence of blades in the mixer, the mixing quality was 86.9 %, and with three blades - 96.1 %. The productivity of the resource-saving feed production line is 2 tons per hour, the grinding module of feed ingredients was 1.80-2.60 mm, the dosing accuracy of the weighing batcher with strain gauges is 0.1 kg, the mixing quality is not lower than 95.0%.

Based on a full-factorial experiment containing 54 studies, by changing three factors: rotation speed (rpm); number of blades; operating time (min), two models were obtained – linear



$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 \tag{7}$$

and quadratic

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3. \tag{8}$$

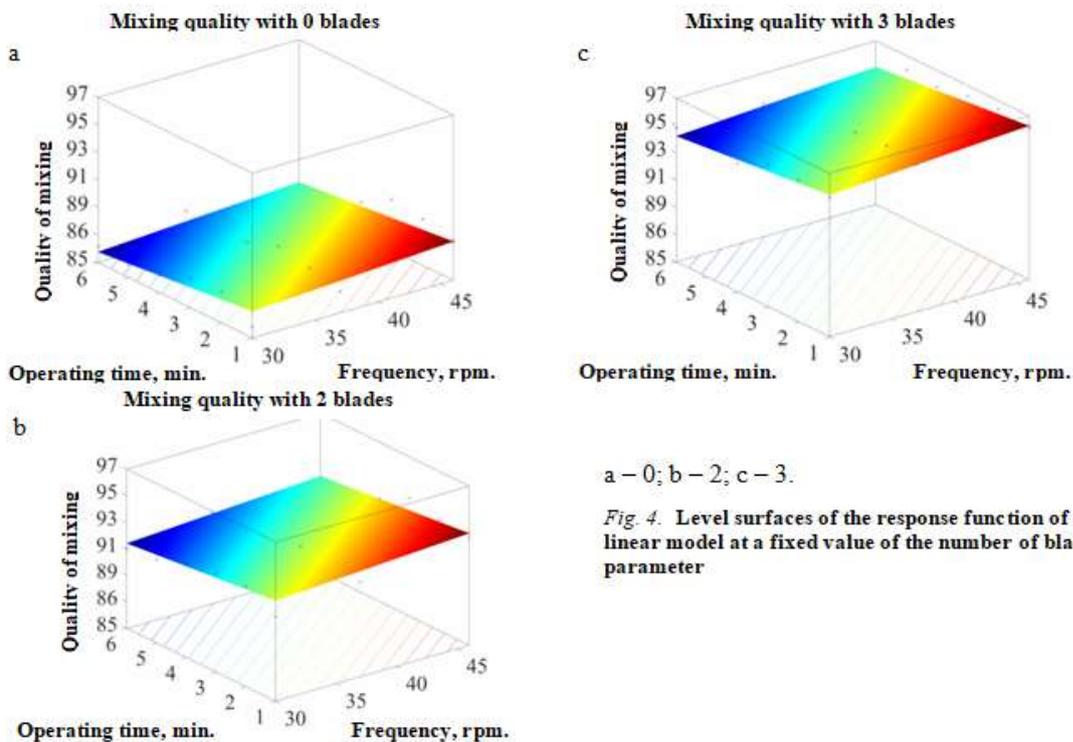
Calculations were performed in the MATLAB mathematical package using the regress function, which allows you to find not only the model parameters themselves, but also confidence intervals for them at a given level of confidence probability (and, if necessary, you can also get other results: the minimum sum of squares of deviations L, various statistics, etc.). We took the confidence probability p=95 %. The results of the calculations were as follows. For the linear model, its coefficients are equal to:

$$\begin{aligned} b_0 &= 85.555191; & b_2 &= 2.819722; \\ b_1 &= 0.054014; & b_3 &= -0.234127. \end{aligned} \tag{9}$$

Confidence intervals for them at the 95% confidence level:

$$\begin{aligned} 84.454801 \leq b_0 \leq 86.655581; & & 2.680337 \leq b_2 \leq 2.959107; \\ 0.027469 \leq b_1 \leq 0.080559; & & -0.335919 \leq b_3 \leq -0.132335. \end{aligned} \tag{10}$$

All factors turned out to be significant: the confidence interval for none of them covers zero. For the linear model, the maximum value of the response function y is always reached at the boundary of the region of permissible values. In our model, y\_max=96.2649, and this value is reached at x1=46 rpm; x2=3 blades; x3=1 min. The response function level surfaces at a fixed value of the parameter x1 or x2 are shown in Fig. 4.



The results for the quadratic model are much more interesting. Its coefficients:



$$\begin{aligned}
 b_0 &= 83.325577; & b_5 &= 0.208056; \\
 b_1 &= 0.076641; & b_6 &= -0.081052; \\
 b_2 &= 2.203267; & b_7 &= 0.000486; \\
 b_3 &= 0.986178; & b_8 &= -0.017381; \\
 b_4 &= 0.000490; & b_9 &= 0.001054;
 \end{aligned}
 \tag{11}$$

and the confidence intervals at the 95% confidence level are as follows:

$$\begin{aligned}
 75.545275 \leq b_0 \leq 91.105880; & & 0.041186 \leq b_5 \leq 0.374925; \\
 -0.326294 \leq b_1 \leq 0.479576; & & -0.142986 \leq b_6 \leq -0.019117; \\
 1.300683 \leq b_2 \leq 3.105852; & & -0.018428 \leq b_7 \leq 0.019401; \\
 0.292309 \leq b_3 \leq 1.680046; & & -0.031194 \leq b_8 \leq -0.003568; \\
 -0.004726 \leq b_4 \leq 0.005705; & & -0.071476 \leq b_9 \leq 0.073584.
 \end{aligned}
 \tag{12}$$

According to this model, the factors  $x_1$ ,  $x_1^2$ ,  $x_1x_2$  and  $x_2x_3$  are insignificant: the confidence intervals for them cover zero. At the same time, the factor  $x_1x_3$  is significant. The largest value of the response function  $y_{\max} = 96.5483$  in this model is achieved at  $x_1 = 46$  rpm;  $x_2 = 3$ ; but  $x_3$  it is no longer at the boundary of its range of variation, but at an intermediate point:  $x_3 = 1,2$  min (with an accuracy of 0.1 min).

The level surfaces of the response function of the quadratic model at a fixed value of the parameter  $x_1$  or  $x_2$  are shown in Fig. 5.

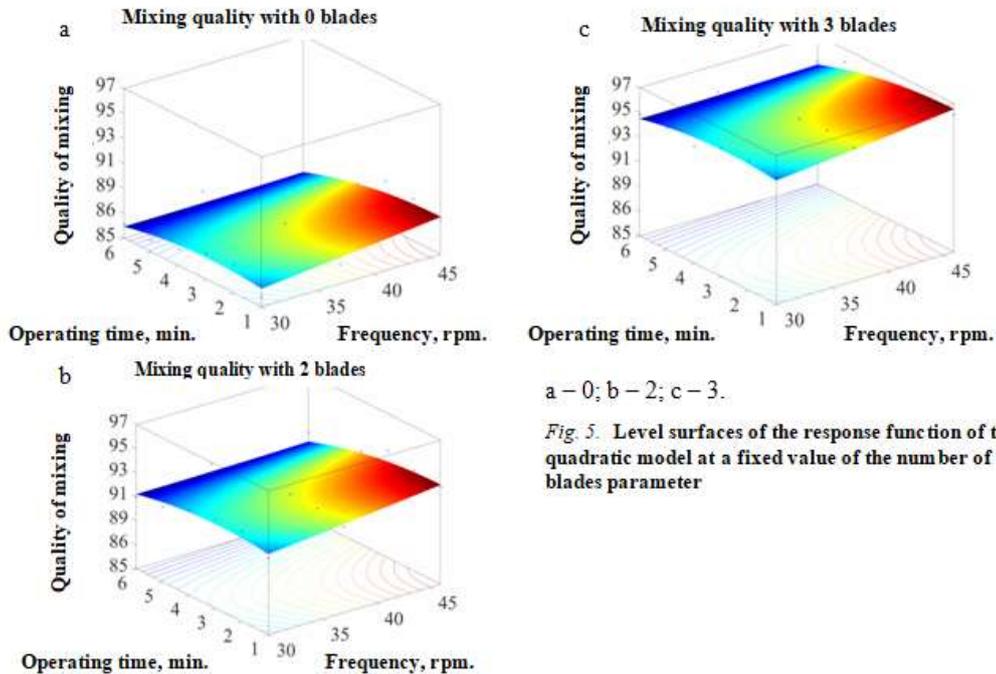
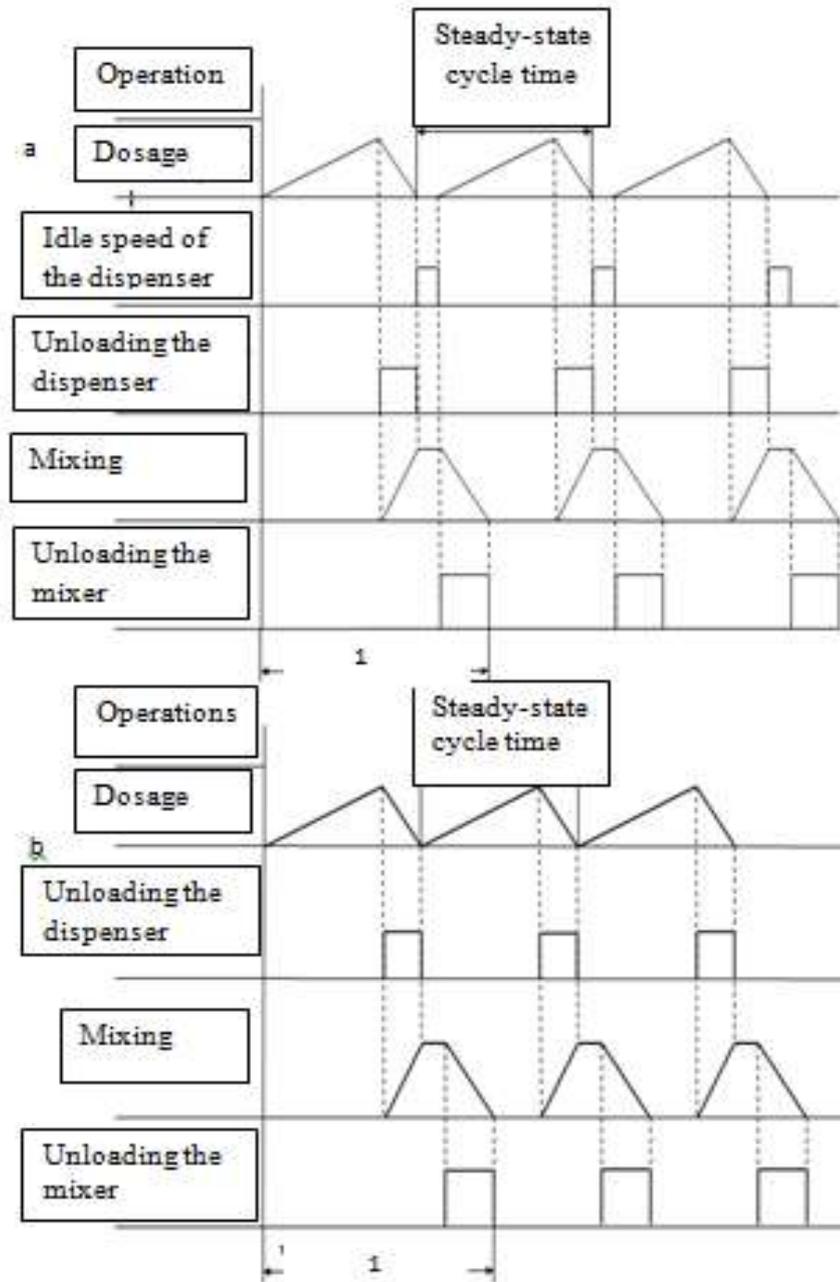


Fig. 5. Level surfaces of the response function of the quadratic model at a fixed value of the number of blades parameter

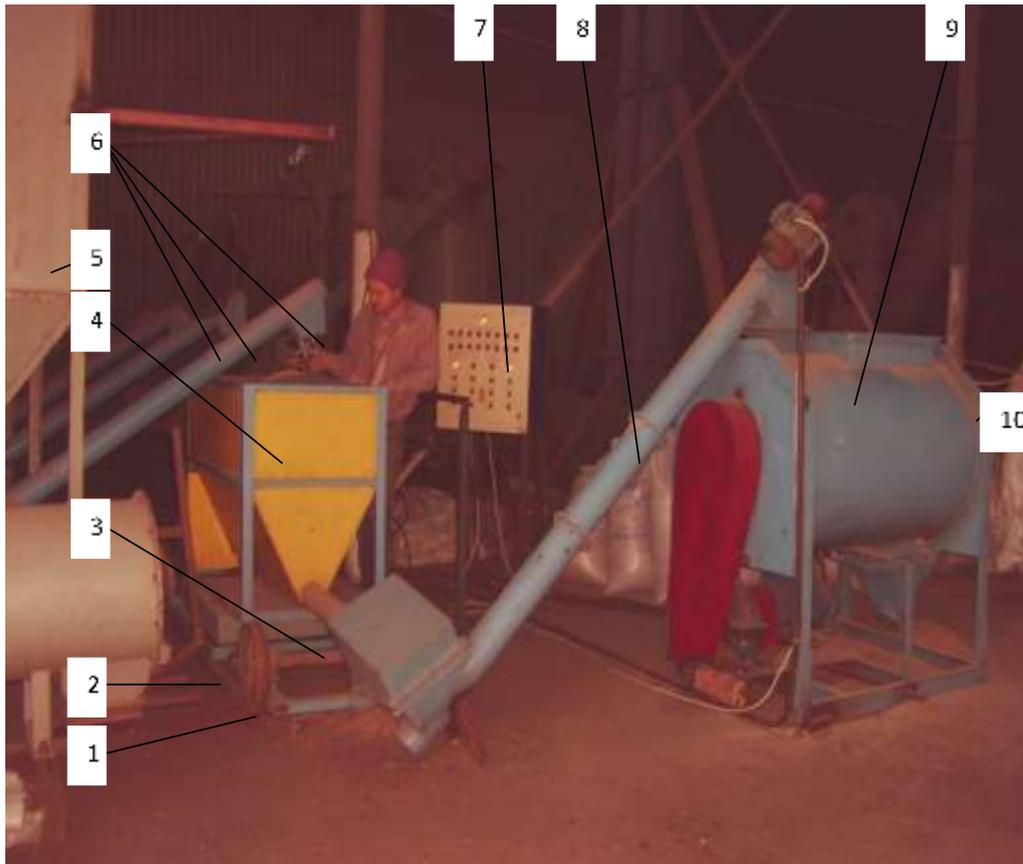
Based on the determination of the time spent on performing operations for the production of compound feed, cycle diagrams were constructed, which are presented in Fig. 6.



**Fig. 6. Cyclediagramsofthefeedproductionprocessoftechnologicallineswith a capacity of 2 tonsperhour for a.s.r. withbackupofweighingdispensersandmixers**

- a - redundancy of weighing batchers and mixers;
- b - redundancy of mixers;
- 1 - time of the initial cycle.

The performance of the unloading augers from the feed ingredient hoppers was determined when loading the feed ingredients into the weigher hopper (Fig. 7). The performance of the unloading augers was  $5.0 \pm 0.06$  tons per hour.



**Fig. 7. Unloading of feed ingredients from the weighing batcher to the mixer**

*1 – mobile platform of the weighing batcher; 2 – platform of strain gauges; 3 – unloading screw of the weighing batcher; 4 – hopper of the weighing batcher; 5 – hopper of feed ingredients; 6 – unloading screws of feed ingredients; 7 – cabinet of start-up and protection equipment; 8 – loading screw; 9 – mixer; 10 – feed unloading screw*

It was established the productivity of the technology of mechanized feed production per hour: according to the technology with flexible connections and loaded redundancy of two weighing batchers and two mixers – 1.974 t/h; using technology with flexible connections and a weighing dispenser, which provides unloading of raw material components in two opposite directions and loaded backup of two mixers – 2.307 t/h.

**Discussion.** Increasing the efficiency of livestock farming at this stage is practically impossible without ensuring reliable production of compound feed for each livestock farm. The use of compound feed produced by powerful specialized compound feed plants and inter-farm compound feed plants has not become widespread mainly due to its high cost and significant transportation costs for transporting grain raw materials and the finished product. The costs of purchasing factory compound feed are often not repaid by the additional increase in livestock production. Therefore, considerable attention is paid to organizing the production of compound feed directly on farms from grain of their own production and purchased protein-vitamin-mineral supplements. This direction allows to significantly reduce transport and overhead costs, to reduce feed consumption in comparison with feeding an unbalanced ration of concentrates. In general, the cost of such compound feeds is 15-30% lower than that produced by specialized enterprises (Sorokin V. 2009).



A well-known line for the production of PVMS and compound feeds almost similar to the equipment we offer, but without loaded backup of mixers, has been tested. According to the developers, its main technical characteristics are: productivity - 1.2 t/h, total power of electric motors - 34.5 kW, dimensions (room): - 6×12×6 m, service personnel - 2 - 4 people (Kosov M. 2020).

At the same time, the solution we proposed for the technology of mechanized production of compound feed using flexible connections and a weighing batcher, which provides unloading of raw material components in two opposite directions and loaded redundancy of two mixers, has a productivity of 2.307 t/h. that is, practically 1.92 times larger and dimensions (room): - 6×12×6 m. The disadvantage of the line without loaded redundancy of mixers is also that it has a high specific material consumption per ton of compound feed produced and at the same time it is possible to produce compound feed of only one recipe, or only a protein-vitamin-mineral supplement.

### **Conclusions:**

1. Two options for resource-saving technology for the production of compound feed and PVMS with flexible connections in farm conditions with loaded redundancy of weighing batchers and mixers and with loaded redundancy of mixers have been developed and implemented.

2. Research into resource-saving technology of mechanized production of compound feeds and PVMS in farm conditions showed that the dosing accuracy is 0.1 kg. It was established that the speed of the mixer shaft has an impact on the mixing quality indicator, so at 30 rpm. mixing quality is 95.18 %, and at 46 rpm. – 96.1 % and 1 min of mixing.

3. Based on a multifactor experiment, polynomial linear and quadratic models were obtained for the quality of mixing of compound feed ingredients due to the factors: rotation speed, number of blades and operating time with a mixer volume of 0.54 m<sup>3</sup>.

4. The time spent on performing operations for compound feed production was determined and two versions of the cycle diagrams of the compound feed production process of technological lines were constructed:

- with loaded backup of weighing dispensers and mixers,
- with loaded backup of mixers.

5. It was established the initial cycle time of the feed production process of technological lines:

- with the backup of weighing dispensers and mixers, the initial cycle time was 9.7 min. and the established cycle time was 6.9 min.

- with the backup of mixers, the initial cycle time of the feed production process of technological lines with a capacity of 2 tons per hour was 9.7 min. and the established cycle time was 5.9 min.

6. It was established that the productivity of technological lines for the production of feed was:

- using technology with flexible connections and loaded backup of two weighing dispensers and two mixers - 1.974 t.

- using technology with flexible connections and a weighing dispenser that provides unloading of raw material components in two opposite directions and loaded backup of two mixers - 2.307 t.

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## **STATE OF ONTOGENETIC ADAPTATION AND MAIN SELECTION-GENETIC INDICATORS OF THE SHEEP POPULATION**

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*The study was conducted in the conditions of a nucleus herd of sheep Kharkiv intrabreed type of the Prekos breed. By differentiating the population of ewes of two generations ( $n=126$  mother-daughter pairs) taking into account the deviations of individual indicators from the average values production of wool and reproductive coefficients, 4 groups were distinguished, each of which is characterized by a different reaction in the organism-environment system: AA (increased in both signs), BB (decreased) and their corresponding combinations - AB and BA. In total, in the maternal and daughter generations of sheep, the proportion of animals in subgroups (AA + AB) was approximately the same, and was 46.8% and 45.3%, respectively, which is evidence of deviations close to the norm in the quality of genotypes, respectively - 3.5% and - 4.7%. According to the indicator of the sum of values in subgroups (AA + BA), the pressure of natural selection in the daughter generation is somewhat higher, compared to the maternal one. The deviation from the norm is -9.5% and -17.5%, respectively. Taking into account the peculiarities of the combination of the specified traits in animals of individual groups, in general, the ontogenetic adaptability of the compared generations to environmental conditions turned out to be the same, and was 1.032. This state of adaptation in the generation of daughters was observed against the background of their probable superiority at 12 months of age over mothers in production of wool by 9.4% ( $p<0.001$ ) and live weight, by 3.9% ( $p<0.05$ ).*

*An improbable decrease in reproductive ability was noted against the background of an increase in live weight and especially production of wool in the generation of daughters. At the same time, the values of phenotypic correlations between live weight and reproductive coefficients in the generation of mothers turned out to be significantly lower than in their daughters and amounted to +0.009 and +0.156 (for the first three years of breeding use) and +0.104 and +0.112 (for the entire life), respectively. Production of wool, on the other hand, had negative correlations, which in the compared generations decreased in the corresponding age periods from -0.163 and -0.149 (in mothers) to -0.063 and -0.007 (in daughters). The values of the reproductive coefficients in sheep of this population are characterized by a low level of heritability: 0.011 - for the first three lambings, and 0.003 - for the entire life period of reproductive use of ewes.*

*It has been proven that new lines are characterized by higher adaptive capacity, and their share in the genealogical structure of the herd is increasing.*

**Keywords:** *adaptability, sheep, reproductive capacity, correlations, production of wool, combination of traits, heritability.*



## СТАН ОНТОГЕНЕТИЧНОЇ АДАПТОВАНOSTІ ТА ОСНОВНІ СЕЛЕКЦІЙНО-ГЕНЕТИЧНІ ПОКАЗНИКИ ПОПУЛЯЦІЇ ОВЕЦЬ

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Дослідження проведено в умовах нуклеусного стада овець харківського внутрішньо породного типу породи прекос. Диференціацією поголів'я вівцематок двох поколінь ( $n=126$  пар мати-донька) з урахуванням відхилень індивідуальних показників від середніх значень настригів та коефіцієнтів репродуктивності, було виділено 4 групи, кожна з яких характеризується різною реакцією в системі організм – довкілля: АА (підвищений за обома ознаками), ББ (знижений) і відповідні їх комбінації - АБ та БА. Сумарно в материнському та дочірньому поколіннях овець частка тварин в підгрупах (АА + АБ) була приблизно однаковою, та складала відповідно 46,8% та 45,3%, що є свідченням близьких до норми відхилень в якості генотипів, відповідно - 3,5 % та - 4,7 %. За показником суми значень у підгрупах (АА+БА) тиск природнього добору в дочірньому поколінні є децю вищим, порівняно з материнським. Відхилення від норми відповідно становить -9,5 % та -17,5 %. З урахуванням особливостей поєднання зазначених ознак у тварин окремих груп, в цілому онтогенетична адаптованість порівнюваних поколінь до умов довкілля виявилася однаковою, та склала 1,032. Такий стан адаптованості у поколінні доньок спостерігався на тлі вірогідної їх переваги у 12-місячному віці над матерями за настригом вовни, на 9,4 % ( $p<0,001$ ) та живою масою, на 3,9 % ( $p<0,05$ ).

Відзначено невірогідне зниження відтворної здатності на тлі збільшення живої маси та особливо настригу вовни в поколінні доньок. При цьому величини фенотипових кореляцій між живою масою та коефіцієнтами репродуктивності у поколінні матерів виявилися значно нижчими, ніж у їх доньок та склали відповідно +0,009 та +0,156 (за три перші роки племінного використання) та +0,104 і +0,112 (за усе життя). Настриги ж вовни, навпроти мали від'ємні кореляції, які в порівнюваних поколіннях зменшувалися у відповідні вікові періоди від - 0,163 та - 0,149 (у матерів) до - 0,063 та - 0,007 (у доньок). Величини коефіцієнтів репродуктивності у овець цієї популяції характеризуються низьким рівнем успадкованості: 0,011 - за перші три ягіння, та 0,003 - за пожиттєвий період репродуктивного використання вівцематок.

Доведено, що нові лінії відзначаються вищою адаптаційною здатністю, а їх частка в генеалогічній структурі стада зростає.

**Ключові слова:** адаптованість, вівці, відтворна здатність, кореляції, настриг вовни, поєднання ознак, успадкованість.

**Introduction.** Changes in the breed composition of the modern sheep population in Ukraine indicate increased competition between imported and domestic breeds of specialized meat production over local breeds of combined meat-wool and wool-meat production. This is evidenced by the creation of a new Dnieper meat breed in Ukraine and the intensive spread of breeding sheep of imported breeds - Merinolandshaf, Texel,



Dorper, Charolais and others, which have a high conversion of feed into meat products and are characterized by high reproductive capacity. The indisputable advantage of domestic sheep breeds remains their high adaptability to various environmental conditions. The latter are experiencing significant deterioration due to increased temperature and decreased precipitation, and the associated deterioration of sheep feeding conditions during the grazing period. An example of this is the current 2024, an extremely dry year, with long periods of extreme temperature increases in the summer. In such conditions, domestic sheep breeds are able to compete with imported ones by directing the breeding process to successfully combine their inherent adaptability with a higher level of productivity.

In this regard, Werf J., Graser H.-U., Frankham R. (2009) note that adaptability is a fundamental characteristic of plant and animal genetic resources, which allows them to survive in their usual environment and adapt to changing conditions. Numerous breeding programs for farm animals are aimed at increasing productivity, but this is often accompanied by a decrease in their adaptability. Therefore, the use of more productive genetic resources is opposed by the question of whether they are adapted to local production systems.

Therefore, animal adaptation requires reaching certain compromises when developing and implementing breeding programs and selecting breeds for breeding in certain environmental conditions (Gaughan, J. B., Sejian, V., Mader, T. L., Dunshea, F. R., 2019).

This is also emphasized by the results of the studies of Sila W., Gachuri C.K. et al. (2021). Summarizing the experience of five years of breeding of Red Masai sheep in the arid region of Kenya after their introduction, they note that in order to ensure a stable long-term increase in their productivity, it is necessary to apply breeding programs that cover large arrays of animals and compare the results of their breeding with indicators of productivity and adaptability in reference herds.

The need to take into account in breeding work a complex of such traits as reproductive ability and productivity of sheep throughout their lives is also emphasized in their studies by Kizilaslan M., Arzik Y., Behrem S. (2024). They consider this to be the key factors for the formation of the stability and profitability of the sheep breeding industry.

So, in modern conditions, an important theoretical and applied task for breeders is to study the patterns of the breed formation process, analyze changes in adaptability and genetic structure of populations under the influence of various breeding methods, assess the combinatorial ability of breeds, types and lines. Artificial populations, which are sheep herds, are complex biological systems, the development of which is aimed primarily at ensuring the required level of production of products of a certain quality. In this regard, assessing the state of such populations and developing methods for managing them should take into account a set of indicators such as: productivity dynamics, magnitude and forms of variability and inheritance of quantitative traits, animal adaptability, as well as the level of financial and energy costs for product production, etc.

As one of the forms of controlling selection changes in the population Kovalenko V.P., Nezhlukchenko T.I., Nezhlukchenko N.V. (2012) use the establishment of an adaptive norm of quantitative traits of typological groups of animals with different parameters and ontogenetic variability in certain technological conditions of maintenance and at different levels of their feeding. In this case, the division of animals into classes (M- - minus, M<sub>0</sub> - modal, M+ - plus) is used according to the most important selection quantitatively measured traits, exterior or linear parameters. Depending on the conditions of the "environment", the adaptability indicators of the selected classes may correspond



to one of the following three levels: optimal, suboptimal, or superoptimal. Adaptability at the optimal level is noted if the viability and reproductive qualities of animals are manifested against the background of their highest productivity.

For dairy cattle herds Polkovnikova O.P. (1992) proposed and modified by Pidpala T.V. (2022) a method of monitoring the state of their adaptation, which consists in determining the group structure of animals in terms of the combination of their deviations from the optimum in terms of milk yield and reproductive ability in adjacent generations.

Such a methodological approach allows us to assess, on the one hand, the impact of selection for milk productivity, and on the other hand, the pressure of natural selection on controlled livestock populations. That is, for the assessment, signs that are negatively correlated with each other are used - the average lactation expectancy of cows and the coefficient of their reproductive ability. The presence of such a connection is emphasized in their studies by Fedorovych V. V., Fedorovych E. I., Mazur N. P., Dyachenko O. B. (2019). In addition, according to Gorelik O. V., Gorelik A.S., Galushina P.S. et al. (2021), the impact of increasing milk productivity by more than 10 thousand kg of milk per cow per year on reducing their reproductive ability increases.

Regarding the sheep breeding industry, especially for sheep of the combined direction of productivity, the leading breeding traits are the shearing of wool and the live weight of animals. The indicators of the reproductive ability of sheep, which ultimately determine the level of selection intensity in herds and affect the possible volumes of mutton production, are to some extent competitive in terms of directing the nutrients of feed to the formation of products, especially wool. This is convincingly traced from the fact that almost all breeds of sheep that produce a large amount of fine wool have a multiplicity of only from 110 to 130 lambs per 100 ewes. High multiplicity, from 180 to 220 or more lambs, is inherent in breeds of sheep mainly with a low level of wool productivity - fur, meat, dairy directions. This is more clearly seen in the example of meat merinos and multi-fetal merinos of the "burula" type, which are significantly inferior in shearing to Australian merinos of all wool types. Kennedy J.P. (1967), studying the genetic and phenotypic relationships between fertility and wool production in two-year-old ewes of the Australian Merino breed of the "peppin" type, emphasized that shearing of unwashed wool and the number of lambs born and weaned from ewes had highly probable in value and negative in direction phenotypic correlations. Genetic correlations also had significant negative indicators and were characterized by significant standard errors.

Therefore, for a long time, research and development of breeding methods have been carried out that would ensure an increase in wool productivity and at the same time improve the reproductive ability of sheep. Now classic studies by Young, S. S. Y. and Turner H. N. (1965) show that the coefficient of heritability of multiparity in ewes of the Australian Merino breed after the first lambing is low and is 0.03, and after the second lambing it increases to 0.35. Among the factors that influence the degree of intrabreed variability of this trait are the age of the ewes, the level and quality of feeding, the insemination season, and the combination of climatic conditions. Despite the low level of inheritance and the influence of phenotypic factors on the degree of variability of this trait, these researchers nevertheless proposed effective breeding techniques that ensured an increase not only in wool productivity, but also in sheep fertility.

Ramos Z., Blair H.T., Barbieri I. et al. (2021) also report on the possibilities of the above-mentioned transformation in nucleus herds of fine-wool sheep in Uruguay, provided that long-term breeding work is carried out. Over a period of more than 20 years, breeders have managed to increase the shearing of washed wool, live weight, and



maintain a high level of reproductive ability of sheep by working to reduce the thickness of the wool. This has become the basis for increasing the economic efficiency of sheep production in general.

Thus, indicators of the reproductive ability of farm animals are indicators not only of the adaptability of populations to environmental conditions, but can also affect the overall efficiency of production. Therefore, constant attention is required to determine the level of animal adaptability in connection with the intensification of selection for a number of other productivity traits that are significant for general production. Given the relevance of the problem under consideration for the livestock industry over a long period of time and the need for its further solution, the purpose of this work was determined by us - to study the correlations between the indicators of reproductive ability and productivity of sheep, indicators of their heritability and to establish the state of adaptability of the sheep population and its individual structural elements under the influence of selection measures.

**Materials and methods of research.** The study was conducted on ewes of the Kharkiv intrabreed type of the Prekos breed in the experimental base of the Institute - the breeding plant, the state enterprise experimental farm "Hontarivka". The analysis involved the results of the assessment of 126 pairs of ewes, which represent a related chain of "mother-daughter". Wool productivity was estimated according to generally accepted methodological techniques (Ibatullin I.I. et al., 2017). In this case, shearings, as well as wool coefficients, were determined. The indicators were taken into account in the year of age. The live weight of sheep at weaning and at 12 months and older was taken into account according to the results of weighing animals with an accuracy of 0.5 kg, and the average daily gains (g/day) of live weight in lambs were determined by the calculation method based on the data of live weight accounting and the growth period.

The signs characterizing the reproduction indicators of ewes were estimated by the reproductive coefficients, defined as the ratio of the number of live and stillborn lambs to the number of reproductive years of each ewe. The contribution of ewes belonging to each of the factory lines to the progress of the population was determined by the ratio of the number of daughters selected for breeding use to their total number obtained at birth.

Adaptation to environmental conditions of two adjacent generations was assessed according to the methods of Polkovnikova O. P. (1992), Pidpala T. V. (2022), modified by us to the conditions of sheep breeding. On generations of related ewes, which form a mother-daughter chain, the average production of wool and animal reproductive coefficients were taken into account. At the same time, two levels of their phenotypic manifestation were distinguished, namely: increased (A) and decreased (B) - deviations from the average values, as well as the corresponding combinations of values: (A-A) - increased, or (B-B) - decreased level for both signs and the corresponding combinations of (A-B) and (B-A) options.

For each generation, the group structure (%) was determined; indicators of ontogenetic adaptation (F) and the values and directions of its change.

The level of adaptation of the generation to the environment was estimated by the coefficient, which takes into account the ratio of the sums of group components (A-A) + (A-B) and (A-A) + (B-A). The relative advantage of animals of the first half-sum of components was considered as a deviation from the optimum in the quality of genotypes, and the second - as an increase in the pressure of natural selection on the herd.

To determine the level of adaptation of sheep of individual lines, the formula modified for the conditions of an artificial population, which is a flock with selection regulated by the breeder, was used:



$$F = B \times P \quad (1)$$

where F is the level of adaptation;

B is the % of selected for further reproduction of the herd of female livestock;

P – the reproductive coefficient of one sexually mature individual.

The average values of the studied traits, their errors and the values of the correlation and inheritance coefficients were determined by biometrics methods using the MS Excel environment (Baranovsky D. I. et al., 2017)

Results of the studies. Changes in productivity under the influence of environmental conditions and selection were determined on two generations of ewes of the Prekos breed, which represent a related chain mother ÷ daughter. The type of birth of animals (from singletons or as part of a multiple litter), live weight indicators, shearing at one year of age, wool coefficients and reproductive coefficients were taken into account.

The studies established that among ewes of the maternal generation, the proportion of animals that came from twins was 21%, while among their daughters the similar indicator was 25%. Despite the mentioned minor differences in the type of birth, qualitative changes occurred in the daughter generation regarding the increase in live weight and wool productivity against the background of a decrease in reproductive ability. Thus, the live weight in the daughter generation increased by 3.9% compared to the indicators of their mothers ( $p < 0.05$ ). Since wool shearing has a positive correlation with live weight, in this case, a significant increase in wool productivity in the daughter generation can be considered as a consequence of selection for both of these characteristics (Table 1).

Table 1

**Characteristics of two generations of ewes according to the main indicators of productivity and reproduction (n – 126 heads in each generation)**

Gene ratio	Live weight		Production of wool		Wool coefficient		Reproduction coefficient	
	M±m, kg	Cv, %	M±m, kg	Cv, %	M±m, g/kg	Cv, %	M±m	Cv, %
M	44,0 ±0,61	15,7	3,72 ±0,08	25,5	85,2 ±1,86	24,4	1,127 ±0,033	33,6
D	45,7 ±0,52	12,7	4,07 ±0,07	19,9	90,1 ±1,84	22,9	1,094 ±0,031	35,3
+/-,%	+3,9 <sup>3</sup>	-	+9,4 <sup>1</sup>		+5,8	-	-2,9	-

Note 1. M – mothers; D – daughters; +/- – ratio of D to M indicators.

Note 2. <sup>1</sup> -  $p < 0,001$ ; <sup>3</sup> -  $p < 0,05$ .

The daughter generation significantly ( $p < 0.001$ ) exceeds their mothers in terms of wool shearing at one year of age, but is inferior to them in terms of the reproductive coefficient, which is calculated by the ratio of the number of lambs obtained from them on average for the entire period of breeding use. That is, an improbable decrease in reproductive ability is observed against the background of an increase in live weight and especially wool shearing in the daughter generation. At the same time, the correlation coefficients between live weight and reproductive coefficients in the mother generation turned out to be significantly lower than in their daughters and amounted to +0.009 and +0.156 (for the first three years of breeding use) and +0.104 and +0.112 (for the entire life), respectively. Production of wool, on the other hand, had negative correlations,



which in the compared generations decreased in the corresponding age periods from - 0.163 and - 0.149 (in mothers) to - 0.063 and - 0.007 (in daughters).

Given the indicated correlations between the traits and the role of the reproductive ability of females in the formation of the population's adaptability to environmental conditions, in order to quantitatively assess the state of ontogenetic adaptation in generations of ewes and the magnitude and directions of its change, the animal population of both generations was differentiated, taking into account the deviations of individual indicators from the average values of shearing and reproductive coefficients (Table 2). In this case, 4 groups were distinguished, each of which is characterized by a different reaction of animals in the organism-environment system.

*Table 2*

**Ratio of animals by combination of shearing and reproductive rate**

Genera tion	all heads	AA		AB		BA		BB	
		n	%	n	%	n	%	n	%
M	126	27	21,4	32	25,4	24	19,1	43	34,1
D	126	13	10,3	44	35,0	28	22,2	41	32,5

It was found that in total in the maternal and daughter generations of sheep the proportion of animals in subgroups (AA + AB) was approximately the same, and was 46.8% and 45.3%, respectively, which is evidence of deviations close to the norm in the quality of genotypes, respectively - 3.5% and - 4.7% (Table 3).

*Table 3*

**Characteristics of two generations of sheep by shearing wool and reproductive performance in the distribution groups**

Performance indicators	Group	Subgroup by trait development	Maternal	Daughter
			M±m	M±m
Production of wool, kg	I	A	4,56±0,13	4,77±0,16
Reproductive rate		A	1,493±0,04	1,469±0,07
Production of wool, kg	II	A	4,48±0,13	4,71±0,10
Reproductive rate		B	0,895±0,04	0,880±0,03
Production of wool, kg	III	B	3,05±0,10	3,48±0,09 <sup>2</sup>
Reproductive rate		A	1,513±0,05	1,463±0,04
Production of wool, kg	IV	B	3,00±0,07	3,55±0,07 <sup>1</sup>
Reproductive rate		B	0,862±0,03	0,805±0,04

*Note* <sup>1</sup> -  $p < 0,001$ ; <sup>2</sup> -  $p < 0,01$ .

The increase in production of wool in sheep of the daughter generation occurred mainly due to the increase in the proportion of animals that combined high shearing with a non-significant decrease in reproductive ability. This is especially noticeable in ewes of groups III and IV. Judging by the sum of the values in the subgroups (AA + BA), the pressure of natural selection in the daughter generation is somewhat higher compared to the maternal one. The deviation from the norm is -9.5% and -17.5%, respectively.

At the same time, the level of productivity and reproduction of animals in individual groups determined a different indicator of their adaptability (Table 4).



Table 4

**Characteristics of two generations of sheep by state of relation (%) to the optimum of wool shearing and reproduction indicators and adaptability in the distribution groups**

Performance indicators	Group	Subgroup by trait development	Generation	
			maternal	daughter
Wool sheared, kg	I	A	122,6	117,2
Reproductive coefficient		A	132,4	139,6
Group adaptability coefficient		-	1,08	1,19
Wool sheared, kg	II	A	120,4	115,8
Reproductive coefficient		B	78,6	84,2
Group adaptability coefficient		-	0,65	0,73
Wool sheared, kg	III	B	82,0	85,38
Reproductive coefficient		A	134,2	140,0
Group adaptability coefficient		-	1,63	1,64
Wool sheared, kg	IV	B	80,6	87,0
Reproductive coefficient		B	76,5	77,0
Group adaptability coefficient		-	0,95	0,89

In general, animals of both generations of group II were characterized by reduced adaptability, in which, against the background of higher by 16-20% against the average level of shearing, there was a decrease in reproductive coefficients by 15-20%. At the same time, the deviation from the norm was higher in the maternal generation.

The best adaptability was also noted for the generation of daughters in group AA and slightly worse only in group BB.

Taking into account the above differences between individual groups, in general, the degree of adaptation of the compared generations of sheep to environmental conditions turned out to be the same and amounted to 1.032 (Table 5).

Table 5

**Assessment of the adaptability of sheep of the compared generations**

Group (combination of traits)	maternal			daughter		
	structure, %	structure, %		structure, %	adaptation coefficient	
		groups	generation		groups	generation
AA(I)	21,4	1,08	0,231	10,3	1,19	0,123
AB(II)	25,4	0,65	0,166	35,0	0,73	0,256
III(BA)	19,1	1,63	0,311	22,2	1,64	0,364
IV(BB)	34,1	0,95	0,324	32,5	0,89	0,289
			1,032			1,032

That is, the increase in shearing did not cause a violation of the ontogenetic adaptability of the daughter generation sheep.

The studies also assessed the magnitude of the correlations between the main indicators of productivity and reproductive ability in ewes of the compared generations (Table 6)



Table 6

**The magnitude of the correlations between the signs of productivity and reproductive ability in related ewes of two generations**

Pairs of traits	Values of $r \pm m_r$ in ewes of two generations	
	mother	daughters
<b>Average daily gain from birth to 90 days of age</b>		
- Number of lambs per ewes for the entire breeding period	0,043±0,089	0,090±0,090
- Reproductive rate for the entire breeding period	-0,112±0,088	-0,061±0,090
<b>Live weight at 12 months</b>		
Number of lambs per ewes for the entire breeding period	-0,114±0,088	-0,132±0,088
- Reproductive rate for the entire breeding period	0,104±0,088	0,112±0,088
<b>Shearing of wool</b>		
- Number of lambs per ewes for the entire breeding period	-0,226±0,085 <sup>2</sup>	0,410±0,074 <sup>3</sup>
- Reproductive rate for the entire breeding period	-0,149±0,087	-0,070±0,089
<b>Wool coefficient</b>		
- Number of lambs per ewes for the entire breeding period	-0,148±0,087	0,432±0,073 <sup>3</sup>
- - Reproductive rate for the entire breeding period	-0,242±0,085 <sup>2</sup>	-0,068±0,090

Note: <sup>1</sup> -  $p < 0,001$ ; <sup>2</sup> -  $p < 0,01$ .

It was found that in both generations, the growth rate of ewes from birth to 90 days of age (weaning), as well as live weight at the age of 12 months, do not have a reliable correlation with the number of lambs and their reproductive coefficients for the entire period of breeding use. At the same time, the shearing of wool and the wool coefficient associated with it and live weight in ewes of the maternal generation have a negative reliable ( $p < 0.01$ ) relationship with the number of lambs and fertility, respectively. In animals of the daughter generation, on the contrary, both indicators of wool productivity with the number of lambs had a highly reliable positive relationship. And with the reproductive coefficient, although the relationship remained negative, its value significantly decreased compared to mothers, and amounted to -0.070 and -0.068, respectively.

According to the results of the regression analysis we performed, it was found that the total number of lambs that can be obtained from ewes of the daughter generation depends on the value of this indicator in their mothers by 34.5% ( $p < 0.001$ ). The total number of reproductive years in mothers also affects the similar indicator in their daughters by 41.3% ( $p < 0.001$ ). At the same time, the dependences between the values of the reproductive coefficients in mothers and their daughters turned out to be low ( $\eta^2 \times = 0.011$ , for the first three lambings, and 0.003 - for the entire period of reproductive use). The relatively higher coefficient of inheritance of the indicator of the number of lambs



obtained from ewes for the first 3 years of their breeding use (0.016) indicates the priority of its use in breeding work with this population of sheep.

A feature of the breeding work in the breeding plant (nucleus) is the use of linear breeding. Therefore, the general picture of its adaptability is formed by animals of individual lines. The degree of adaptability of ewes belonging to the existing line 9e89/108 and the new lines 1579/1625 and 4464/4487 was determined based on the results of their lambing in 2021 and the indicators of further selection and natural loss of ewes obtained from them. It was established (Table 7) that 101 ewes were obtained from ewes belonging to the line 1579/1625, with intra-line selection to rams of the same line.

Table 7

**Level of selection of ewes of different lines**

Lineage of ewes received	Total lambs, heads	Including		From the total number of ewes left for reproduction	
		rams	ewes	heads	%
1579/1625	184	83	101	54	53,5
4464/4487	354	177	177	90	50,8
9e89/108	110	55	55	27	49,1
-	648	315	333	171	51,4

Of these, after sorting for sale at 90 days of age according to growth rate, type of birth, correspondence to the type of herd and line, as well as due to natural attrition from birth to one year of age, 53.5% of the total number of offspring remained for further reproduction. Similar indicators in line 4464/4487 were 177 and 50.8%, respectively. The share of the herd selected for further reproduction in both new lines was higher compared to animals of the line 9e89/108, which has existed for 25 years, by 4.4 and 1.7 absolute percent, respectively. The average fertility of ewes of the same lines was also taken into account (Table 8). The average yield of lambs per lambing of a sexually mature female (reproductive coefficient) was the highest in line 1579/1625. Their advantage over ewes of other lines was 5.8% and 9.4%, respectively.

Table 8

**Fertility of ewes of different lines**

Lineage	Total lambs produced, head.	Number of sexually mature ewes, head.	Offspring produced per 1 ewe, head.			
			on average	by lambs		
				1-3	4-6	7 and more
1579/1625	184	144	1,28	1,23	1,46	1,08
4464/4487	354	294	1,21	1,21	1,31	1,17
9e89/108	110	94	1,17	1,31	1,12	0,92

Taking into account that the potential for multiple births in sheep is revealed when they reach 4-6 years of age, the indicated advantage of animals of this age of a given line increases by 11.5% and 30.4%, respectively. For animals of the existing line, the peak of multiple births appears from the first to the third lambing, while in new lines it noticeably shifts to 4-6 years of age of animals. At the same time, relatively greater multiple births are maintained in them even at an older age. Taking into account the values of the percentage of herd repair and multiple births of ewes, the adaptability of animals of the



evaluated lines was calculated. Having the corresponding adaptability coefficient of 0.684, ewes of line 1579/1625 exceeded animals of line 4464/4487 by 11.6% (absolute indicator 0.613) and line 9e89/108 by 19.2% (versus 0.574). This indicates that new lines are more competitive and their share in the genealogical structure of the herd is growing.

**Discussion.** Most of the existing publications cited in the introductory part of this article indicate that the indicators of the reproductive ability of sheep directly, and taking into account their complex interaction with other breeding traits, affect the efficiency of sheep production.

Regarding the indicators of the live weight of sheep, most publications show a positive relationship with the signs of reproductive ability. At the same time, a number of researchers (Ferguson M. B., Thompson A. N., Gordon D. J. et al., 2011; Slavova P., Laleva S., Popova Y., 2015) emphasize that only the influence of live weight and their fatness before insemination and during the reproductive cycle is significant on the reproductive ability of ewes. At the same time, the optimal live weight of ewes is considered to be the one that corresponds to the average value of this trait for the breed, and fatness is at the level of 2.5 to 3.5 points.

Michels H., Decuyper E., Onagbesan O. (2000), also noting the positive relationship between live weight in sheep with such an indicator as ovulation frequency, once point out its absence with the level of animal fatness, due to the state of interaction of the genotype with the environment. They also did not find any connection between the live weight of ewes and prenatal mortality of lambs among the breeds and lines they compared.

The features of the interaction in the genotype-environment system regarding changes in live weight under the influence of acute feed stress were revealed by Amiri S., Puillet L., Huau C. et al. (2023). The results of their study indicate that the stress response they established can be used as a criterion for assessing the lifespan of goats, the nature of lactation and the combination of milk productivity and reproductive ability. According to their data, longer-lived dairy goats better maintain the stability of live weight under stress, have a more even lactation curve and better milk quality, while their stress-sensitive peers are better adapted to the redistribution of feed nutrients to ensure such physiological functions as pregnancy and rapid achievement of the peak of lactation against the background of a sharp decrease in live weight in the first half of it. As a result, the duration of use of such animals is significantly shorter.

Research Ramos Z., Garrick D. J., Blair H. T. et al. (2023) prove that the thickness of the wool has no definite connection, while the indicators of live weight and the area of the muscle "eye" in one-year-old sheep are positively moderately or strongly correlated with most indicators of reproductive ability in already adult animals.

One of the important indicators that determines the live weight of adult sheep is the average daily gain during the first 12 months of their life. Ben Salem M., Rekik M., Ben Hamouda N. et al. (2009) conducted an experiment to determine the effect of changes in the live weight of young sheep during 200 days after weaning from their mothers on their subsequent reproductive ability. They found that in young sheep of the Barbary breed with superfine merino wool in desert conditions, due to a decrease in live weight after weaning, there is a significant delay in the onset of sexual maturity at the age of 13 months. However, due to the high phenotypic plasticity of sheep of this breed, improving the level of animal feeding allowed at the age of 18 months to obtain a lamb yield of about 80% regardless of the degree of previous loss of live weight.

The studies of Duman M., Şekeroğlu A., Aksoy Y. (2024) on the contrary prove that the reduced growth intensity of sheep of the Akaraman breed under the influence of environmental factors is one of the reasons not only for the decrease in live weight and



survival of lambs, but also for some deterioration in the subsequent reproductive qualities of adult sheep.

Significant variability from -0.36 to +0.11. correlations between maternal additive effects on live weight of lambs and direct additive effects on reproductive traits of sheep are noted in their studies by Walkom S. F., Brien F. D., Hebart M. L. et al. (2015).

The results of our research indicate that the average daily growth rates of ewes during their growth period from birth to 90 days of age do not have significant phenotypic correlations with the number of future lambs, and with the reproductive coefficients of ewes for the entire period of their breeding use.

Judging by the presence of negative correlations, larger at the age of 12 months are subsequently characterized by a shorter period of reproductive use. However, at the same time, the average multiplicity tends to increase, especially in the generation of daughters. As evidenced by the corresponding correlation coefficient +0.112, versus +0.104 in mothers. So, in general, the correlation dependence we found between the live weight of sheep and their reproductive qualities is consistent with most of the above publications. However, in our studies, its level has the character of a trend that persists in the compared generations, which may be a feature of the Prekos sheep breed of combined meat-wool direction of productivity. With an increase in the average level of the trait in the generation of daughters, the level of correlation of this trait with the coefficient of reproduction of animals throughout life increases somewhat.

Unlike live weight, the relationship between wool shearing and the associated wool coefficient was found to be closer in both generations of ewes. In ewes of the maternal generation, it had a negative probable ( $p < 0.01$ ) character with both the number of lambs and the reproductive coefficient. In animals of the daughter generation, on the contrary, a highly probable positive relationship was established between both pairs of traits, which is a consequence of directional selection. At the same time, a significant increase in wool shearing with a moderate increase in live weight in the daughter generation did not significantly affect the wool coefficient indicator. This is evidence of the preservation in the compared generations of the type characteristic of sheep of the combined meat-wool direction.

The presence of an average level of negative correlation between wool shearing and the number of lambs born is indicated in their work by Ramos Z., Garrick D. J., Blair H. T. et al. (2023). However, the researchers note that despite this complexity, it is still possible to simultaneously carry out selection to improve wool shearing and the reproductive ability of sheep. They consider the use of appropriately developed indices for the assessment and selection of animals that successfully combine these traits as a means of implementation. The complexity of selection even for the main indicator, which is associated with the adaptive ability of animals - with fertility, is due to its low level of heritability. According to Mokhtari M.S., Rashidi A., Esmailizadeh A.K. (2010) the number of lambs at birth and at weaning in Kermani ewes has low heritability indices, respectively 0.01 and 0.03 and repeatability 0.08 and 0.10.

Also, the heritability coefficients of fertility of meat sheep of Texel, Shropshire, Oxford Down and Suffolk breeds varied only in the range from 0.04 to 0.06, the absolute indicators of which were from 1.36 to 1.58 lambs on average per lambing. (Maxa J., Norberg E., Berg P., Pedersen J., 2007).

According to Kalaydzhiev. G. I. (2022), sheep of the Stara Zagora breed, with average phenotypic fertility values for the first lambing of 1.13, the second - 1.25 and the third - 1.29, had the corresponding heritability indices of the trait 0.183; 0.149 and 0.137. Despite the low level of heritability of the indicators of the reproductive ability of sheep, Kizilaslan M., Arzik Y., Behrem S. (2024). note that further studies of their correlations



with other breeding traits can significantly improve the sheep breeding systems in the region of fine-wool sheep breeding. Therefore, they propose to identify and include the most important lifetime indicators of reproductive ability and productivity of sheep in breeding programs.

The heritability coefficients determined by us also indicate insignificant dependencies between the values of the reproductive coefficients in mothers and their daughters. They turned out to be low,  $\eta^2_x = 0.011$  - for the first three lambings, and 0.003 - for the entire period of reproductive use of ewes. The coefficient of heritability in generations of the indicator of the number of lambs obtained for the first three reproductive years of ewes turned out to be somewhat higher - 0.016 ( $p < 0.05$ ). Despite this, the methodological approach applied by us in the conditions of the nucleus for breeding sheep of the Prekos breed proves that along with the increase in wool productivity in the daughter generation, the state of ontogenetic adaptability in it remains stable, since the correlations between shearing and the main signs of reproductive ability change significantly in it and are somewhat strengthened with the live weight of animals at 12 months of age. Differentiation of the population of two generations of ewes of the Prekos breed, taking into account the deviations of individual indicators from the average values of wool shearing and reproductive coefficients and the selection of animals with AA and AB combinations of traits is an effective breeding tool for improving both traits.

### **Conclusions.**

1. Differentiation of the population of two generations of ewes of the Prekos breed, taking into account the deviations of individual indicators from the average values of wool shearing and reproductive coefficients, did not reveal a deterioration in the state of ontogenetic adaptability in the daughter generation against the background of a probable increase in wool productivity ( $p < 0.001$ ) and live weight ( $p < 0.05$ ) over animals maternal generation.

2. Ewes of the line 1579/1625 with an adaptation coefficient of 0.684 exceed animals of the line 4464/4487 by 11.6% (absolute indicator 0.613) and the line 9e89/108 by 19.2% (versus 0.574).

3. The values of the reproductive coefficients of this population of sheep are characterized by a low level of heritability, 0.011 - for the first three lambings, and 0.003 - for the entire period of reproductive use of ewes.

4. The indicators of live weight at the age of 12 months of animals are positively weakly correlated, while the shearing of wool and wool coefficients also have weak, but negative correlations with the main indicators of the reproductive ability of ewes. Against the background of a likely increase in wool productivity and, to a lesser extent, live weight, these connections are somewhat strengthened in the daughter generation.

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**INFLUENCE OF SCIENTISTS-HIPOLOGISTS OF THE  
INSTITUTE OF LIVESTOCK OF NAAS ON THE HORSE  
BREEDING OF UKRAINE: A REVIEW**  
(to the 95th anniversary of the Livestock farming institute of NAAS  
and the 90th anniversary of the Department of horse breeding)

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*The article is devoted to the review of the formation and activities of the scientific structural unit on horse breeding of the Livestock farming institute of the NAAS, as a scientific center on horse breeding, which carries out scientific support of the industry, starting from 1944 and until now. In particular, the scientific influence of the employees of the scientific unit on horse breeding in Ukraine is analyzed. The personalities of scientists who worked in the department (laboratory) of horse breeding of the institute at different periods, their personal contribution to the horse breeding industry are considered.*

*The achievements of the scientists-hipologists of the institute in breeding and technological research, the creation of new breeds, types, lines of horses, the improvement of the horse feeding system, elements of horse training, the study of the features of local horse populations in different regions of Ukraine are highlighted.*

*The directions of scientific work, the created breeds, types and lines of horses, breeding, technological developments, research on genetics, feeding, biotechnology, economics in horse breeding are considered.*

*In the article, the authors partially used archival research by Victoria Vyacheslavivna Kunets, in particular, information from her historical and bibliographic publication "Scientific achievements of scientists in the development of domestic horse breeding and horse breeding", for which they express their sincere gratitude to her.*

*Keywords: Livestock farming institute of the NAAS, horse breeding, scientists-hipologists, scientific support, breeds, lines, types*

**ВПЛИВ НАУКОВЦІВ-ШОЛОГІВ ІНСТИТУТУ  
ТВАРИННИЦТВА НААН НА КОНЯРСТВО УКРАЇНИ: ОГЛЯД  
(до 95-річчя Інституту тваринництва НААН та 90-річчя відділу  
конярства)**

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*Стаття присвячена огляду формування і діяльності наукового структурного підрозділу з конярства Інституту тваринництва НААН, як наукового центру з конярства, що здійснює науковий супровід галузі, починаючи з 1944 року і понині. Зокрема проаналізовано науковий вплив співробітників*



*наукового підрозділу на конярство України. Розглянуто особистості науковців, які у різні періоди працювали у відділі (лабораторії) конярства інституту, їх особистий внесок у конярську галузь.*

*Висвітлено досягнення науковців-інологів інституту у селекційних і технологічних дослідженнях, створенні нових порід, типів, ліній коней, удосконаленні системи годівлі коней, елементів тренінгу коней, вивченню особливостей локальних популяцій коней у різних регіонах України.*

*Розглянуті напрями наукової роботи, створені породи, типи і лінії коней, селекційні, технологічні розробки, дослідження з генетики, годівлі, біотехнології, економіки у конярстві.*

*У статті автори частково використали архівні дослідження Вікторії В'ячеславівни Кунець, зокрема – відомості з її історико-бібліографічного видання «Науковий доробок учених у розвиток вітчизняного конярства та кіннозаводства» за що висловлюють їй щире вдячність.*

**Ключові слова:** *Інститут тваринництва НААН, конярство, кіннозаводство, науковці-інологи, науковий супровід, породи, лінії, типи*

In 2024, the Institute of Livestock of the National Academy of Agrarian Sciences of Ukraine will celebrate its 95th anniversary and the 90th anniversary of the creation of its scientific unit - the Department of Horse Breeding - a modern leading breeding center for horse breeding. The purpose of this article is to highlight the main areas of scientific research of the Department of Horse Breeding and its achievements.

**Research methods.** The article highlights the authors' own scientific achievements, memories of the formation of the team and scientific topics, the main results of research from the laboratory's scientific reports during its period of activity, while using historical, bibliographical methods and systematization of materials.

The prerequisite for the establishment in 1944 of the Department of Horse Breeding at the Ukrainian Research Livestock farming institute (UNDIT, then - the Scientific Research Institute of Forest-Steppe and Polissya of the Ukrainian SSR (NIILIP), now - the Institute of Livestock of the NAAS) after the liberation of the Kharkiv region from German occupation was the need for a rapid and qualitative improvement of the horse population, which at that time had an important function in agriculture and the army. The first part-time head of the Department of Horse Breeding was Candidate of Agricultural Sciences Yuriy Yuriyovych Podoba, who in 1945 headed the Department of Small Animal Husbandry (Kunets, 2014). The Horse Breeding Department was initially staffed by scientists whose field of work had not previously been related to horse breeding. The main tasks of the Horse Breeding Sector, according to the Regulations on the UNDIT, were:

- methodological guidance of selection and breeding work in the field of horse breeding in state breeding nurseries and state stables for individual types of horses (trotters, draft horses, riding horses);

- study and implementation of methods of selection and breeding of horses in order to improve their quality and accelerate reproduction;

- study of issues of crossbreeding of horses in order to establish the direction of selection and breeding work with crossbreed animals, improve and compare breeds and justify their zoning;

- development and implementation of methods and techniques for feeding, caring for and keeping horses in production;

- study and implementation of methods of using horses for work in production;



- development of individual issues in the field of horse breeding on the instructions of the management.

The first scientific report on the research work of the Horse Breeding Department was compiled for 1945 by Yevhen Yosypovych Senkovsky (a research associate of the Horse Breeding Department since January 15, 1945) on the topic: "Study of horse breeding resources in the main regions of the Ukrainian SSR." The materials of this report were partially published in the journal "Equestrianism" (Senkovsky, 1946). At that time, E. Y. Senkovsky was an experienced specialist in horse breeding, having worked as the manager of the "Narodna Ferma" stud farms, a zootechnician in various farms, and an inspector of horse breeding in the Kharkiv region. Since September 1941, he was authorized to evacuate animals from the Kharkiv region, in particular, he organized the evacuation of horses from Derkul stud farm No. 63 in 1941-1942. (Kunets et al., 2009)

The fastest possible restoration and further development of horse livestock and breeding was one of the main requirements for livestock breeders in the first post-war years. In connection with the significant arrival of horses from Germany and Hungary in 1945 as reparations and the beginning of the creation of a Ukrainian horse breed, the horse breeding department was expanded, the management Since March 1, 1946, it has been carried out by the front-line soldier Professor Vasyl Kuzmich Klyuchnikov, in the past - a teacher and head of the Kharkiv Dairy and Zootechnical Institute (Kharkiv State Zoo-Veterinary Academy) and the Kharkiv Agricultural Institute (Kharkiv National Agrarian University named after V.V. Dokuchaev). Since June 26, 1946, Ivan Dmitrievich Manakov and technician Sergei Servych Shevchenko were enrolled in the department.. Professor S. V. Afanasyev (author of the "Album of Horse Breeds", 1953) also worked in the department for a short time. In 1947, the department of horse breeding was fully staffed. Mikhail Ivanovich Novikov was hired as a senior research fellow, and the technician was Fedir Kuzmich Musienko, an experienced zootechnician, formerly the head of the equestrian unit of the Yagilnytsky stud farm No. 19 (Kuners, 2014).

The scientists of the department of horse breeding widely promoted the industry, provided practical assistance to stud farms, stud farms, collective farms, state farms and other farms, gave lectures, held talks for all categories of specialists, and distributed literature. The achievements of the industry by the scientists of the department were exhibited at exhibitions.

In 1947, the department employee E. Y. Senkovsky published the monograph "Feeding and Care of Horses" (Senkovsky, 1947) and the first dissertation in the department was defended by Ivan Dmitrievich Manakov on the topic "Foreign draft horses as the basis for creating a draft type of working horses" (Manakov, 1947).

In 1947, scientists from the horse breeding department conducted a survey of horse populations in the western regions of Ukraine. In this expedition, significant attention was paid to horses of the Hutsul and Polissya breeds (Manakov, 1948).

In 1948, senior researcher Mykhailo Ivanovych Novikov joined the department, and in 1948, his position was taken by Viktor Oleksiyovych Solovyov, previously the head of the horse breeding department of the Kyiv Research Station of Animal Husbandry (Kunets, 2014).

In 1948, the department conducted two scientific topics; "Study of breed resources in the Ukrainian SSR" (head V. K. Klyuchnikov) and "Improvement of breed and breeding qualities of horses of the Krasnogradsk, Pyriatyn and Romensk Autonomous Okrugs of the Ukrainian SSR" (head I. D. Manakov). In the same year, the first postgraduate student in the specialty "Equestrianism" was accepted at the institute - Fedor Kuzmich Musienko, under the scientific supervision of V. Klyuchnikov (Kunets, 2014). A significant event in the further work of the Department of Horse Breeding was the



enrollment in postgraduate studies in 1950 of Dmitry Andriyovych Volkov - the future founder of his own scientific school of scientists and horse breeders (Ionov, 2014; Pomitun & Tkachova, 2019). This year Dmitry Andriyovych would have turned 105 years old. His enormous contribution to science and the field of horse breeding will be discussed in more detail below. In the same year, V.K. Klyuchnikov was recalled to the position of Deputy Minister of Agriculture of the Ukrainian SSR and the horse breeding department was headed by I. D. Manakov (Kunets, 2014). Thus, the responsible executors of the scientific topic were the head of Manakov I.D. and graduate student Volkov D.A., the scientific topic concerned mainly the improvement of draft horses. In just 5 years, scientists formed more than 20 valuable mother families in a new draft type. Special attention was paid to the technology of growing, feeding, training and testing draft young stock, which became the basis of D.A. Volkov's PhD thesis (Volkov, 1953).

In 1953, the head of the horse breeding department and at the same time the deputy director for scientific work of the institute was appointed an experienced specialist-hipologist Mykola Mikhailovich Burlakov, who had worked in production for many years, including being the director of the State Stud Farm No. 49, the director of the Alma-Ata All-Union Trust of Stud Farms of the USSR National Committee for the Study of Stud Farms and the Ukrainian Trust of Stud Farms of the USSR National Committee for the Study of Stud Farms. In 1954, M.M. Burlakov was recalled to the Ministry of Agriculture of the USSR and Dmitry Andriyovich Volkov became the head of the department (Kunets, 2014; Pomitun & Tkachova, 2019). In 1956, the horse breeding department was joined by an experienced specialist in horse breeding and organizer of stud farms – Pavel Pavlovich Volkov and Anna Stepanivna Filimonova – previously a lecturer in animal husbandry at the school for training heads of collective farms and technicians of the Institute of Experimental Veterinary Medicine. The work of the Horse Breeding Department in 1958 was aimed at checking the state of breeding and selection, conditions for keeping breeding stock and raising breeding young stock in stud farms (D. A. Volkov, P. P. Volkov), as a result of which priority measures were developed and implemented for the systematic conduct of work on the qualitative improvement of the horse population of the republic (Volkov D. & Volkov P., 1958).

Since 1960, the Horse Breeding Department has been managing breeding work to improve the Oryol and Russian trotters, purebred horse breeds, the creation of the Ukrainian horse breed group and the Novooleksandrivskyy heavy-duty type of horses. During this work, new lines and mother families in breeds were created, and horse keeping and training systems were developed.

In 1967, the chief judge (and later the director of the Kharkiv State Hippodrome) Mykola Mykolayovych Ponomarenko entered the postgraduate program of the Institute of Horse Research and Training, who conducted important experimental studies on the influence of training intensity and liveliness of mares on their reproductive ability and the quality of their offspring, which were covered in his candidate dissertation (Ponomarenko, 1971). In 1972, significant personnel changes took place at the institute, and the horse breeding department also underwent changes. D.A. Volkov was appointed deputy director for scientific work of the institute, and only G. S. Filimonov remained in the department (Kunets, 2014). But already at the end of 1973, a promising young specialist, Alexander Aleksandrovich Novikov, was enrolled in the department. The subject of the department's work at that time concerned the development and implementation of methods for improving the breeding and sporting qualities of factory-bred horses. Volume I of the Ukrainian Horse Breeding Code was compiled and materials for its approval were prepared. During these years, the Horse Breeding Department was



reorganized into a laboratory attached to the Small Animal Breeding and Horse Breeding Department under the leadership of V. V. Myros (Kunets, 2014).

The equine laboratory continued to be replenished with experienced personnel who entered postgraduate studies under the leadership of Dmitry Andriyovych Volkov, forming his scientific school (Ionov, 2014; Pomitun & Tkachova, 2019). In 1953, the equine laboratory was replenished with a practicing horseman from the Strelka Stud Farm, Oleksandr Mykhailovych Latka, later a recognized expert on the Ukrainian horse breed. In 1981, Tamara Ivanivna Volkova was enrolled in the laboratory staff, in the future a specialist in horse feeding. In 1985, Violetta Stanislavivna Slizh, a former trotter rider at the Kharkiv State Hippodrome, who received the second prize in the All-Union Student Essay Competition named after S.M. Budyonny, entered the postgraduate study. She became one of the first scientists at the institute to start working with computer technology and software, and created the first database of Ukrainian horse breeds. In 1987, an experienced zootechnician, Olga Mykhailivna Sobol, entered graduate school, studying and implementing genetic indicators of trotting horse agility – later a lecturer at Kherson State Agrarian University. In 1978, the head of the production department (later director) of the Kharkiv State Hippodrome, Vasyl Oleksiyovych Lebedev, entered the institute's graduate school, who substantiated and implemented individual elements of the trotting horse testing system at the hippodromes, which increased their agility (Lebedev, 1989)

With the acquisition of independence by Ukraine, reforms took place in all sectors of the economy, including agriculture. In particular, horse breeding was very difficult during this period, the number of horses began to decline rapidly, stud farms, racetracks, and equestrian complexes were in a difficult state. In the conditions of the reduction of the horse breeding industry and economic decline, scientists of the horse breeding laboratory received a responsible task - to preserve the gene pool of horses in Ukraine. The first scientific project on horse breeding in independent Ukraine was the research project "To improve the gene pool of breeds and develop a highly efficient technology for breeding horses for agricultural production and equestrian sports" (D. A. Volkov, O. O. Novikov, V. S. Slizh, O. M. Latka). Scientists studied the current technologies in stud farms and identified the weakest elements, which were refined and introduced into the new technology. The most effective elements of horse training technology were studied. An assessment of the quality of movements at different gaits was developed (V. S. Slizh, D. A. Volkov), research was conducted on the experimental substantiation of the technology of growing, training and testing young Ukrainian horse breeds in stud farms (D. A. Volkov, V. S. Slizh, O. M. Sobol). In 1993, an experienced horseman-practitioner Serhiy Vyacheslavovich Lyutykh entered graduate school. His research was aimed at developing breeding directions with draft horses, he studied milk and working productivity, and is currently working on the problems of sports horse breeding.

A new important transformation of the equine sector of the institute took place in 2000 - the equine laboratory again acquired a separate status due to the expansion of demand for scientific support for equine breeding of all areas of productivity in new market conditions (order No. 243/k dated October 23, 2000). Oleksandr Oleksandrovich Novikov was appointed head of the laboratory.

Taking into account the contribution of the Livestock farming institute of the NAAS to the practice of domestic horse breeding, the order of the Ministry of Agrarian Policy of Ukraine No. 165/54 dated June 9, 2003 approved the institute as a scientific breeding center for equine breeding.

According to the plan of scientific research and contractual topics, a redistribution of scientific workloads was carried out among the laboratory staff, to which scientists,



laboratory assistants and technicians from other departments of the institute were transferred; Anatoly Andriyevich Bondar, Iryna Volodymyrivna Tkachova, Valentyna Andriyivna Tverdokhlib, Valentyna Viktorivna Yatsutsenko, Victoria Alekseevna Kuraksyna, Kateryna Volodymyrivna Gdanska, Valentyna Fedorivna KovalKoval. At the same time, the laboratory was replenished with talented graduate students: N. V. Volgina, O. V. Bondarenko, T. O. Kovaleva, N. O. Laryna, O. A. Chorna, O. O. Kornienko, A. V. Andriychuk, I. V. Bilous. Most of them defended their dissertations on time and joined the ranks of scientists-hipologists and specialists in horse breeding. Over the entire period of the scientific division of horse breeding, its employees defended 13 candidate and 4 doctoral dissertations. With the expansion of scientific topics in horse breeding, other scientific departments of the institute were connected to the work: the biotechnology center (O. B. Sushko, O. V. Tkachev), the genetics laboratory (V. I. Rossokha, G. M. Tur, T. L. Voroshina, N. V. Shkavro, T. V. Kovaleva, L. T. Dobrodeeva, O. O. Aleshchenko, O. V. Boyko, O. A. Zaderikhina, O. V. Brovko, O. L. Grebenyuk), the feeding laboratory (V. M. Kandyba), the economics laboratory (O. L. Kukla), the analytical laboratory (L. M. Fedotova, T. O. Bredykhina, S. S. Varchuk). The Livestock farming institute of the NAAS was the coordinator of the scientific program "Equestrianism", in which the following took part: the Institute of Animal Breeding and Genetics, Ternopil and Mykolaiv Institutes of Animal Husbandry, Transcarpathian State Veterinary Service and Ivano-Frankivsk State Veterinary Service.

In 2007, the head of the laboratory O.O. Novikov passed away prematurely and the laboratory of horse breeding was headed by Iryna Volodymyrivna Tkachova (in the future - the director of the institute). Further work of the laboratory was aimed at preserving the gene pool and biological diversity of horses, developing effective breeding and technological methods that contribute to the realization of the genetic potential of horses in various areas of economic use. Currently, the unit carries out scientific topics in the coordination network of the Institute of Animal Breeding and Genetics named after M.V. Zubets of the NAAS for the research project "Genetic Conservation".

In 2018, the institute underwent staff optimization and changes in the structure of the institute, as a result of which the horse breeding laboratory entered the newly created Department of Breeding and Technological Research in Small Animal Husbandry and Horse Breeding as a scientific sector and breeding center for horse breeding.

And today, in extremely difficult war conditions, equine scientists continue to work for the preservation and improvement of Ukrainian horse breeding.

#### **Breeding research, creation of new types and breeds of horses.**

The limited and heterogeneous horse resources that remained after the occupation of Ukraine required significant efforts of specialists to successfully solve the problem of creating a new breed of horses and qualified assistance from scientific institutions. Employees of the Horse Breeding Department - Head V. K. Klyuchnikov, Candidate of Agricultural Sciences I. D. Manakov and Senior Researcher E. I. Senkovsky during 1946 conducted a survey of pedigree horses that survived the war in Poltava, Kharkiv, Sumy, Kirovohrad regions. The expedition survey covered 4076 horses, including the breeding stock of the Krasnograd, Kirovohrad, Pyriatyn and Pryluky stud farms. It was established (Senkovsky, 1946) that in most areas, pedigree horses are very scattered (2-3 heads each) on collective farms. Only in some breeding farms were they gathered in more or less significant groups (20-50 heads). In the Novoukrainsk district of the Kirovograd region, which was saturated with pedigree horses before the war, single individuals remained. The organization of farms in this area was initially carried out at the expense of a small number of local horses and imported mares of the Karabair breed, which, according to scientists, did not meet the task of breeding a new breed. Based on the data from the



survey and assessment of the population, a meeting of specialists was held at the Livestock farming institute and urgent measures were outlined to restore pedigree horse breeding. It was decided to import thoroughbred and high-blooded stallions into the network of state stud farms to reduce the heterogeneity of the horse massif and improve the breed, caliber and exterior indicators. On the recommendations of scientists, a significant number of riding horses of domestic origin and Western European breeds, which arrived from military units and as reparations, were gradually collected in stud farms and horse farms. The need to breed a large agricultural horse of the riding-sled type (universal working productivity) was determined instead of a highly specialized thoroughbred riding breed (Volkov D., Volkov P., 1958).

On April 16, 1953, Mykola Mikhaïlovich Burlakov was appointed head of the horse breeding department, who had previously headed the Kharkiv Regional Department of Agriculture, and even earlier - in 1945-1951 he was the director of the Ukrainian Trust of Horse Breeding Plants of the People's Commissariat of Land of the USSR. The time of his leadership of the department coincided with the rapid disbandment of the cavalry and the direction of horse breeding for sports (Kunets, 2014).

In connection with the transfer of M. M. Burlakov, on June 2, 1954, a front-line veteran, Candidate of Agricultural Sciences Dmitry Andriyovych Volkov was appointed as Deputy Director for the Scientific Part of the Institute, Head of the Horse Breeding Department. He was entrusted with the methodological and scientific management of breeding work to create the Ukrainian riding breed group of horses. He developed long-term breeding plans for 1961-1965 and 1966-1970, which gradually reduced the number of stallions of the original breeds and increasingly used stallions of the desired genealogical complex of domestic selection. In accordance with the long-term plans, stud farms carried out measures to improve horses of the Ukrainian riding breed group by means of targeted selection and selection, organization of sports training, evaluation of stud stallions by the quality of offspring, selection of progenitors and establishment of genealogical lines, maternal families.

Based on the materials of the survey of the livestock of the newly created breed group in 1970, D.A. Volkov and the department staff developed a long-term plan for breeding work with horses of the Ukrainian riding breed group for 1970-1980. The main task of the breeding work was to create a new breed of riding-sled horses suitable for use in equestrian sports (in particular, in its classical types: show jumping, dressage, eventing, style-chasing), as well as for use in agriculture and the improvement of local horses. The certificate of the Scientific Research Livestock farming institute of the Forest-Steppe and Polissya of the Ukrainian SSR dated May 7, 1971 on the mass survey of the horse population indicated that 95% of the dams met the requirements of the breed group type. By origin, these were crosses of three and four breeds, obtained as a result of crossing the Hungarian, Trakehner, Hanoverian breeds with purebred horse breeds and were largely fixed by further mating of crosses of the desired type with each other. In 1974, the employees of the Horse Breeding Department compiled the first volume of the State Stud Book of the Ukrainian Horse Breed Group. In 1978-1979, the employees of the Institute, as part of the State Commission, conducted a control survey of the horse population, took measures to organize breeding work on breeding horse farms and developed long-term work plans for each stud farm. In 1986, the employees of the Horse Breeding Department were pared in accordance with the then current regulations materials for the approbation of the Ukrainian horse breed and submitted for consideration to the State Agricultural Industry of the Ukrainian SSR and the State Agricultural Industry of the USSR.

Further selection work was carried out guided by a single plan of breeding work, developed by specialists of the horse breeding department, which made it possible to



combine the positive qualities of the original breeds and obtain horses that surpass them in a set of selection characteristics. Of great importance in this work was the creation of an initiative Breeding Council for the breed and the opening of an international auction in Kyiv for the sale of horses of the new breed. In September 1990, a group of experts, which included prominent scientists of the time - Professor Yu. N. Barmintsev, Professor O. A. Balakshin and others, conducted a survey of the basic farms - the originators of the creation of the new breed. Based on the results of the survey, specialists from the horse breeding department prepared materials for the approval of the breeding achievement, approved by the State Commission of the Council of the USSR Ministry of Food and Procurement. The new breed was given the name "Ukrainian riding horse".

The process of creating and improving horses of the domestic sports breed would have been impossible without the authors and main executors of the breeding programs – scientists of the institute and horse breeders: D. A. Volkov, O. A. Kalantary, P. A. Deineka, V. O. Peresady, M. B. Ignatieva, A. S. Filimonova, V. I. Kulikov, V. V. Stashevsky, P. Ya. Belan, N. T. Bobkova, V. P. Shimshyrt, P. M. Krymtsov, B. I. Moskalenko, I. G. Kravchenko, I. E. Gotlib. Later, scientific support for the work with the Ukrainian horse breed was carried out by Slizh V. S., Novikov O. O., Latka O. M., Bondarenko O. V., Tkachova I. V.

Currently, the Ukrainian horse breed, the main purpose of which is to be used in classical equestrian sports, ranks first among other breeds of Ukraine in terms of the number of breeding stock. The leading farms for its breeding are LLC "Kharkiv Horse Breeding Plant", branches of the State Enterprise "Equestrianism of Ukraine": Lozov Horse Breeding Plant No. 124, Oleksandrijsk Horse Breeding Plant No. 74, Dnipropetrovsk Horse Breeding Plant No. 60.

At the same time as the breeding of the Ukrainian horse breed, scientists of the horse breeding department were engaged in the improvement of heavy draft horses, the need for which was very significant in the post-war period. The prerequisite for working with draft horses was the importation of draft horses of Western European origin, mainly Belgian Ardennes, to the Dibrivsky Stud Farm in 1868. For a long time, breeders of local and improved mares crossed them with Belgian mountain Ardennes, and to a lesser extent with Brabancons and Percherons. By 1920, several breed types had formed in the Russian Ardennes breed, the smallest of which and the most uniform in proportional build were the Dibrivsky Ardennes. In 1923, draft horses of the Dibrivsky type were transferred to the Novooleksandrivsky Stud Farm, and in 1929, the stock of draft horses from the Mariupol People's Farm was transferred to this plant.

Until 1941, the main goal of working with heavy draft horses at the Novooleksandrivsky Stud Farm was to breed larger horses with correct exterior and high working productivity.

In the post-war years, heavy draft horses were sent to Ukrainian stud farms for reparations and were carefully studied. According to the recommendations of scientists from the horse breeding department, further work with heavy draft horses was based on crosses of lines with the simultaneous use of inbreeding. As a result, a type of horse was obtained, which was characterized by small stature, a wide barrel-shaped body, on low, dry, correctly set legs, with a light head, a short wide neck, a long, bifurcated, wide, slightly drooping croup.

Under the methodological guidance of D. A. Volkov, in 1948 the creation of the Novooleksandrivsky (Ukrainian) type of heavy draft horse began, officially approved on December 31, 1970. (Order No. 437 of the USSR Ministry of Agriculture). Even before the approval of the new type of heavy draft horses, starting from the selection plans



approved in 1960, its transformation into a new breed began (Volkov D. A., Kemarska M. S., Kalantar A. A.).

Further intensive use of the complexes obtained at the Dibrivsky and Novooleksandrivsky stud farms, the creation of genealogical lines and maternal families, as well as appropriate training and testing of the working qualities of horses made it possible to carry out complex work on creating a new heavy draft breed based on the heavy draft type. The decisive factors in this work were the organization and conduct of expert assessment of the breeding nucleus, the selection and intensive use of the best stallions-breeders, selection for improving the type of body structure, assessment of the quality of the offspring, testing them for working productivity. A certain role in the selection work was played by a comparative two-stage assessment of line continuations and breeding stallions by offspring and the quality of daughters used in the reproductive composition. The selection was aimed at the maximum development of the main genealogical lines and branches through the best stallions (Pavlovskii & Tkachova, 2019). The Novooleksandrivskaya heavy-duty breed was approved as a selection achievement in 1997 (Author's Certificate No. 1262 dated 28.11.1997 r.). The team of authors included D. A. Volkov (scientific supervisor), M. S. Kemarska, O. A. Kalantar, I. P. Goroshko and other highly qualified breeders. Under the leadership of Professor D. A. Volkov, scientific and methodological support of breeding work with the Novooleksandrivky Draft breed was carried out by A. S. Filimonova, S. V. Lyutikh, I. V. Tkachova, N. V. Volgina, O. O. Kornienko, S. S. Pavlovsky.

Modern horses of the Novooleksandrivky breed differ significantly from analogues of the Russian heavy draft breed, in particular its Ural type. They are more massive, have a strong constitution, are well adapted to the conditions of maintenance and use in different regions of Ukraine, are characterized by high working capacity, early maturity, fertility and milk yield (up to 4000 liters of milk). More than 20 representatives of the Novooleksandrivky heavy-duty breed were awarded the titles of champions and record holders in express cargo delivery and draft endurance.

With the participation of scientists from the horse breeding laboratory at the institute, research was conducted to study the quality of mares' milk, its nutritional value and the necessary technological conditions for the production of high-quality koumiss (S.V. Lyutykh, L.M. Rosso). State standards were developed for the requirements for the quality of mares' milk and koumiss.

Considerable attention in the scientific research of the institute was paid to the improvement of the purebred riding horse breed, which has been bred in Ukraine for more than two centuries. At different periods of time, breeding work on the improvement of horses of this breed was carried out according to selection programs developed under the methodological guidance of scientists from the horse breeding department (laboratory): D. A. Volkov, O. O. Novikov, I. V. Tkachova, T. O. Kovaleva. Every year, scientists evaluated purebred young horses at Ukrainian racetracks, made suggestions for selection plans, and developed long-term breeding plans for stud farms.

Oleksandr Oleksandrovich Novikov, head of the horse breeding laboratory in 1998-2007, was particularly interested in purebred horse breeding. As part of research on purebred horse breeding, changes in the parameters of horse selection by breed and by stud farm and lineal affiliation of the generation were studied in terms of racing class and liveliness. In stallions and dams used over the past 20 years, age variability and heredity indicators were studied, and based on these data, a new method of selecting dams for stallions by liveliness classes and a breeding program for working with purebred horses in Ukraine were proposed. The results of the research became the basis of the scientist's PhD thesis (Novikov, 2002).



Scientists of the Institute's Horse Breeding Department have conducted many years of research on the assessment of lines of purebred horse breeds by liveliness, distance, and the number of victories in races of various levels according to the international classification. As a result, the modern direction of selection is the specialization of lines in the breed depending on the presence of certain qualities of stayers, sprinters, and distancers. For this purpose, a methodology has been developed for assessing the reproductive composition of the breed by liveliness indicators with its division into classes; high, medium, and quiet, which makes it possible to take into account the performance index of horses when selecting breeding young stock and compiling selections (D. A. Volkov, O. O. Novikov).

At different stages of work with the breed, studies were conducted aimed at assessing the intensity of selection, features of variability, heredity, and repeatability of liveliness indicators in generations in terms of lines and individual stud farms. In order to determine the constant influence of individual stud stallions and the nature of their compatibility in various genealogical complexes, the horse breeding laboratory annually evaluates stallions by the quality of their offspring. The main indicator is the stallion's "success index", the number of victories and prize places in traditional prizes received by the offspring, and the total amount of winnings by the offspring.

Extensive research work has been carried out to improve the Oryol breed of horses. Resources have been studied and plans for breeding work on the breed to improve and create new lines have been developed, breeding traits have been studied and standards have been developed for individual stud farms (D. A. Volkov, P. P. Volkov, V. V. Galas, M. M. Ponomarenko, O. M. Sobol, I. V. Tkachova, O. O. Kornienko).

In recent years, active work has been carried out to create and test the domestic genealogical line of the Zapad Orlov trotting breed (Tkachova & Frolova, 2020).

An urgent task of breeding work with trotting breeds is to further increase the agility of horses both over short and long distances. In this regard, the question arises about the directions of breeding work with trotting horse breeds in the future. The basis of further work is purebred breeding of horses of each breed separately with the maximum use in breeding work of agile stallions-sprouts, who also have a clearly expressed body type of their breed. It is certain that in this case it will be necessary to obtain high-class stallions and dams and ensure their effective use in breeding work.

For a long time, in Ukraine, with the scientific and methodological support of scientists from the Livestock farming institute of the NAAS, work has been underway to improve the domestic prize trotter (D.A. Volkov, P.P. Volkov, V.V. Galas, M.M. Ponomarenko, O.M. Sobol, N.V. Volgina, I.V. Tkachova, O.O. Kornienko). The idea of creating a domestic trotting breed belongs to the luminaries of equine science at the institute - D.A. Volkov and O.O. Novikov, but it was their students and followers who had to implement it. In 2014, the first program for breeding horses of the Ukrainian trotting breed group was developed, and in 2016, materials on its testing were submitted for consideration (I.V. Tkachova, O.O. Kornienko). Given the proven genetic uniqueness of the newly created breeding achievement, it was decided to carry out work on transforming it into a new breed - the Ukrainian Trotting Horse (Tkachova & Yusyuk-Omelnytska, 2023). It is currently being tested and there is a gratifying hope that the 95th anniversary of the Livestock farming institute of the NAAS and the 90th anniversary of the Horse Breeding Department will be marked by the approval of a new breeding achievement - the Ukrainian Trotting Horse.

#### **Studies of local horse populations.**

In addition to pedigree horses, considerable attention was paid to local horses from different regions of Ukraine, as well as horses of local breeds imported from other



countries, which had excellent adaptive qualities, were unpretentious, strong and adapted to agricultural work. Thus, in 1946, scientists from the Department of Horse Breeding conducted a survey of Mongolian horses imported to the Kharkiv region in 1944 (Kunets, 2014).

In 1948-1951, scientists from the Department of Horse Breeding conducted research on the horse population in the regions of Polissya of the Ukrainian SSR, which made it possible to identify horses of the Polissya breed, and conducted experiments on improving local Polissya horses as heavy draft horses in Sumy and Chernihiv regions. The results obtained were included in the candidate's dissertation of graduate student F. K. Musienko "The Horse of Polissya of Ukraine and Methods of Its Improvement" (Musienko, 1951).

In the conditions of the mountainous region of Crimea, in 2009-2013, experiments were conducted to study herd horse breeding as a separate ecosystem. The analysis of the horse composition of the largest equestrian tourist bases of the mountainous Crimea determined the main parameters of horse selection, important for equestrian tourism: morphological and physiological features, ethology and temperament, exterior and interior indicators, breed and pedigree, as a unifying factor of the complex of selection parameters. The organizational and technological parameters of equestrian tourism and the morphometric characteristics of the mass of horses employed in equestrian tourism were studied, and a methodology for selecting horses for equestrian tourism based on these characteristics was developed (I. V. Tkachova, I. V. Bilous) (Tkachova et al., 2009). The adaptive, physiological, morphological and hematological indicators of Crimean-type horses of different sex and age groups were assessed at altitudes (from 300 to 700 m above sea level) in conditions of distance runs of 18 and 32 km (Andriichuk et al., 2015).

In 2015, within the framework of the implementation of the international cross-border program IPBU.03.01.00-18-751/11-00 "Utworzenie Polsko-Ukraińskiego Centrum Hodowli i Promocji Konia Huculskiego" ("Creation of a Polish-Ukrainian Center for Breeding and Promotion of the Hutsul Horse"), an inventory of 614 horses was conducted in the Transcarpathian region in order to identify horses of the Hutsul breed and the most typical of them, a photo archive and database were formed (I. V. Tkachova).

In the western regions of Ukraine, the Torian breed of draft horses is widespread, whose representatives were not registered in the state breeding register and were bred chaotically in small farms. During 2017-2019, at the initiative of the Horse Breeding Center of the Livestock farming institute of the NAAS (I. V. Tkachova), meetings were held with the leadership of the Tory Horse Breeding Association (Estonia), an expeditionary survey of the Tory horse population of Ukraine and an expert assessment of the horses. As a result of the work carried out, it was established that the Tory horses of Ukrainian selection meet the breed standard, and the Livestock farming institute of the NAAS received permission to organize its own pedigree register of Tory horses of Ukrainian selection, the first volume of which was published in 2021 in English (Tkachova et al., 2021).

Horse breeding paid significant attention to horse breeding technology. This direction was developed back in 1949 with the approval of the scientific topic of the horse breeding department "Development of a system for raising, training and testing pedigree horses in the State Stud Farms of the Ukrainian SSR" (head I. D. Manakov). The development of foals, selection of young animals, research groups were formed, and the results of training draft horses were studied (Kunets, 2014).

Based on the study of the technology of raising horses in stud farms and their assessment of sporting qualities depending on bloodline, lineage, selection methods and



body structure, the parameters of the desired (standard types) horses were established, as well as an experimentally substantiated and developed system of early sports training of young horses of the Ukrainian horse breed for dressage and show jumping, which ensures the development of motor skills and the ability to overcome obstacles 120-150 cm high (D. A. Volkov, V. S. Slizh).

Elements of the technology of systematic education of foals of the purebred horse breed were developed (T. O. Kovaleva), which contribute to improving work with young horses during handling, training and, accordingly, increase performance during hippodrome tests.

Separate elements of the technology of testing trotting horses were developed (V. A. Lebedev).

The relationship between physiological and biochemical indicators with the functional state of horses of sports breeds, changes in the content of oxidative stress markers and the activity of antioxidant defense enzymes in the dynamics of physical exertion was studied. A patent for a utility model (Pat. 146831 Ukraine, MPK (2021.01) A61V 5/00) "Method for determining the level of fitness of sports horses by hematological, biochemical parameters and markers of oxidative stress" (I.V. Tkachova, A.V. Andriychuk) and a monograph "Antioxidant mechanisms of adaptation of horses in the process of physical training" (jointly with Polish colleagues) were obtained.

Regulatory documentation has been prepared that regulates the technology of horse breeding based on the initial requirements for technological, organizational-economic, zoohygienic and ethological standards for breeding, keeping, training and testing horses, developed using previous developments, developed standards and recommendations (I. V. Tkachova).

#### **Improvement of the horse feeding system**

Given the great importance of the level of horse feeding for the manifestation of their genetic potential, the horse breeding department (laboratory) conducted scientific research on the organization of the feed base, studying the nutritional value of rations for different sex and age groups of horses, creating original feed premixes, protein-vitamin-mineral supplements and compound feeds.

Even at the beginning of the operation of the institute, the horse breeding department paid much attention to the organization of the feed base in the conditions of various farms. Thus, in 1949, in the conditions of the Pyriatyn and Krasnograd state breeding nurseries of the Kharkiv region (a total of 74 breeding farms), scientists of the horse breeding department developed plans for the organization of the feed base, adjusted crop rotation schemes, the structure of crops for uninterrupted supply of feed. Since 1950, D.A. Volkov has been engaged in issues of feeding heavy draft young animals, improved rations, and organized early feeding of foals. Guided by the need of horses for basic nutrients and biologically active substances, in 1993-1997, premix recipes for different sex and age groups were developed and experimentally substantiated. The effectiveness of the developed recipes was tested in the diets of breeding dams and foals from weaning to 2 years of age in scientific and production experiments at leading stud farms. A patent of Ukraine was obtained for the developed premixes (№ 20409 A dated 15.07.97). In 1996, a "sports" version of the experimental premix was developed, which was tested on sports horses of the dressage and competition groups of the Mykolaiv Equestrian Base "Kolos", as well as horses of the Ukrainian national equestrian team.

In 1997-1998, a scientific and production experiment was conducted at the Lozivsky Stud and the Olimpiysky Stud Farm in Mykolaiv to study the effect of a protein-vitamin-mineral supplement (PVMD) on the development of foals of the Ukrainian horse breed. The studies established that the inclusion of PVMD in the diet has a positive effect



on the development of foals after weaning (V. M. Kandyba, T. I. Volkova, O. O. Novikov).

In 2001-2005 in Lymarivsky and Stryletsky Stud, the positive effect of the developed compound feed with the inclusion of premixes on the reproductive ability of stallions-breeders and dams was studied. In the Stryletsky stud farm, the effectiveness of compound feed with the inclusion of a vitamin-mineral premix for suckling foals and young horses in training was also studied. Feeding the developed compound feed to suckling foals of purebred riding breeds as part of the diet has a beneficial effect on growth and development, provides a significant improvement in the performance of horses and keeps them in good prize-winning order (V. M. Kandyba, T. I. Volkova, O. O. Novikov).

A feed additive for horses during periods of intensive physical exertion has been developed. It based on oat bran-based feed with the addition of bee honey (mass fraction – 15.0%), vegetable oil enriched with vitamin D, feed chalk. The biologically active substance in the feed additive is bee honey. The energy and nutritional value of the feed additive was calculated (I. V. Tkachova)

In co-authorship with the Department of Animal Husbandry of the NAAS, a monograph for a wide range of horse breeding specialists “Physiology of Nutrition and Standardization of Horse Feeding” was published.

The development and implementation of a computerized system of breeding accounting in breeding, prize, sports and draft horse breeding (V.S. Slizh, I.V. Tkachova) ensured an increase in the rate of accumulation of informative material on horse breeding and the quality of statistical data processing. Unified forms of breeding accounting in horse breeding have been developed, databases have been created for each breed, which accumulate information on the origin, development, working capacity and breeding use of horses, registers, catalogs, state books of breeding horses are maintained, and the reproductive composition is assessed by the quality of offspring. The Livestock farming institute of the NAAS currently stores and constantly updates the most informative database of horse breeding in Ukraine.

Only in the last decade, the institute's hippologists have developed:

- The program for breeding horses of the Ukrainian riding breed until 2020;
- The program for breeding horses of the Novooleksandrivky Draft breed until 2020;
- The program for breeding horses of the Ukrainian trotting breed group until 2020;
- The program for preserving the gene pool of horses in Ukraine until 2025.
- Methodological recommendations have been developed and implemented:
- Selection and genetic parameters of horses in the genetic passporting system";
- Standardized feeding of horses of different areas of use;
- Evaluation of physiological indicators of sports and prize horses by the volume and intensity of physiological loads;
- Effective formation and use of mother families in the Ukrainian riding breed;
- Regulations on testing horses of the sports direction of work capacity.

The Horse Breeding Center actively cooperates with the State Enterprise "Equestrianism of Ukraine", the State Enterprise "Agency for Identification and Registration of Animals", the Public Organization "Association of Horse Breeders of Ukraine", the Public Organization "Association of Subjects of Stud Business in Horse Breeding", engaged in breeding horses of the Ukrainian riding breed", the Public Organization "Association of Horse Racing and Racing", the Public Organization "Association of the Draught Horse", the employees of the center are members of the



working group on horse breeding and expert commissions of the Ministry of Agrarian Policy and Food of Ukraine.

Since 2010, the employees of the horse breeding sector have supported international cooperation with the Institute of Biology and Earth Sciences of the Pomeranian University in Slupsk (Republic of Poland) on conducting joint research and implementing a research project on the study of adaptation mechanisms of sport horses, as well as with the Tory Horse Breeding Association (Estonia), which coordinates the Ukrainian-Estonian commission for assessing the breeding value of Tory horses.

The employees of the horse breeding sector carry out advisory activities, provide practical assistance (conducting boning, expert assessment of horses, description of signs, rationing of rations, clarification of pedigrees, assistance in identifying and registering horses, adjusting training elements, etc.) and provide consultations to farms on issues of breeding, keeping, feeding horses.

The diverse requirements for horses necessitate the study of methods for obtaining the most desirable types and groups of horses that meet certain requirements, the development of methods, programs and business projects that will ensure the maintenance of the competitiveness of breeds in Ukraine, taking into account social and economic conditions. The priority in scientific research is given to the main direction of horse breeding - stud horse breeding, which is able to meet the needs for horses of different breeds in a multi-structured market economy. At the same time, the task is set in the near future to reach the world level on the basis of breeding and technological developments to increase the competitiveness of horses of domestic breeding. This is expected to be achieved both through the intensification of the breeding process in breeds and the development and implementation of progressive scientifically based energy-saving technologies for breeding, training, testing of pedigree horses, immunogenetic and cytogenetic diagnostics, promising methods for assessing work capacity, feed production and feeding technology, optimal ration structure, and the economics of horse breeding production at the level of the latest world achievements.

An important issue also remains the preservation and further development of horse breeds with a limited gene pool. Competition between foreign-bred horses and domestic ones, well-adapted to the economic and climatic conditions of different regions of Ukraine, as well as socio-economic restructuring, changes in ownership forms, and the economic crisis, have made the problem of preserving valuable, small-numbered breeds of domestic breeding particularly important. The fate of horse breeds that have traditionally been bred on the territory of Ukraine for many years depends on the solution to this issue: Orel and Russian trotters of the established domestic type, purebred riding, Tory, Arabian, and especially breeds created in Ukraine, which are our national heritage: Hutsul, Ukrainian Trotter, Novooleksandrivky Draft, newly created Ukrainian Scientists-hypologists of the Livestock farming institute of the NAAS are working on these issues.

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**ACTIVITY OF ANTIOXIDANT ENZYMES IN THE CARDIAC  
AND HEPATIC TISSUES OF RAINBOW TROUT  
(*ONCORHYNCHUS MYKISS* WALBAUM) FED A DIET  
SUPPLEMENTED WITH B-GLUCANS**

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*This study investigates the effects of dietary  $\beta$ -glucans on the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx)] in the hepatic and cardiac tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum). Supplementation with  $\beta$ -glucans significantly modulated catalase (CAT) and glutathione peroxidase (GPx) activities in both tissues, suggesting enhanced antioxidant defence mechanisms. In the hepatic tissue, a 264.6% increase in CAT activity ( $p < 0.05$ ) and a 311% increase in GPx activity ( $p < 0.05$ ) was observed, indicating enhanced hydrogen peroxide detoxification and oxidative stress resistance. A similar enhancement of antioxidant capacity was observed in the heart, with an increase in cardiac CAT activity of 135.7% ( $p < 0.05$ ). Although changes in superoxide dismutase (SOD) activity were not statistically significant, a trend of decreased hepatic SOD activity and increased cardiac SOD activity was observed, potentially reflecting tissue-specific oxidative defense strategies. Glutathione reductase (GR) activity decreased in both tissues, albeit not significantly, suggesting possible adaptations in the glutathione cycle. The findings of this study emphasise the tissue-specific modulation of antioxidant pathways by dietary  $\beta$ -glucans and their potential role in enhancing oxidative stress resistance in aquaculture species. The data suggest that dietary  $\beta$ -glucans induce tissue-specific modulations of antioxidant enzyme activities in rainbow trout, potentially enhancing oxidative resilience in liver and heart tissues. These effects are likely to arise from the bioactive properties of  $\beta$ -glucans, which are known to influence immune and oxidative defence pathways. It is recommended that future studies focus on long-term feeding trials and the inclusion of additional biomarkers to facilitate a comprehensive understanding of the physiological effects of  $\beta$ -glucan supplementation.*

**Keywords:** Rainbow trout, *Oncorhynchus mykiss*,  $\beta$ -glucans, antioxidant enzymes, catalase, glutathione peroxidase, superoxide dismutase, oxidative stress, hepatic tissue, cardiac tissue.



## АКТИВНІСТЬ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ У СЕРЦЕВІЙ ТА ПЕЧІНКОВІЙ ТКАНИНАХ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS WALBAUM*), ЯКУ ГОДУВАЛИ ДІЄТОЮ, ДОПОВНЕНОЮ В-ГЛЮКАНАМИ

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У цьому дослідженні ми вивчали вплив дієтичних  $\beta$ -глюканів на активність ключових антиоксидантних ферментів [супероксиддисмутази (SOD), каталази (CAT), глутатіонредуктази (GR) і глутатіонпероксидази (GPx)] у печінці та серцевій тканині райдужної форелі (*Oncorhynchus mykiss Walbaum*) після 14-денного періоду перорального прийому добавок з  $\beta$ -глюканами. Добавка  $\beta$ -глюканів значно змінила активність каталази (CAT) і глутатіонпероксидази (GPx) в обох тканинах, що свідчить про покращення антиоксидантних механізмів захисту. У печінці було зафіксовано збільшення активності CAT на 264,6% ( $p < 0,05$ ) та GPx на 311% ( $p < 0,05$ ), що вказує на покращену детоксикацію перекису водню та підвищену стійкість до окиснювального стресу. Подібне посилення антиоксидантної активності спостерігалось і в серці, з підвищенням активності CAT в серцевій тканині на 135,7% ( $p < 0,05$ ). Хоча зміни в активності супероксиддисмутази не були статистично значущими, спостерігалась тенденція до зменшення активності SOD у печінці та збільшення її активності у серці, що потенційно відображає тканинно-специфічні стратегії окиснювального захисту. Активність глутатіонредуктази знижувалася в обох тканинах, хоча й незначно, що може вказувати на можливі адаптації в циклі глутатіону. Результати цього дослідження підкреслюють тканинно-специфічну модуляцію антиоксидантних шляхів дієтичними  $\beta$ -глюканами та їх потенційну роль у підвищенні стійкості до окиснювального стресу. Дані свідчать, що  $\beta$ -глюкани у складі раціону райдужної форелі спричиняють тканинно-специфічну модуляцію активності антиоксидантних ферментів, що потенційно підвищує окиснювальну стійкість печінки та серцевої тканини у цього виду риб. Ці ефекти, ймовірно, зумовлені біоактивними властивостями  $\beta$ -глюканів, відомих своєю здатністю впливати на імунні та окиснювальні захисні шляхи. Майбутні дослідження зосередимо на довгострокових випробуваннях  $\beta$ -глюканів в годівлі лососевих риб та включенні додаткових біомаркерів для повного розуміння фізіологічних ефектів добавок з  $\beta$ -глюканами.

**Ключові слова:** Райдужна форель, *Oncorhynchus mykiss*,  $\beta$ -глюкани, антиоксидантні ферменти, каталаза, глутатіонпероксидаза, супероксиддисмутаза, окиснювальний стрес, печінка, серцева тканина

**Introduction.** The supplementation of aquaculture diets with bioactive compounds has emerged as a key strategy to improve the health and growth performance of farmed fish (Torres-Maravilla E. et al., 2024). Among these compounds,  $\beta$ -glucans – naturally occurring polysaccharides found in the cell walls of fungi, yeasts and cereals – have gained considerable attention due to their immunostimulatory and antioxidant



properties (Singla A. et al., 2024). The incorporation of  $\beta$ -glucans in aquafeeds has been extensively studied for their role in boosting the immune system and improving resistance to pathogenic challenges (Rodrigues M. V. et al., 2020). However, their influence on the activity of antioxidant enzymes in vital organs, such as the heart and liver tissues of fish, remains underexplored.

Rainbow trout (*Oncorhynchus mykiss* Walbaum), an economically important species in aquaculture, serves as a valuable model for studying the effects of dietary supplementation on fish physiology. As ectothermic organisms, fish are particularly susceptible to oxidative stress caused by environmental and metabolic factors, which can disrupt cellular homeostasis and affect overall health (Uiuu P. et al., 2021; Banae M. et al., 2023). The liver, as the primary organ responsible for metabolism and detoxification, and the heart, which is critical for circulatory function, are particularly vulnerable to oxidative damage (Cichoż-Lach H. and Michalak A., 2014; Allameh A. et al., 2023). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play a pivotal role in mitigating the deleterious effects of reactive oxygen species (ROS), thereby maintaining cellular integrity (Jomova K. et al., 2023).

It has been hypothesised that dietary  $\beta$ -glucans may modulate oxidative stress responses by increasing the activity of antioxidant enzymes. This modulation is hypothesised to be facilitated by the activation of signalling pathways that regulate cellular redox balance (Yu C. et al., 2021). Despite the encouraging evidence from studies in other animal models, the specific effects of  $\beta$ -glucans on the enzymatic antioxidant defence systems in fish tissues are not yet fully understood. Investigation of these effects is crucial for optimising aquafeed formulations and improving the sustainability of aquaculture practices.

The present study aims to evaluate the activity of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx)] in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with  $\beta$ -glucans. By elucidating the biochemical responses to  $\beta$ -glucan supplementation, this research aims to provide insights into the potential health benefits and mechanisms of action of  $\beta$ -glucans. The findings will contribute to the development of functional aquafeeds designed to improve the resilience and performance of farmed fish under varying environmental conditions.

#### **Materials and methods.**

**Fish and experimental design.** Thirty healthy rainbow trout (*Oncorhynchus mykiss*), with an average weight of  $55.9 \pm 2.1$  g, were selected for the experiments. The fish were housed in an indoor system equipped with a freshwater supply, adequate aeration and an internal power filter. Water quality parameters were maintained at a temperature of  $16 \pm 2$  °C, dissolved oxygen levels of  $12 \pm 0.5$  ppm and pH between 7.4 and 7.6. During a 14-day acclimation period, the trout were fed a commercial basal diet at 1.5% of body weight (BW) four times a day.

Subsequent to the acclimation period, the fish were randomly divided into two groups and housed in aerated 250 L square tanks containing dechlorinated tap water, with each group occupying one tank (15 fish per tank). Throughout the feeding trial, the photoperiod conditions remained naturally occurring. The study was conducted at the Department of Salmonid Research, National Inland Fisheries Research Institute (Rutki, Poland).

The feeding trial lasted for a period of 14 days, during which the control group (n = 15) received a basal diet, while the  $\beta$ -glucan group (n = 15) received a diet supplemented with the Yestimun<sup>®</sup> powder at 1% of the basal diet (containing 85%  $\beta$ -1,3/1,6-glucans,



Leiber GmbH, Bramsche, Germany). The basal diet was supplemented with 1% Yestimun<sup>®</sup> powder (1 kg per 99 kg, w/w). This insoluble, highly purified preparation contains natural polysaccharides, including  $\beta$ -1,3/1,6-D-glucans, derived from brewer's yeast (*Saccharomyces cerevisiae*), which typically contains approximately 30%  $\beta$ -glucans on a dry weight basis.

Throughout the feeding trial, survival and weight gain were meticulously monitored. At the conclusion of the 14-day period, the fish were euthanised by decapitation, and the liver and heart tissues were collected for further analysis. It is noteworthy that the experiments were performed in duplicate.

**Hepatic and cardiac tissue isolation.** Tissue samples were collected from the fish post-decapitation, with one fish utilised for each homogenate preparation. The liver and heart were then excised, weighed and rinsed in ice-cold buffer. The tissues were then minced and washed with ice-cold 100 mM Tris-HCl isolation buffer in order to remove any residual blood. The tissues were then homogenised in 10 volumes (v/w) of isolation buffer and subsequently subjected to a centrifugation process at 3,000 g at 4°C for a duration of 10 minutes. The resulting clarified upper layer was stored in a -22°C freezer for subsequent analyses of enzymatic activity and oxidative stress biomarkers. The isolation buffer comprised 100 mM Tris-HCl, adjusted to pH 7.2 with HCl.

**Biochemical assays.** All enzymatic assays were performed at  $23 \pm 1^\circ\text{C}$  using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The initiation of the enzymatic reactions was facilitated by the addition of the homogenate suspension. The detailed assay conditions are described below, and each sample was analysed in duplicate. Protein concentrations in the samples were determined by the Bradford M. M. method (1976) using bovine serum albumin as the standard.

**Superoxide dismutase activity.** Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was evaluated by its ability to dismutate superoxide radicals generated during the auto-oxidation of quercetin in an alkaline environment (pH 10.0), according to the method described by Kostiuk V. A. et al. (1990). The reaction was initiated by adding 0.1 mL of quercetin (1.4  $\mu\text{M}$  dissolved in dimethylsulfoxide) to the reaction mixture. Absorbance at 406 nm was recorded immediately following the addition of quercetin and again after a period of 20 minutes. SOD activity was expressed in units per milligram of tissue protein.

**Catalase activity.** Catalase (CAT, E.C. 1.11.1.6) activity was determined by monitoring the reduction of  $\text{H}_2\text{O}_2$  levels in the reaction mixture, measured spectrophotometrically at 410 nm, as described by Koroliuk M. A. et al. (1988). The reaction was initiated by adding 0.1 mL of the homogenate sample to the incubation medium consisting of 2 mL of 0.03%  $\text{H}_2\text{O}_2$  solution and 1.0 mL of 4% ammonium molybdate dissolved in 12.5 mM  $\text{H}_2\text{SO}_4$  solution (used as a blank). The absorbance of the reaction mixture was compared with the blank. One unit of catalase activity was defined as the amount of enzyme required to decompose 1  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  per minute per milligram tissue protein.

**Glutathione reductase activity.** Glutathione reductase (GR, E.C. 1.6.4.2) activity was measured by the method described by Glatzle D. et al. (1974). The assay quantifies NADPH consumption spectrophotometrically in the presence of oxidised glutathione (GSSG). GR catalyses the reduction of GSSG while oxidising NADPH, resulting in a decrease in absorbance at 340 nm. The reaction mixture contained 2.4 mL of 67 mM sodium phosphate buffer (pH 6.6), 0.2 mL of 7.5 mM GSSG and 0.1 mL of the homogenate sample. The oxidation rate of NADPH was monitored at 340 nm and quantified on the basis of the molar extinction coefficient of  $6.22 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ . GR activity was expressed as nmol NADPH oxidised per minute per milligram tissue protein.



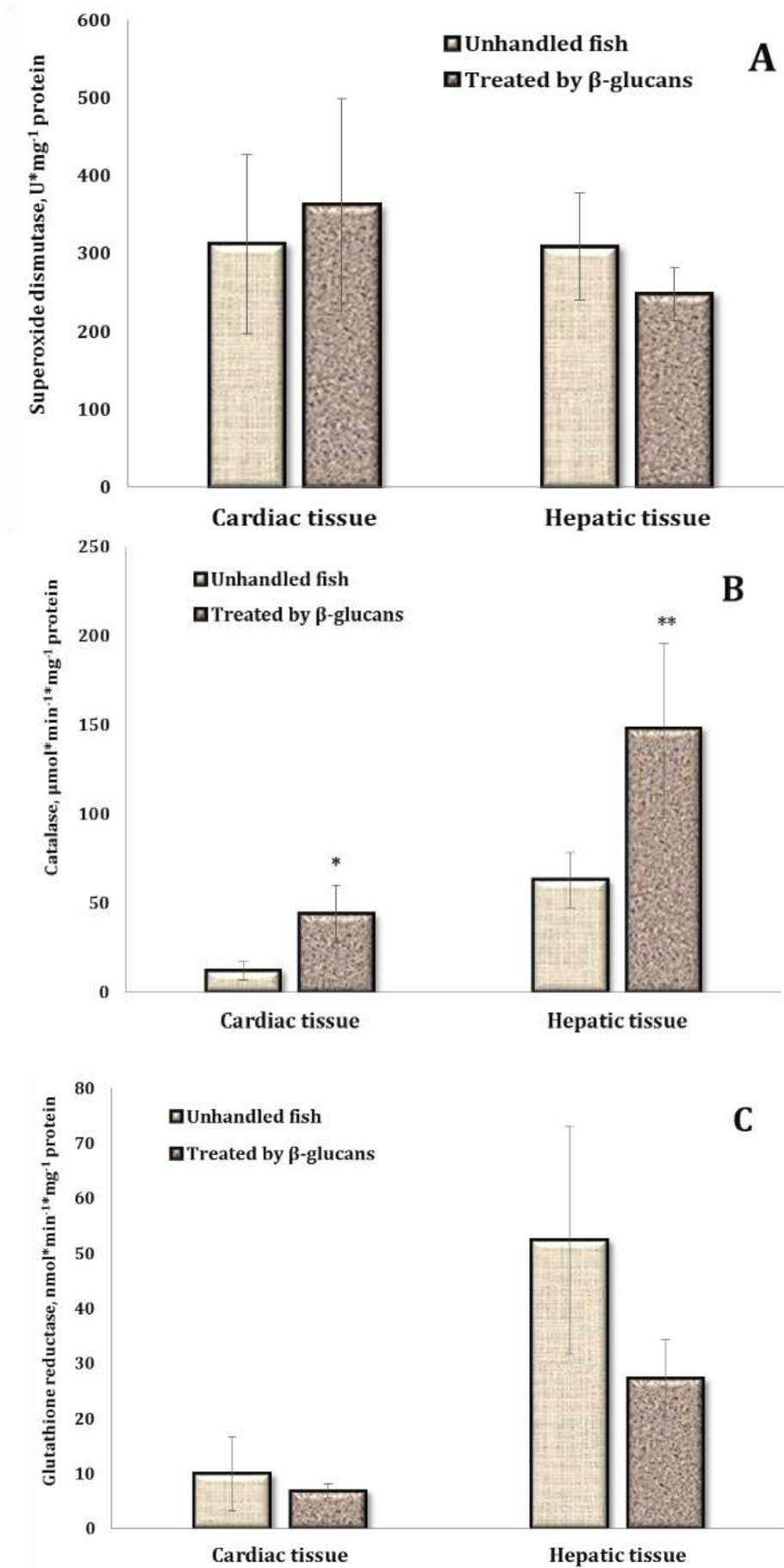
**Glutathione peroxidase activity.** Glutathione peroxidase (GPx, E.C. 1.11.1.9) activity was determined by measuring the non-enzymatic consumption of reduced glutathione (GSH) in a reaction mixture containing 5,5-dithiobis-2-nitrobenzoic acid (DTNB), as described by Moin V. M. (1986). The test mixture consisted of 0.8 mL 0.1 M Tris-HCl buffer containing 6 mM EDTA and 12 mM sodium azide (pH 8.9), 0.1 mL 4.8 mM GSH, 0.2 mL homogenate sample, 1 mL 20 mM t-butyl hydroperoxide and 0.1 mL 0.01 M DTNB. The rate of GSH oxidation was monitored spectrophotometrically at 412 nm. GPx activity was expressed as nmol of GSH oxidised per minute per milligram tissue protein.

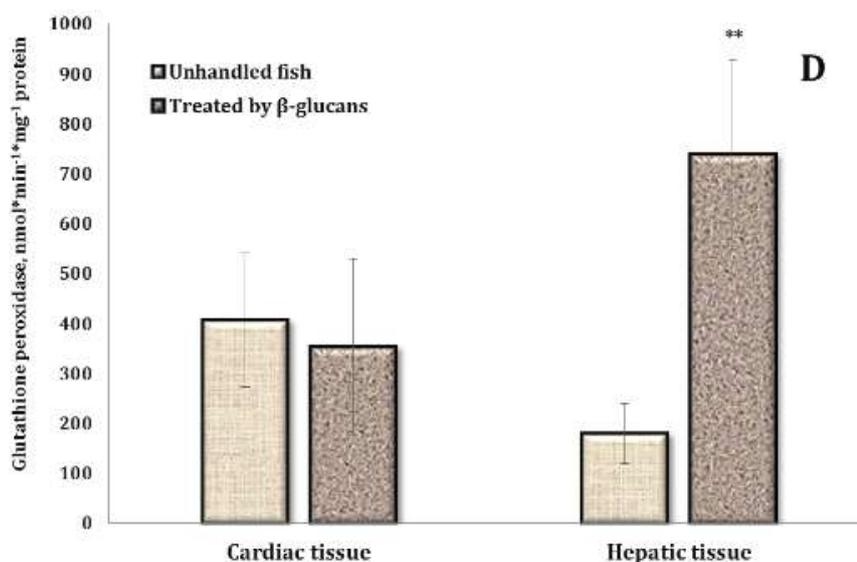
**Statistical analysis.** Basic statistical analyses were performed using the Statistica 13.3 software package (TIBCO Software Inc., USA). Homogeneity of variance was assessed using Levene's test for equality of error variances, while normality of data was assessed using the Kolmogorov-Smirnov test. The results obtained are presented as the mean  $\pm$  standard deviation (S.D.). The determination of significant differences between means was conducted through the implementation of a multiple range test, with a minimum significance level of  $P < 0.05$ . Comparisons between the control and experimental groups were made using multivariate analysis of variance (MANOVA) followed by Bonferroni's post-hoc test. Statistical differences were considered significant at  $P < 0.05$ . For data sets that did not follow a normal distribution, logarithmic transformation was applied according to Zar J. H. (1999).

**Results.** The activity of key antioxidant enzymes in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with  $\beta$ -glucans is shown in Figure 1.

The results of the present study demonstrated that the administration of low doses of  $\beta$ -glucans induced a decrease in SOD activity in the hepatic tissue of rainbow trout to ( $247.83 \pm 33.90$  U $\cdot$ mg $^{-1}$  protein), in comparison to the untreated trout ( $309.17 \pm 68.99$  U $\cdot$ mg $^{-1}$  protein) (by 19.8%,  $p > 0.05$ ). In the cardiac tissue, an increase in SOD activity was observed, reaching ( $362.84 \pm 136.13$  U $\cdot$ mg $^{-1}$  protein) in the experimental group compared to ( $312.56 \pm 115.05$  U $\cdot$ mg $^{-1}$  protein) in the untreated trout (by 16.1%,  $p > 0.05$ ) (Fig. 1A). The administration of low doses of  $\beta$ -glucans resulted in a statistically significant increase in CAT activity (Fig. 1B). In the hepatic tissue of rainbow trout, CAT activity was increased to ( $148.88 \pm 48$   $\mu$ mol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) in comparison to the untreated trout ( $62.83 \pm 15.3$   $\mu$ mol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 264.6%,  $p < 0.05$ ). In the cardiac tissue, CAT activity increased to ( $43.75 \pm 16.08$   $\mu$ mol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) compared to the untreated trout ( $12.0 \pm 4.89$   $\mu$ mol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 135.7%,  $p < 0.05$ ) (Fig. 1B).

The results obtained demonstrated that the administration of low doses of  $\beta$ -glucans induced a decrease in GR activity in the cardiac tissue of rainbow trout to ( $6.73 \pm 1.3$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein), in comparison to the untreated trout ( $9.88 \pm 6.73$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 31.9%,  $p > 0.05$ ). In the hepatic tissue, GR activity was decreased to ( $27.2 \pm 7.1$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) compared to the untreated trout ( $52.31 \pm 20.7$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 48%,  $p > 0.05$ ) (Fig. 1C). The administration of low doses of  $\beta$ -glucans resulted in a modification in GPx activity (Fig. 1D). In the hepatic tissue of rainbow trout, GPx activity was increased to ( $738.45 \pm 189$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) in comparison to the untreated trout ( $179.66 \pm 60.52$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 311%,  $p < 0.05$ ). In the cardiac tissue, GPx activity was reduced to ( $352.32 \pm 177.43$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) in comparison to the untreated trout ( $406.24 \pm 133.88$  nmol $\cdot$ min $^{-1}$  $\cdot$ mg $^{-1}$  protein) (by 13.3%,  $p > 0.05$ ) (Fig. 1D).





**Fig. 1. The activity of key antioxidant enzymes [superoxide dismutase (SOD, A), catalase (CAT, B), glutathione reductase (GR, C), and glutathione peroxidase (GPx, D)] in the cardiac and hepatic tissues of rainbow trout fed a diet supplemented with β-glucans.**

*The results are expressed as mean ± S.D.*

*\* and \*\* – Differences between the control and experimental groups were analyzed with MANOVA and Bonferroni's post-hoc test. Differences were considered significant at  $P < 0.05$ .*

**Discussion.** The results of this study offer insight into the modulatory effects of dietary β-glucans on antioxidant enzyme activities in the hepatic and cardiac tissues of rainbow trout. A notable finding was the differential response of superoxide dismutase (SOD) activity between hepatic and cardiac tissues. The decline in hepatic SOD activity in trout treated with β-glucans suggests a potential reduction in oxidative stress levels or a shift in the oxidative defence strategy within the liver. Conversely, the observed increase in cardiac SOD activity suggests enhanced superoxide detoxification, which could be a compensatory mechanism to counteract oxidative stress in cardiac tissue. While the changes in SOD activity did not reach statistical significance, the observed trends merit further investigation to elucidate tissue-specific responses to β-glucan supplementation.

The marked increase in catalase (CAT) activity in both hepatic and cardiac tissues following β-glucan supplementation underscores the pivotal function of this enzyme in mitigating oxidative stress by degrading hydrogen peroxide. The marked increase in hepatic CAT activity (by 264.6%,  $p < 0.05$ ) compared to the control group underscores the pivotal role of the liver in detoxification processes. A similar increase in cardiac CAT activity (135.7%,  $p < 0.05$ ) suggests a robust increase in antioxidant capacity, which may protect against oxidative damage in cardiac tissue. The findings suggest that β-glucans stimulate hydrogen peroxide detoxification pathways, thereby enhancing tissue resistance to oxidative stress.

Conversely, a decrease in glutathione reductase (GR) activity was observed in both liver and heart tissues, though these changes did not attain statistical significance. This decline could be indicative of alterations in the glutathione cycle, potentially reflecting an adaptive response to dietary β-glucans. The pronounced decrease in hepatic GR activity (by 48%) suggests that the hepatic glutathione pool may be differentially



modulated by  $\beta$ -glucan supplementation. However, further studies are required to clarify the implications of this finding for cellular redox balance.

Glutathione peroxidase (GPx) activity exhibited divergent responses in liver and heart tissues. The marked increase in hepatic GPx activity (by 311%,  $p < 0.05$ ) indicates an increased use of reduced glutathione for peroxide detoxification, thus highlighting the central role of the liver in oxidative defence. Conversely, the modest decrease in cardiac GPx activity suggests that other antioxidant systems may compensate for peroxide detoxification in the heart. These observations underscore the tissue-specific modulation of the glutathione system by  $\beta$ -glucans.

In our previous study (Tkachenko H. et al., 2023), we explored the impact of dietary  $\beta$ -glucans on lipid peroxidation in the hepatic and cardiac tissues of three fish species: rainbow trout (*Oncorhynchus mykiss*), European whitefish (*Coregonus lavaretus*), and graylings (*Thymallus thymallus*), following a 14-day supplementation period. The results indicated a significant reduction in 2-thiobarbituric acid reactive substances (TBARS) levels, a marker of lipid peroxidation, in both tissues for rainbow trout and European whitefish. In a similar manner, graylings that were fed low doses of  $\beta$ -glucans for a period of 14 days exhibited decreased TBARS levels in both hepatic and cardiac tissues. These findings suggest that dietary  $\beta$ -glucans can effectively enhance the antioxidant defence systems in these fish species, mitigating oxidative stress by reducing lipid peroxidation. This antioxidative effect appears consistent across the examined species and tissues, demonstrating the potential of  $\beta$ -glucans as a beneficial dietary supplement in aquaculture. The hypothesis that low-dose  $\beta$ -glucan supplementation enhances antioxidant capacity in fish is supported by the observed reduction in TBARS levels in key tissues such as the liver and heart. These results are consistent with the broader understanding of  $\beta$ -glucans as functional feed additives with both health-promoting and growth-enhancing properties in aquaculture species (Tkachenko H. et al., 2023).

It is well established that dietary  $\beta$ -glucans possess immunostimulatory and antitumour properties, primarily through the activation of mucosal immune system cells via specific  $\beta$ -glucan receptors (Nakashima A. et al., 2018; Zhong X. et al., 2023). The receptors that have been identified include dendritic cell-associated C-type lectin-1 (Dectin-1), complement receptor 3 (CR3), differentiation cluster 11b (CD11b)/CD18,  $\alpha$ M $\beta$ 2-integrin, macrophage differentiation antigen-1 (Mac-1), lactosylceramide (LacCer), and scavenger receptors (SRs) (Zhong X. et al., 2023).  $\beta$ -glucans and mannans derived from yeast cell walls have been identified as alternatives to antibiotics for the prevention and treatment of animal diseases, as well as the mitigation of the emergence and spread of antibiotic-resistant bacterial pathogens (Liu Y. et al., 2021; Bar-Dagan H. et al., 2023).

When administered orally,  $\beta$ -glucans are absorbed through the gastrointestinal tract, taken up by tissue macrophages, and fragmented. These fragments are then transported to the bone marrow and reticuloendothelial system, where they are released and subsequently taken up by other immune cells, triggering various immunological responses (Barton C. et al., 2016; Singh R. P. and Bhardwaj A., 2023). These characteristics have prompted research into the utilisation of  $\beta$ -glucan particles as vaccine platforms against invasive fungal diseases (Vetvicka V. et al., 2020). Additionally,  $\beta$ -glucan particles are being explored as oral vaccine delivery systems, functioning both as carriers and adjuvants (Mirza Z. et al., 2017; Wu Y. et al., 2023).

As a group of polysaccharides with inherent immunostimulatory characteristics,  $\beta$ -glucans present promising opportunities for developing novel vaccine adjuvants. Their compatibility, safety, and tolerability render carbohydrate structures such as



polygalactans, fructans,  $\beta$ -D-glucans,  $\alpha$ -D-glucans, D-galactose, and D-glucose attractive candidates for use as vaccine adjuvants and immunomodulatory (Colaço M. et al., 2022; Liang X. et al., 2024). Key factors influencing the toxicological and adjuvant properties of  $\beta$ -glucan-based formulations include the particle size and the method of antigen encapsulation or surface adsorption (Colaço M. et al., 2022; Jesus S. et al., 2024).

Experimental research has illuminated the mechanisms of immune activation by  $\beta$ -D-glucans, particularly the roles of dectin-1 and C3-iCR3 receptors. In order to optimise the therapeutic application of  $\beta$ -glucans, it is essential to thoroughly define biologically active molecules and to comprehensively characterise glucans from various sources chemically and biologically (Tsoni S. V. and Brown G. D., 2008; Mata-Martínez P. et al., 2022).

It is noteworthy that the physicochemical properties of  $\beta$ -glucans include antioxidant activity, enabling them to scavenge reactive oxygen species (ROS). In addition to their role in disease prevention,  $\beta$ -glucans have a significant role in the human diet as a source of fibre, which has been demonstrated to reduce cholesterol absorption, enhance digestive processes, and stimulate the production of short-chain fatty acids in the intestines (Kofuji K. et al., 2012; Nakashima A. et al., 2018).

In order to evaluate the capacity of  $\beta$ -glucans as natural antioxidants, an investigative approach was undertaken by Song H. S. and Moon K. Y. (2006) that entailed the examination of their antioxidant properties through the utilisation of five distinct *in vitro* methods. These methodologies encompassed the assessment of lipid peroxidation value (POV), nitric oxide (NO) scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, and  $\beta$ -carotene diffusion assay. The  $\beta$ -glucans evaluated encompassed extracts from *Saccharomyces cerevisiae* KCTC 7911, along with both water-soluble and water-insoluble forms derived from the yeast mutant *S. cerevisiae* IS2. In the POV test, the antioxidant activities of all  $\beta$ -glucans were either comparable to or exceeded that of vitamin C, a well-known antioxidant. It is noteworthy that both  $\beta$ -glucan and water-insoluble mutant  $\beta$ -glucan exhibited 2.5 times the potency of vitamin C at a dosage of 2 mg. Conversely, in the NO and DPPH tests, which evaluate radical scavenging capacity, vitamin C exhibited approximately 100 times greater activity than the  $\beta$ -glucans. Notably, the  $\beta$ -glucans exhibited higher radical scavenging activity than N-acetyl-L-cysteine (NAC), a recognised radical scavenger, in the DPPH test. Furthermore, the water-insoluble mutant  $\beta$ -glucan demonstrated 2.6-fold and 5-fold greater activity than the water-soluble  $\beta$ -glucan in the NO and DPPH tests, respectively, suggesting that all  $\beta$ -glucans possess the capacity to effectively scavenge radicals such as NO and DPPH. In the reducing power and  $\beta$ -carotene diffusion assays,  $\beta$ -glucans exhibited lower antioxidant profiles in comparison to vitamin C. Nevertheless,  $\beta$ -glucan and the water-insoluble mutant  $\beta$ -glucan displayed marginal reducing power and significant  $\beta$ -carotene diffusion activity. These findings underscore the differential antioxidant potentials of the various  $\beta$ -glucan forms. The results obtained demonstrate that  $\beta$ -glucans, particularly the water-insoluble mutant form, possess notable antioxidant properties and could serve as effective natural antioxidants. The activity of  $\beta$ -glucans across a range of assays indicates their potential for application in health and nutrition as a viable alternative to synthetic antioxidants (Song H. S. and Moon K. Y., 2006).

The collective evidence suggests that dietary  $\beta$ -glucans induce tissue-specific modulations of antioxidant enzyme activities in rainbow trout, potentially enhancing oxidative resilience in liver and heart tissues. These effects are likely to arise from the bioactive properties of  $\beta$ -glucans, which are known to influence immune and oxidative defence pathways. Future studies should focus on long-term feeding trials and the



inclusion of additional biomarkers to fully understand the physiological effects of  $\beta$ -glucan supplementation.

**Conclusions.** The present study demonstrates that dietary supplementation with low doses of  $\beta$ -glucans significantly influences antioxidant enzyme activities in the hepatic and cardiac tissues of rainbow trout. The marked increases in CAT and GPx activities in the liver and heart highlight the potential of  $\beta$ -glucans to enhance oxidative defence mechanisms. Conversely, the observed decreases in SOD and GR activities reflect a complex, tissue-specific response to  $\beta$ -glucan supplementation. The findings of this study provide valuable insights into the role of dietary  $\beta$ -glucans in modulating oxidative stress and support their use as a functional feed additive to improve fish health and resilience.

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## IGESTIBILITY OF NUTRIENTS AND ENERGY SUPPLY OF ANIMALS UNDER THE INFLUENCE OF UNIFORMITY OF MIXING FEED

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*The article presents data from studies of the influence of the factor of uneven mixing of the feed mixture (corn silage, alfalfa hay and compound feed) on the apparent digestibility of nutrients throughout the gastrointestinal tract of young cattle (9 heifers of the Ukrainian red-motley breed). The studies used a method of modeling the consumption of unevenly mixed rations by animals, where 25 % of the ration consisted of high-energy components of the mixture in the second experiment, and feeds with a low energy concentration in the third. It was found that when consuming a low-concentrate feed mixture with an energy concentration in the diet of 8.9 MJ/kg dry matter and a crude protein concentration of 9.7 % with a mixing uniformity of 75 % compared to the control diet (100 % mixing uniformity), there were implausible changes in the consumption of nutrients by individual animals. Fluctuations in the apparent digestibility of nutrients throughout the gastrointestinal tract of young cattle ranged from 2 % to 6 % when consuming a feed mixture with a mixing uniformity of 75 %. On the high-nutrient diet, the digestibility of crude protein and crude fiber increased by 14% and 9 %, respectively. On the low-nutrient diet, there was a significant ( $p < 0.05$ ) decrease in digestibility by 5 % for dry matter and organic matter and nitrogen-free extractives, and for crude protein (by 12 %), compared to the high-nutrient diet. The provision of animals with energy and protein significantly increased ( $p < 0.01$ ) on the high-nutrient diet by 34.7 % and 11.5 %, respectively. Consumption of the low-nutrient diet had practically no effect on energy content. When feeding a feed mixture with a 75 % mixing uniformity on a low-nutrient diet, a significant increase in alkaline phosphatase activity was observed by 42 %, a high-nutrient diet significantly affected the increase in erythrocyte content by 7.9 %, while most hematological and biochemical blood parameters of heifers fluctuated within the physiological norm for animals of this age.*

**Keywords:** digestibility, feed mixture, energy available for exchange, mixing uniformity, hematological and biochemical blood parameters.



## ПЕРЕТРАВНІСТЬ ПОЖИВНИХ РЕЧОВИН І ЗАБЕЗПЕЧЕНІСТЬ ТВАРИН ЕНЕРГІЄЮ ЗА ВПЛИВУ РІВНОМІРНОСТІ ЗМІШУВАННЯ КОРМОСУМІШІ

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*У статті наведені дані досліджень впливу фактору нерівномірного змішування кормосуміші (силос кукурудзяний, сіно люцернове та комбікорми) на видимому перетравності поживних речовин по всьому шлунково-кишковому тракту молодняка великої рогатої худоби (9 теличок червоно-рябої української породи). У дослідженнях використовували метод моделювання споживання нерівномірно змішаного раціону тваринами, де 25 % раціону складало у другому досліді високоенергетичні компоненти суміші, а у третьому – корми з низькою концентрацією енергії. Встановлено, що при споживанні нізкоконцентратної кормосуміші при концентрації енергії в раціоні 8,9 МДж/кг сухої речовини і концентрації сирого протеїну 9,7 % з рівномірністю змішування 75 % в порівнянні з контрольним раціоном (100 % рівномірність змішування) відбувалися невірогідні зміни в споживанні поживних речовин окремими тваринами. Коливання видимої перетравності поживних речовин по всьому шлунково-кишковому тракту молодняка великої рогатої худоби становили від 2 % до 6 % при споживанні кормосуміші з рівномірністю змішування 75 %. На високопоживному раціоні переваримість сирого протеїну та сирого клітковини зросла на 14 % і 9 %, відповідно. На нізкопоживному раціоні спостерігалось вірогідне ( $p < 0,05$ ) зниження перетравності на 5 % для сухої і органічної речовини та безазотистих екстративних речовин, для сирого протеїну (на 12 %), в порівнянні з високопоживним. Забезпеченість тварин енергією і протеїном значно збільшувалась ( $p < 0,01$ ) на високопоживні раціони на 34,7 % і на 11,5 %, відповідно. Споживання нізкопоживного раціону практично не впливало на вміст енергії. При згодовуванні кормосуміші 75 %-вої рівномірності змішування на нізкопоживному раціоні спостерігалось вірогідне збільшення активності лужної фосфатази на 42 %, високопоживний раціон вірогідно вплинув на збільшення вмісту еритроцитів на 7,9 %, тоді як більшість гематологічних і біохімічних показників крові теличок коливалася в межах фізіологічної норми для тварин даного віку.*

**Ключові слова:** перетравність, кормосуміш, доступна для обміну енергія, рівномірність змішування, гематологічні і біохімічні показники крові.

**Introduction.** One of the main advantages of using feed mixtures is the leveling of differences in the consumption of diet components by individual animals. This property of the feed mixture is directly related to the mixing uniformity of its components. Finding out the optimal mixing uniformity is important from the point of view of saving resources and fully providing productive animals with nutrients.

In the case of poor-quality feed mixture preparation with insufficient mixing or separation of feeds during distribution, selective eating of feeds by individual animals may occur (Amaral-Phillips D. M, et al., Grant R. J. et al, 1990, Kononoff P. J. et al, 2003a and 200b). The uniformity of mixing significantly depends on the degree of



grinding of roughage (Baran M., 1980., Clauss M. et al, 2011, Tafaj M. et al, 1999). Our studies and data from other authors indicate that from a physiological point of view, there are optimal sizes of roughage particles, deviations from which in the direction of increase cause a decrease in dry matter intake and a slowdown in the rate of fractional outflow from the rumen, and deviations in the direction of decrease cause a decrease in the number of chewing movements during chewing and the volume of saliva (Guozhong D., et al., 2011, Lechner I. et al., 2013., Rolf S. et al., 2018), disrupt the specific layered structure of the rumen contents and its motility, contribute to increased acidity in the rumen, and are often accompanied by abomasum displacement and digestive disorders (Kaiyue P. et al., 2022, Golder H. M. et al., 2014, Krause K. M. et al., 2002 and 2003). Finding the optimal size of coarse feed grinding when preparing a feed mixture is a compromise between the negative impact of overgrinding and the negative consequences of insufficiently uniform mixing (Beauchemin K. A. et al., 2003, Maekawa M. et al., 2002, Maulfair D. D. et al., 2011, Vibart R. E. et al., 2010). In our opinion, the provision of animals with nutrients with the same uniformity of mixing of the feed mixture, but with different energy content, can differ significantly (Vasylevskiy M. V. and Yeletska T. O., 2012).

**Purpose of the work.** To investigate the effect of consuming a feed mixture with a mixing uniformity of 75% by young cattle on the apparent digestibility of nutrients in the diet and the content of energy available for exchange in order to ensure the effective use of feed nutrients and their transformation into livestock products.

**Materials and methods.** To study the effect of the uniformity of feed mixture mixing on the consumption of nutrients and the energy actually received by the animals from the diet, three experiments were conducted on 9 heifers of the red-and-white breed. The animals were kept tethered, fed twice a day with free access to individual automatic drinkers. The diet consisted of corn silage, alfalfa hay, and mixed feeds.

The studies were conducted using the method of modeling the consumption of an unevenly mixed diet by animals. For this purpose, the content of individual feeds in the main diet and their ratios were calculated. In the first experiment (control), the animals received all the feeds in the form of a feed mixture, which corresponded to a mixing uniformity of 100%. Then, the ratio of feeds was calculated at a uniformity of 75%. For this purpose, it was assumed a priori that 75% uniformity is the actual consumption by animals of a diet in which 75% is represented by 100% uniformly mixed feed mixture, in which the ratio of all components corresponds to the diet of the first experiment. The remaining 25% was replaced in the second experiment with high-energy components of the mixture, and in the third with feeds with a low energy concentration. The content of available energy for metabolism (AEE) was calculated based on apparently digestible nutrients using energy coefficients (Nehring K., 1975). Statistical processing was performed using the method of variance analysis and the method of paired related data (Vasilevskij N. V., 2007). The case was considered probable when the error was less than 5%. Since the purpose of our research was to find the boundary of the zone of influence of negative parameters, the probability analysis was also carried out in the zone of tendency to probability, when the error was in the range from 20% to 5%.

**Research results.** The amount of feed consumed is presented in Table 1. The consumption of dry and organic matter in our experiments was at the level of 3.5–4.0 kg, respectively.



Table 1

**Average consumption of dietary nutrients by animals, (M ± m), n = 3**

<b>Experiments</b>	<b>1</b>	<b>2</b>	<b>3</b>
DM, g	3396 ± 148	4406 ± 52	3728 ± 26
Cv, %	7,55	2,05	1,20
OM, g	3193 ± 135	4140 ± 47	3501 ± 23
Cv, %	7,31	1,97	1,15
CFt, g	62,27 ± 1,95	67,38 ± 0,57	69,87 ± 0,41
Cv, %	5,43	1,46	1,02
CP, g	320 ± 12	463 ± 4	340 ± 2
Cv, %	6,39	1,57	1,07
CF,g	1145 ± 44	1486± 17	1248 ± 8
Cv, %	6,65	1,93	1,12
NFE, г	1666 ± 77	2124 ± 26	1843 ± 13
Cv, %	8,01	2,11	1,22
Ca, г	29,79 ± 1,34	46,84 ± 1,74	31,03 ± 0,25
Cv, %	7,76	6,43	1,39
P, г	8,59 ± 0,40	12,24 ± 1,62	9,04 ± 0,07
Cv, %	8,07	22,87	1,29

Note: Here and below: DM – dry matter, OM – organic matter, CF – crude fat, CP – crude protein, CF – crude fiber, NFE – nitrogen-free extractives, Cv – probability coefficient.

The largest spread for the main nutrients was observed in the first experiment (Cv = 6.99 ÷ 7.63%), a significant decrease in the probability coefficient compared to the control was in the second (5.50 ÷ 5.57%) and third experiments (0.71 ÷ 1.00%). Calculation of the significant difference according to Student's t-test in the first and second experiments between the consumption of dry and organic matter, crude protein, crude fiber, nitrogen-free extractives showed a significant difference (p < 0.05 ÷ 0.01) in the consumption of these substances, the change in the consumption of crude fat was at the trend level (Table 2).

There was also a trend level (0.2 > p > 0.05) difference in the consumption of dry and organic matter, crude protein, crude fiber, nitrogen-free extractives between the first and third experiments. A significant difference in the consumption of crude fat was established (p < 0.05). When comparing nutrient intake in the second and third experiments, a significant difference was established for all indicators (p < 0.05 ÷ 0.01). Using the Student's t-test for the same indicators, but by the method of pairwise related dry matter intake, showed a significant difference in the intake of crude fiber and nitrogen-free extractives, for other nutrients - at the level of the trend between the first and third experiments.



Table 2

**Difference in nutrient intake of diets with different mixing uniformity**

<b>The difference between experiments</b>	<b>1 та 2</b>	<b>1 та 3</b>	<b>2 та 3</b>
<b>DM g</b>	<b>-1010,6</b>	<b>-332,2</b>	<b>678,43</b>
t <sub>1</sub>	-6,44**	2,21*	11,66***
t <sub>2</sub>	-5,20**	2,72*	9,86***
<b>OM, g</b>	<b>-946,9</b>	<b>-308,01</b>	<b>638,93</b>
t <sub>1</sub>	-6,63**	2,25*	12,15***
t <sub>2</sub>	-5,87**	2,76*	10,28***
<b>CFt, g</b>	<b>-5,11</b>	<b>-7,60</b>	<b>-2,49</b>
t <sub>1</sub>	2,51*	3,81**	-3,56**
t <sub>2</sub>	2,26*	4,93**	2,93**
<b>CP, g</b>	<b>-142,99</b>	<b>-20,03</b>	<b>122,97</b>
t <sub>1</sub>	11,43***	1,67	26,25***
t <sub>2</sub>	-10,09***	2,06*	22,19***
<b>CF, g</b>	<b>-341,38</b>	<b>-103,3</b>	<b>238,09</b>
t <sub>1</sub>	-7,27**	2,31*	12,91***
t <sub>2</sub>	-6,39**	35,92***	10,93***
<b>NFE, g</b>	<b>-457,5</b>	<b>-176,8</b>	<b>280,64</b>
t <sub>1</sub>	-5,63**	2,26*	9,71***
t <sub>2</sub>	-5,00**	2,76**	8,20***
<b>Ca, g</b>	<b>-17,05</b>	<b>-1,24</b>	<b>15,81</b>
t <sub>1</sub>	-7,78**	0,91	9,00***
t <sub>2</sub>	-6,50**	1,14	8,43***
<b>P, g</b>	<b>-3,66</b>	<b>-0,45</b>	<b>3,21</b>
t <sub>1</sub>	-2,19*	1,11	1,98*
t <sub>2</sub>	-2,00*	1,35	1,94*

Note. t<sub>1</sub> – calculated indicator based on variance analysis; t<sub>2</sub> – calculated indicator based on pairwise related data on SR consumption; \* – probability of differences between the indicators of the experiments at the trend level; \*\* – probability of differences between the indicators of the experiments at the p < 0.05 level; \*\*\* – probability of differences between the indicators of the experiments at the p < 0.01 level.

Such differences between the intakes in the experiments caused a difference in the characteristics of the actually consumed rations (Table 3). It was determined that all these indicators significantly differed between the second and third experiments. A significant difference was established in the concentration of crude protein between the first and second and the first and third experiments. It was established that the excretion of nutrients with feces differed significantly only in the content of nitrogen-free extractives (p < 0.05) in the first and second and in the first and the third experiments.



Table 3

Characteristics of rations, (M±m), n=3

Experiments	Exchangeable energy (EE), MJ EA	Concentration EE, MJ/kgDM	Feeding level, MJ/W <sup>0,75</sup>	Concentration CP, %	CP/EE, g/MJ
1	30,09 ± 2,24	8,86 ± 0,66	0,59 ± 2,41	9,42 ± 0,07	10,70 ± 0,57
2	40,54± 6,47**1,2	9,20 ± 2,66	0,77± 6,07*1,2	10,50± 45,99***1,2	11,43 ± 2,51
3	31,89± 0,80***2,3	8,55± 0,64**2,3	0,61± 0,27***2,3	9,11 ± 4,62***1,2; **1,3;***2,3	10,62± 0,11***2,3

Note: \* - probability of differences between experimental indicators at the trend level; \*\* - probability of differences between experimental indicators at the p <0.05 level; \*\*\* - probability of differences between experimental indicators at the p <0.01 level.

Based on the data on the consumption and excretion of nutrients, their digestibility was calculated (Fig. 1).

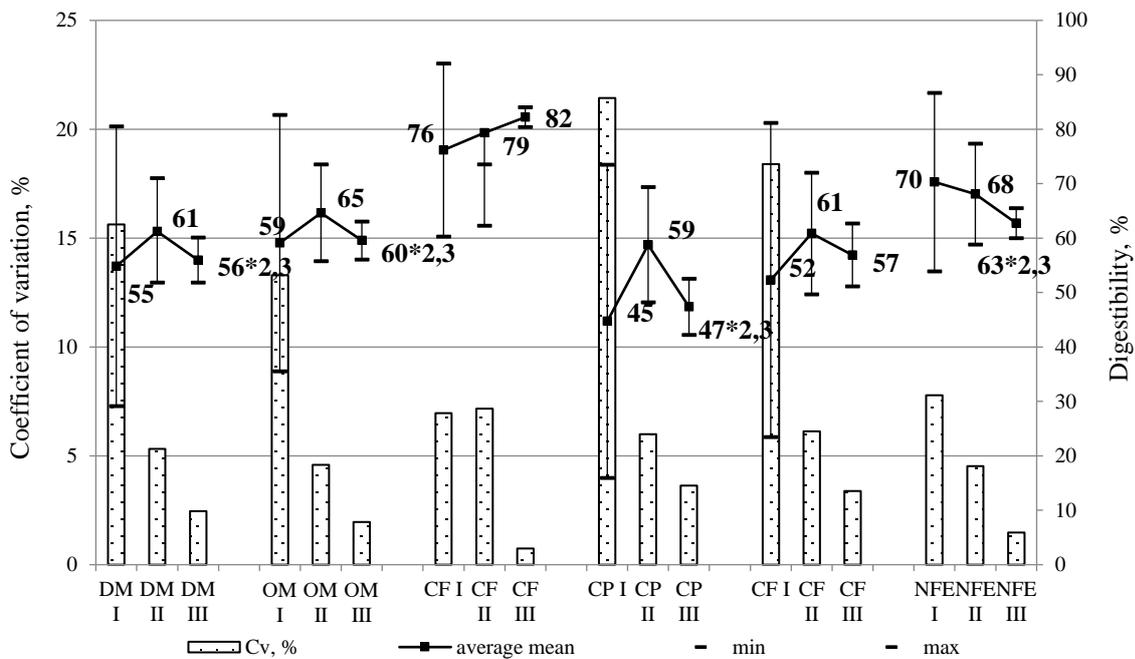


Fig. 1. Nutrient digestibility of diets with 75 % uniformity of mixing.

The fluctuations of this indicator between experiments ranged from 2 % to 6 %. But in the second experiment, compared to the control, the digestibility of crude protein and crude fiber increased by 14 and 9 %, respectively. The apparent digestibility throughout the gastrointestinal tract of the experimental animals due to the uneven mixing of the components in the second and third experiments, compared to the control, did not significantly change. When comparing the second and third experiments, a decrease in digestibility by 5 % was observed for dry matter, organic matter and nitrogen-free extractives, as well as a significant decrease in digestibility of crude protein (by 12 %) (p <0.05).

It is known that the physiological state of the animal, its age, feeding conditions, productivity and other factors affect the composition of the animal's blood. Thus, the



results of biochemical studies (Table 4) of selected blood samples showed that the content of total protein, as the main indicator of its use in the body, was higher in the third experiment, in the second experiment this indicator had an intermediate value.

The content of albumins in the serum of heifers for all experimental groups of animals was within the normal range and was approximately the same - 39 ÷ 40 %. The content of globulins went beyond the limits of physiological fluctuations (29 ÷ 49 %) - an increase of 22.7 % in the first experiment, 23.1 % - in the second and 25.2 % - in the third. It can be noted that the urea content was within the physiological range (3.7 - 4.13 mmol / l).

*Table 4*

**Biochemical indicators of blood of experimental animals, (M±m), n = 3**

Tests		1	2	3
Total protein, g/l		62,73±0,31	66,87±1,89	69,67±0,94
Albumin,%		40,37±0,95	40,17±0,27	39,13±0,96
Total globulins,%		59,63±0,95	59,83±0,27	60,87±0,96
a/g		0,67±0,03	0,70±0,01	0,63±0,03
globulins, %	α-1	5,0±1,26	4,37±0,82	2,77±0,33
	α-2	14,63±3,36	14,80±0,95	14,90±2,60
	β	14,83±1,31	15,67±1,72	16,70±1,47
	γ	25,17±0,17	25,03±0,55	26,50±0,87
Glucose, mol/l		4,50±0,12	4,80±0,19	4,90±0,17
lysozyme activity, 10 <sup>-3</sup> g/l		0,70±0	1,39±0,61	0,70±0,12
alkaline reserve, mg%		254,67±18,42	227,67±18,41	272,33±22,32
Urea, mmol/l		3,77±0,23	3,80±0,06	3,70±0,15
AST, units/l		40,00±1,15	41,33±0,88	38,00±1,15
ALT, units/l		27,67±2,03	32,33±0,67	31,67±1,33
Creatinine, kmol/l		156,17±1,56	142,60±0,60* <sup>1,2</sup>	150,87±2,14* <sup>2,3</sup>
Alkaline phosphatase, units/l		211,33±22,67	214,67±26,69	300,00±5,77* <sup>1,3; *2,3</sup>

*Note.* \* – probability of differences between the indicators of the experiments at the p <0.05 level.

The glucose content (the norm for young cattle is 2.5–3.5 mol/l) in the experimental animals increased – from 4.5 mol/l in the first experiment to 4.8 mol/l in the second and to 4.9 mol/l in the third experiment. The alkaline reserve of blood serum, according to our data, was: 255 mg%, 228 and 272 mg%, respectively. This is below the norm (460 – 580 mg%): in the first experiment – by 44.6 %, in the second – by 50.4 % and in the third – by 40.9 %, respectively. A significant increase in alkaline phosphatase activity was found by 42 % in the third experiment (before control) and by 40 % between the second and third experiments.

Analysis of hematological parameters (Table 5) showed that in all experiments an increase in the content of erythrocytes was observed, in the third experiment the number of leukocytes increased slightly. The hemoglobin content in the blood of animals ranged from 121.67 to 129.33 g/l.



Table 5

**Hematological parameters of the blood of experimental animals, (M±m), n=3**

Tests		1	2	3
Erythrocytes, 10 <sup>12</sup> /g/l		7,63±0,69	8,23±0,62* <sup>1,2</sup>	8,43±0,09
Hemoglobin, g/l		121,67±5,61	129,33±8,65	128,33±5,78
Leukocytes, g/l		8,73±1,16	9,73±0,16	12,47±3,03
Phagocytic	Activity, %	52,00±9,24	42,67±7,42	50,67±1,33
	index	3,40±0,12	4,70±0,46	4,07±0,62
	number	1,73±0,30	2,03±0,50	2,07±0,27
	capacity	5,53±0,86	6,80±1,66	7,07±0,50
Neutrophils, %	young	0	0	0
	rodnucleate	3,33±0,88	2,67±0,67	4,33±1,86
	segmentnucleate	34,67±4,33	32,67±2,93	27,00±9,61
Eosinophils		4,0±0,58	6,33±1,20	7,67±2,19
Basophils		0,33±0,33	0,67±0,67	0,67±0,33
Monocytes		0	0	0
Lymphocytes		56,00±4,62	55,33±3,39	58,33±6,69
Monocytes		1,33±0,33	2,33±0,88	2,00±1,00

Note. \* – probability of differences between the experimental indicators at the  $p < 0.05$  level.

**Discussion.** Optimal uniformity of mixing ensures the same consumption of all components of the diet by all animals, which should ensure, on the one hand, the same nutritional value of the feed in all parts of its volume, and on the other hand, it is unacceptable to grind coarse feed too densely. The use of mixtures of heterogeneous composition for feeding animals significantly reduces their productive effect (Raab L., 2009, Maekawa M. et al, 2002). Feeding young cattle with a feed mixture with a mixing uniformity of 75 % in our studies had a slight effect on nutrient intake. As for the apparent digestibility of nutrients throughout the gastrointestinal tract of experimental animals, it varied depending on the nutritional value of the diet. Thus, an increase in the digestibility of crude protein and crude fiber was observed when feeding a highly nutritious diet, while feeding a low-nutrient diet led to a decrease in the digestibility of dry and organic matter, crude protein, and nitrogen-free extractives. Feeding highly nutritious diets significantly increased the provision of animals with energy and protein. And when consuming a low-nutrient diet by young cattle, there was practically no effect on energy content, which is consistent with our previous studies, where we indicated that the consumption of high- and low-nutrient diets with increasing uneven mixing will lead to significant differences in the intake of energy and nutrients from the feed by individual animals (Yeletska T.O., 2014).

The indicators of most blood metabolites were within normal limits, and only some of them slightly deviated beyond the physiologically permissible values. Thus, an increase in the content of globulins may indicate an increase in the protective function of the body, and an increase in the content of glucose may be associated with an increase in the somatotropic function of the pituitary gland and other hyperglycemic hormones. (Yousefinejad S. et al., 2021). An indicator of the state of the blood plasma buffer system is the alkaline reserve. A decrease in this indicator, according to our data, indicates a limited buffer capacity to counteract acidification, which can be explained by the same type of feeding with a predominance of corn silage in the diet, imbalanced mineral nutrition and insufficient level of physiological mechanisms for regulating acid-base balance due to long-term feeding of animals with unbalanced diets, which is indicated in



the works (Nasrollahi S. M. et al., 2019, Lukashynskyi V., 1987). The increase in alkaline phosphatase activity when replacing 100% of the uniformly mixed feed mixture with feed with an unevenly mixed fourth fraction is probably the result of inadequate feeding with carbohydrate-containing feeds with easily absorbable bases (Utama D.T. et al., 2018). As a result of the analysis of hematological indicators, it was found that as a result of the experiment, both in the first and in the second and third experiments, moderate erythrocytosis and physiological leukopenia were observed. A slight lymphocytosis was also observed. This can be explained by the fact that in young growing organisms, which have significant growth energy, the percentage of lymphocytes is always higher than in adults. The hemoglobin content in the blood of animals corresponds to the physiological norm for a given age (Peredriy N.N. et al., 2017).

### Conclusions

1. It was found that when consuming a low-concentrate feed mixture with an energy concentration in the diet of 8.9 MJ/kg dry matter and a crude protein concentration of 9.7 % with a mixing uniformity of 75 % compared to the control diet (100 % mixing uniformity), there were improbable changes in the consumption of nutrients by individual animals.

2. When consuming a feed mixture with a mixing uniformity of 75 %, the fluctuations in the apparent digestibility of nutrients throughout the gastrointestinal tract of young cattle ranged from 2 % to 6 %. On a highly nutritious diet, the digestibility of crude protein and crude fiber increased by 14 % and 9 %, respectively. On the low-nutrient diet, there was a significant ( $p < 0.05$ ) decrease in digestibility by 5 % for dry matter, organic matter and nitrogen-free extractives, for crude protein (by 12 %), compared to the high-nutrient diet.

3. The energy and protein intake of animals increased significantly ( $p < 0.01$ ) on the high-nutrient diet by 34.7 % and 11.5 %, respectively. Consumption of the low-nutrient diet had virtually no effect on energy content.

4. Consumption of a 75 % uniform feed mixture led to a significant increase in phosphatase activity by 42 % on the low-nutrient diet. Feeding a highly nutritious diet significantly increased the content of erythrocytes by 7.9 %, while most hematological and biochemical blood parameters fluctuated within the physiological norm for a given age of animals.

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## **GENETIC ANALYSIS OF LINES OF HORSES OF THE HUTSUL BREED OF DOMESTIC SELECTION**

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*The article presents the results of an extended study and analysis of the immunogenetic profile of lines of populations of horses of the Hutsul breed in Ukraine.*

*Considerable work is being carried out to preserve, improve and reproduce small breeds of horses, one of which is the Hutsul breed.*

*The distribution of genetic variability, genetic structure between lines of the Hutsul breed of horses and the transmission of alleles to descendants from the ancestors were studied using certification by polymorphic blood groups.*

*A comparison of gene frequencies according to the spectrum of alleles of the main D-system of blood groups of the lines of the Hutsul horse breed of domestic selection was carried out. It was established that in the Goral line (stag-breeding stallion 85 Pedro) the highest frequency of the Ddk allele ( $q=0.667$ ). High frequency of the Ddk allele in the Grobi line from the heir of the breeding stallion Grobi XV-10 SK/Aster ( $q=0.500$ ), and throughout this line ( $q=0.431$ ). In the Gurgul line the highest frequency of the Dcgm allele ( $q=0.357$ ). High frequency of the Dcgm allele in the lines of Ousora (Poland) (stag-breeding stallion 52 Warnik 255) and Gurgul (stag-breeding stallion 81 Orlyk 024) ( $q=0.400-0.529$ ), respectively. The Ousor line from the heir of the stud stallion 82 Ousor Vulkan is characterized by a high frequency of the Ddg allele ( $q=0.500$ ) and throughout this line ( $q=0.435$ ).*

*The highest genetic indicators were obtained in the Gurgul line, the level of polymorphism ( $N_e = 3.960$ ), the lowest in the Goral line ( $N_e = 2.051$ ). The Goral line (stable stallion 85 Pedro) has the highest expected homozygosity coefficient ( $C_a = 0.488$ ), the lowest in the Gurgul line ( $C_a = 0.253$ ).*

*The differences identified in the spectrum of active alleles of polymorphic blood group systems indicate that each studied genealogical line of horses has a certain "genetic passport", which creates a practical information base for keeping lines and the breed as a whole in Ukraine under genetic control.*

**Keywords:** horses, lines, Hutsul breed, alleles, frequency, genotypes, genetic indicators

## **ГЕНЕТИЧНИЙ АНАЛІЗ ЛІНІЙ КОНЕЙ ГУЦУЛЬСЬКОЇ ПОРОДИ ВІТЧИЗНЯНОЇ СЕЛЕКЦІЇ**

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*В статті викладено результати розширеного вивчення та аналізу імуногенетичного профілю ліній популяції коней гуцульської породи в Україні.*

*Проводиться значна робота по збереженню, вдосконаленню та відтворенню малочисельних порід коней, однією із них є гуцульська порода.*



Розподіл генетичної мінливості, генетичної структури між лініями гуцульської породи коней та передача алелів нащадкам від родоначальників вивчали за допомогою атестацію за поліморфними групами крові.

Здійснено порівняння генних частот за спектром алелів основної D-системи груп крові ліній коней гуцульської породи вітчизняної селекції. Встановлено, що у лінії Горала (жеребець-плідник 85 Педро) найвища частота алелю  $D^{dk}$  ( $q=0,667$ ). Висока частота алелю  $D^{dk}$  у лінії Гробі від спадкоємця жеребця-плідника Гробі XV-10 SK/Астор ( $q=0,500$ ), а по всій цій лінії ( $q=0,431$ ). В лінії Гургула найвисока частота алелю  $D^{csm}$  ( $q=0,357$ ). Висока частота алелю  $D^{csm}$  у лінії Оусора (Польца) (жеребець-плідник 52 Варнік 255) та Гургула (жеребець-плідник 81 Орлик 024) ( $q=0,400-0,529$ ) відповідно. Для лінії Оусора від спадкоємця жеребця-плідника 82 Оусор Вулкан характерна висока частота алелю  $D^{dg}$  ( $q=0,500$ ) та по всій цій лінії ( $q=0,435$ ).

Найвищі генетичні показники були одержані в лінії Гургула рівень поліморфності ( $Ne - 3,960$ ), найнижчий в лінії Горала ( $Ne - 2,051$ ). В лінії Горала (жеребець-плідник 85 Педро) найвищий коефіцієнт очікуваної гомозиготності ( $Ca - 0,488$ ), найнижчий в лінії Гургула ( $Ca - 0,253$ ).

Виявлені відмінності за спектром діючих алелів поліморфних систем груп крові вказують, що кожна досліджена генеалогічна лінія коней володіє певним «генетичним паспортом», що створює практично інформаційну базу для ведення ліній та в цілому породи в Україні під генетичним контролем.

**Ключові слова:** коні, лінії, гуцульська порода, алелі, частота, генотипи, генетичні показники

The Hutsul horse breed is one of the few unique breeds in Ukraine. With a limited gene pool of populations of this breed, it is a national achievement in horse breeding of domestic selection. Unfortunately, little attention is now paid to it, as to all breeds in Ukraine, responding to the issues of society and the requirements of the time. But with the preservation and thorough genetic study of its small number in Ukraine and in European countries, where the foreign gene pool of this population is located, we will be able to research and transfer experience to other generations of our society in parallel.

As a result of studying the genetic structure of Hutsul horses, it was established that the lines arose at the beginning of the 17th century, which originated from the crossing of horses of southern and Mongolian origin in harsh mountain conditions. According to the outstanding ancestors, the lines of Goral, Gurgul, Grobi, Ousor, Polyana and Pietrosu were formed.

According to the State Stud Book Volume II (Holovach M. Y., 2013), the first line of the Hutsul breed was the line of the stallion Goral in the Lviv region, which was the main one and from it came 7 stallions and 70 mares. In parallel, another line of the stallion Grobi was established in Bukovina. From it came at that time 6 stallions and 28 mares, which were recorded in the registration book.

In the period between the First and Second World Wars, new lines were emerging, one of them was the Gurgul line (Romanenko G.V. (2018). During the same period, the lines already had more licensed offspring of stallions, from the Goral line - 18, Grobi - 13, Gurgul - 47. Now, in the post-war period, three genealogical lines of Goral, Grobi and Gurgul were recognized in this breed.

Today, there are representatives of 5 lines of the Hutsul breed in Ukraine: Gurgul, Grobi, Goral, Ousora, Pietrosu (Golovach M. Y., 2010).

From many years of selection work, genetic analysis and certification of the Hutsul horse breed by blood group (Zaderykhina O. A., 2017, Rossokha V. I., 2010),



monitoring, (Podoba B. E., 2012) researchers determine that The gene pool of the Hutsul breed combines the features inherent in riding and trotting breeds.

It is also extremely important in this work that in-depth immunogenetic analysis provides a real opportunity not only to track the movement of markers across generations, but also to control the inheritance of genes from the ancestor to his valuable successors. As it was studied in the works of various researchers (Popadiuk S.S., 2019; Podoba B. E., 2013; Efimenko M. Ya., 2005; Parasochka I. F., 2007), not only to use the latest PCR methods (Behl R., 2007; Georgescu S.E., 2011; Račkauskaitė A., 2021; Szilvia Kusza, 2013; Stachurska A., 2014; Xu L.X., 2012), but also in parallel immunogenetic methods for blood groups, which complement each other (Mihók S., 2005).

The level of polymorphism is an important integral indicator that determines the number of actively acting alleles in the line (Altukhov Yu. P., 1996). So are other indicators of the genetic situation in the animal population.

Due to the high working capacity of horses of this breed in specific mountainous and foothill areas of the Carpathian region, the development of rural tourism and methods of hippotherapy of a number of nervous diseases, and taking into account the balanced temperament, endurance, well-developed herd instinct, authentic need for contact with humans, horses of the small Hutsul breed need to be studied and the gene pool preserved. One of the components of scientific support for the preservation of the gene pool is the identification of the gene structure according to polymorphic blood group systems.

**The purpose** of the research is to investigate genetic markers and genetic indicators of the lines of the Hutsul breed of horses of Ukrainian selection.

**Materials and methods of research.** Immunogenetic studies of the D-system of blood groups were performed on 96 blood samples of the Hutsul breed of horses of domestic selection.

Immunogenetic certification of horses by blood groups was carried out according to generally accepted methods (Burkat V. P., 2010; Hopka M. V., 2007; Rossokha V. I., 2016), (SOU 01.22-37-528: 2006.)<sup>ii</sup>. Serological testing was used to determine erythrocyte alleles of complex polymorphic systems A and D, and monofactor systems C and K. For differentiation of erythrocyte alleles, monospecific serum reagents were used, which were identified with international standards (DSTU ISO 8531:2015)<sup>iii</sup>.

According to the results of testing and determination of genotypes, an analysis of the immunogenetic structure of the studied horses of 4 lines of progenitor stallions was carried out by antigens and alleles of blood groups, and the transmission of marker alleles to the descendants of these genealogical lines.

Genetic analysis of the frequency of occurrence of alleles of polymorphic blood groups and genetic indicators (level of polymorphism (Ne); coefficient of expected (Ca) and actual homozygosity (H) for all loci; indicator of homozygosity realization (W); deficiency of homozygotes (Def); information index (I), observed (Ho) and expected (He) heterozygosity; unbiased expected heterozygosity (uHe); Wright's fixation index F (Fis)) of genealogical lines as a whole, as well as for the descendants of stud stallion of these

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<sup>ii</sup> SOU 01.22-37-528: 2006. Livestock. method for determining blood groups, polymorphic blood proteins and expertise of the origin of pedigree horses. Kyiv: Ministry of Agrarian Policy of Ukraine, 2006. 26 p. (Standard of the Ministry of Agrarian Policy of Ukraine)

<sup>iii</sup> DSTU ISO 8531:2015. Livestock.horses.methods for obtaining and storage of anti-erythrocyte immunodiagnostics for identification of horses by polymorphic blood group systems. [Valid from 2017-07-01]. Kyiv, 2015. 17 p. (Information and documentation).



lines of the Hutsul breed were calculated by generally accepted methods using the programs "Excel 2003", "GenAIEx 6.502" and "Statistica".

**Research results.** We have 5 lines, namely Grobi, Ousora, Gurgul, Goral and Pietrosu. The last line of Pietros continues with a descendant of the stud stallion 86 Pers, bay-piebald, born in 2003 (sire 211 Baca, dam 23 GKr Perunka, owner of the National Breeding Center "Plemkonetsentr"). Only two mares are certified in this line. It is not possible to trace the stallions.

To date, we have studied only the first four lines.

The Grobi line is the first branched line with which work is being carried out in Ukrainian stud farms. It comes from the stallion Grobi, light bay, born in 1895 (in Bukovina).

His descendant is a stallion-breeder 84 Pegazik UA210000251, light bay, born in 2001 (father 10 GRz Lunak, mother 138 GRz Pelagia), place of birth Poland (PL), owner of the National Breeding Association "Plemkonetsentr". And the second descendant of Grobi XV-10 SK/Astor, bay, 2011 (sire 4566 Grobi XV/ Grobi XII) birthplace and owner of the NVA "Plemkonetsentr".

The second line of Ousor from a bay stallion born in 1929 (in Bukovina). His descendants in the Ousor line are the stud stallions 82 Ousor Vulkan HL050870000, mouse-colored, born in 2005, (father 3653 Ousor, mother 730 Grobi XXI-36), birthplace Hungary (H), owner FG "Polonynske gospodarstvo" and the second descendant stud stallion 52 Varnik 255, bay, born in 2002, (father 25 GRz Lotnik, mother 77 GRz Wartka), birthplace Poland (PL), owner FG "Zarechie".

The third line is Gurgul, born in 1924 (in Eastern Slovakia). His descendants in the Gurgul line are the stud stallions 74 Merkur Gu E 2-8, bay, born in 1985, (father Gurgul Edo II, mother 16 Dubrava Gu V-5), birthplace Czechoslovakia, owner Yaremche National Park, the second descendant 81 Orlyk 024, bay, born in 2004, (father 54 Viter 048, mother 560 Ghilka 051), birthplace FG "Polonynske Gospodarstvo", owner FG "Polonynske Gospodarstvo" and the third descendant 69 Lukash Gu E-2-8, bay, born in 1984, birthplace Czechoslovakia, owner SFG "Maximov", who unfortunately died in 2001. He has a descendant of the stud stallion 47 Borzy, igrene suit, born in 1995, owner of the KVI "Varto" from which he has 2 offspring stallions and 1 mare. And mainly from 69 Lukash only heiresses.

And the fourth line of Goral from a Karak stallion, born in 1899 (in the Hutsul region). His descendants in the Goral line are the stud stallion 85 Pedro UA210000250, bay suit, born in 2002, (father 77 GKr Sputnik, mother 72 GKr Panienko), place of birth Poland (PL), owner of the NVA "Plemkonetsentr".

So we have 5 lines, namely Grobi, Ousora, Gurgul, Goral and Pietrosu. The last line of Pietros continues with the offspring of the stallion-breeder 86 Pers, bay-piebald, born in 2003 (father 211 Baca, mother 23 GKr Perunka, owner of the National Breeding Association "Plemkonetsentr"). Only two mares have been certified in this line. It is not possible to trace the stallions.

Today, these four lines are large and remain the leading ones in the Hutsul horse breed (table 1). They have breeding qualities and a large number of stallions-breeders. And the Gurgul line is slowly passing through the daughters of the stallion-breeder 81 Orlyk 024 into the Ousor line through the stallion-breeder 82 Ousor Vulkan.



Table 1

Number of certified stallions and mares by genealogical line branches

Line	Total horses in line, head.	Including		The most promising branches in the lines	Of them, the main production stock of horses, head.						
		Breeding stallions	Mares		total	Including					
						sons	grandchildren	great-grandchildren	daughters	granddaughters	great-granddaughters
Groby	2	2	-	84 Pegasic	63	31	2	-	30	-	-
				Groby XV-10 SK/Astor	7	3	-	-	4	-	-
Ousora	2	2	-	82 Ousor Vulkan	52	16	2	-	34	-	-
				52 Varnik 255	17	5	-	-	12	-	-
Gurgula	3	3	-	81 Orlik 024	46	17	-	-	29	-	-
				74 Merkur Gu E 2-8	22	3	8	-	2	9	-
				69 Lukas Gu E-2-8	13	1	2	-	9	1	-
Gorala	1	1	-	85 Pedro	24	9	-	-	15	-	-

The largest number of certified offspring of stallions and mares in the Gurgul line is 81 animals, the average in Grobi is 70 animals and in Ousor is 69 animals. The fewest animals have been certified in the Goral line – 24 (Table 2).

Thus, the largest number of offspring of stallions is traced in the Grobi line from the stud stallion 84 Pegazik – 33 and the Ousor line from the stud stallion 82 Ousor Vulkan – 18.

The Ousor line from the heir of the stud stallion 82 Ousor Vulkan is characterized by a high frequency of the Ddg allele ( $q=0.500$ ) and throughout this line ( $q=0.435$ ) (Table 3). The highest frequency of the Ddk allele is in the Goral line (stud stallion 85 Pedro) ( $q=0.667$ ). The high frequency of the Ddk allele is in the Grobi line from the heir of the stallion-stud stallion Grobi XV-10 SK/Aster ( $q=0.500$ ) and throughout this line ( $q=0.431$ ).

The highest frequency of the Dcgm allele in the entire Gurgul line ( $q=0.357$ ). High frequency of the Dcgm allele in the Ousor (Poland) (stag-breeding stallion 52 Warnik 255) and Gurgul (stag-breeding stallion 81 Orlyk 024) lines ( $q=0.400-0.529$ ) respectively.

The highest frequency of the Dd allele in the Grobi line from the heir of the Grobi XV-10 SK/Astor ( $q=0.500$ ).



Table 2

**The number of certified stallions-breeders and mares in the section of branches of genealogical lines**

Lines	Branch	Number of active						total
		Stallions			Mares			
		sons	grandchildren	great-grandchildren	daughters	granddaughters	great-granddaughters	
Groby	84 Pegasic	31	2	-	30	-	-	63
	Groby XV-10 SK/Astor	3	-	-	4	-	-	7
Total in the line		34	2	-	34	-	-	70
Ousora	82 Ousor Vulcan	16	2	-	34	-	-	52
	52 Varnik 255	5	-	-	12	-	-	17
Total in the line		21	2	-	46	-	-	69
Gurgula	81 Orlik 024	17	-	-	29	-	-	46
	74 Merkur Gu E 2-8	3	8	-	2	9	-	22
	69 Lukas Gu E-2-8	1	2	-	9	1	-	13
Total in line		21	10	-	40	10	-	81
Gorala	85 Pedro	9	-	-	15	-	-	24

The low frequency of the Ddk allele ( $q=0.022$ ), Dd ( $q=0.022$ ) was in the Ousor line, and the lowest Dd ( $q=0.018$ ) in the Gurgul line.

The Dd and  $D^{bcm}$  alleles are absent in the Goral line. And the Dad and  $D^{cegm}$  alleles are absent in all lines of the Hutsul horse breed.

The Ousor line from the heir of the stud stallion 82 Ousor Vulkan is characterized by a high frequency of the Ddg allele ( $q=0.500$ ) and throughout this line ( $q=0.435$ ) (Table 3). The highest frequency of the Ddk allele in the Goral line (student stallion 85 Pedro) ( $q=0.667$ ). The high frequency of the Ddk allele in the Grobi line from the heir of the stud stallion Grobi XV-10 SK/Aster ( $q=0.500$ ) and throughout this line ( $q=0.431$ ).

The highest frequency of the Dcgm allele throughout the Gurgul line ( $q=0.357$ ). High frequency of the Dcgm allele in the Ousor lines (Poland) (student stallion 52 Warnik 255) and Gurgul (student stallion 81 Orlyk 024) ( $q=0.400-0.529$ ), respectively.

The highest frequency of the Dd allele in the Grobi line from the heir of the stallion-breeder Grobi XV-10 SK/Aster ( $q=0.500$ ).

The low frequency of the Ddk allele ( $q=0.022$ ) Dd ( $q=0.022$ ) was in the Ousor line, and the lowest Dd ( $q=0.018$ ) in the Gurgul line.



Table 3

**Distribution of gene frequencies of D-system blood group alleles by lines of Hutsul horses, M±m**

Lines		Aleli							
		ad	dg	dk	cgm	bcm	de	d	cegm
Grobi (Poland)	84 Pegasik (sire Lunak) (n=33)	0,000	0,258± 0,054	0,424± 0,061	0,106± 0,038	0,076± 0,033	0,121± 0,040	0,015± 0,015	0,000
	Grobi XV-10 SK/Astor (sire 4566 Grobi XV/ Grobi XII) (n=3)	0,000	0,000	0,500± 0,204	0,000	0,000	0,000	0,500± 0,204	0,000
	<b>Total by line (n=36)<sup>1</sup></b>	0,000	0,236± 0,050	0,431± 0,058	0,097± 0,035	0,069± 0,030	0,111± 0,037	0,056± 0,027	0,000
Ousora (Poland)	82 Ousor Vulkan (sire 3653 Ousor) (n=18)	0,000	0,500± 0,083	0,000	0,222± 0,069	0,056± 0,038	0,222± 0,069	0,000	0,000
	52 Varnik 255 (sire 25 GRz Lotnik) (n=5)	0,000	0,200± 0,127	0,100± 0,095	0,400± 0,155	0,200± 0,127	0,000	0,100± 0,095	0,000
	<b>Total by line (n=23)<sup>2</sup></b>	0,000	0,435± 0,073	0,022± 0,022 <sub>1</sub> ***	0,261± 0,065	0,087± 0,042	0,174± 0,056	0,022± 0,022	0,000
Gurgula (Czechoslovakia)	81 Orlik 024 (sire Viter 048) (n=17)	0,000	0,177± 0,065	0,000	0,529 ± 0,086	0,029 ± 0,029	0,235 ± 0,073	0,029 ± 0,029	0,000
	74 Merkur Gu E 2-8 (sire Gurgul Edo II) (n=11)	0,000	0,318± 0,099	0,318 ± 0,099	0,091 ± 0,061	0,046 ± 0,044	0,227 ± 0,089	0,000	0,000
	<b>Total by line (n=28)<sup>3</sup></b>	0,000	0,232± 0,056	0,125 ± 0,044* <sub>*1</sub>	0,357 ± 0,064* <sub>1</sub>	0,036 ± 0,025	0,232 ± 0,056	0,018 ± 0,018	0,000
Gorala (Poland)	85 Pedro (sire 77 GKr Sputnik) (n=9) <sup>4</sup>	0,000	0,056± 0,054 <sub>2</sub> **	0,667 ± 0,111* <sub>**2, **3</sub>	0,167 ± 0,088	0,000	0,111 ± 0,074	0,000	0,000

Note: \* –  $P \leq 0.05$ ; \*\* –  $P \leq 0.01$ ; \*\*\* –  $P \leq 0.001$ ; 1 – 4 – lines of Hutsul horses here and below: 1 – Grobi line, 2 – Ousor line, 3 – Gurgul line, 4 – Goral line



The Dd and Dbcm alleles are absent in the Goral line. And the Dad and Dcegm alleles are absent in all lines of the Hutsul horse breed.

No significant difference between the values of gene frequencies of the D-system alleles of the blood groups of the Hutsul horse breed was found between the Ousor line and the Gurgul line. There is also no statistically significant difference between the Grobi line and the Goral line.

In general, no statistically significant differences were found between the allele indices. But there were differences that should be noted.

Thus, statistically significant (significant) differences were found in the Dcgm allele frequencies ( $P \leq 0.05$ ) between the Grobi line and the Gurgul line.

When comparing the Ddk allele gene frequencies ( $P \leq 0.01$ ) between the Gurgul line and the Grobi line, differences were found at a high level. The same trend was found between the Gurgul line and the Goral line, respectively.

According to the Ddg allele gene frequencies ( $P \leq 0.01$ ) between the Ousor line and the Goral line, differences were found at a high level.

In comparing the gene frequency indices for the Ddk allele ( $P \leq 0.001$ ) between the Ousor line with the Grobi line and Ousor from Goral, respectively, differences were found at the absolute level of statistical significance.

Table 4 presents stallions-breeders with homozygous and heterozygous genotypes according to the D-system of blood groups, which have the largest sample of offspring and have passed immunogenetic certification at Livestock farming institute of the NAAS.

*Table 4*

**Genotypes according to the D-system of stud stallions of the Hutsul breed, admitted for breeding use**

LINE	Breeding stallions from which the largest number of offspring was obtained	Genotypes	Number of offspring in the line			Total offspring by lines
			stallions	mares	total	
GROBY	84 Пегазік	D <sup>dg/dk</sup>	33	30	63	70
	Гробі XV-10 SK/Астор	D <sup>cgm/d</sup>	3	4	7	
OUSORA	82 Оусор Вулкан	D <sup>de/dg</sup>	18	34	52	69
	52 Варнік 255	D <sup>cgm/dg</sup>	5	12	17	
GURGULA	81 Орлик 024	D <sup>cgm/de</sup>	17	29	46	81
	74 Меркур G E 2-8	D <sup>de/de</sup>	11	11	22	
	69 Лукаш Gu E-2-8 (пав у 2001 р.)	-	3	10	13	
GORALA	85 Педро	D <sup>dk/de</sup>	9	15	24	24



So we see that in the Gurgul line there is a homozygous offspring of the stallion-breeder 74 Merkur G E 2-8.

And we state that in the Gurgul line one of the Dde alleles is inherited, because it is in the offspring of the stallions 81 Orlyk 024 and 74 Merkur G E 2-8. And in the Ousor line one of the Ddg alleles is transmitted.

A study was conducted on the inheritance of alleles of 4 lines of Hutsul horses (Table 5). Thus, in the Grobi line, the heir of the stallion-stud stallion 84 Pegazik (n=33), the Ddg allele was transmitted to his 14 offspring of stallions, and the Ddk allele to 19 offspring. But in this line, the heir of the stallion-stud stallion Grobi XV-10 SK/Astor (n=3), the Dd allele was transmitted to 3 offspring, and the Dcgm allele was not transmitted.

Table 5

**Transmission of marker alleles to offspring from stud stallions of the Hutsul breed**

LINE	Nickname and number according to DKPK	n, heads	Alleles		Number of offspring of stallions				$\chi^2$
			I	II	with allele I	%	with allele II	%	
OUSURA	82 Ousor Vulcan	18	de	dg	6	33	12	67	11,845
	52 Varnik 255	5	cgm	dg	4	80	1	20	
GROBY	84 Pegasik	33	dk	dg	19	58	14	42	17,082
	Groby XV-10 SK/Astr	3	cgm	d	0	0	3	100	
GURGULA	81 Orlik 024	17	de	cgm	3	18	14	82	9,134
	74 Merkur G E 2-8	11	de	de	5	45	0	0	
GORALA	85 Pedro	9	de	dk	1	11	8	89	2,250

In the Ousor line, the heir of the stallion-stud stallion 82 Ousor Vulkan (n=18), the Dde allele was transmitted to 6 offspring of stallions, and the Ddg allele to 12 offspring. In the same line, in the heir of the stallion-stud stallion 52 Varnik 255 (n=5), the Ddg allele was transmitted to one offspring, and the Dcgm allele was transmitted to 4 offspring.

In the Gurgul line, in the heir of the stallion-stud stallion 81 Orlyk 024 (n=17), the Dde allele was transmitted to 3 offspring, and the Dcgm allele to 14 offspring. In the same line, in the heir of the stallion-stud stallion 74 Merkur Gu E 2-8 (n=11), the Dde allele was transmitted to only 5 offspring.



But in the Goral line, in the heir of the stallion-stud stallion 85 Pedro (n=9), the Ddk allele was transmitted to 8 offspring, and the Dde allele to one.

A genetic and population analysis of 4 lines of stallions-stud stallion of the Hutsul breed was conducted.

Genetic indicators of genealogical lines of the Hutsul breed were calculated by lines as a whole, as well as by the descendants of the stallions-stud stallioning of these lines (Table 6).

Table 6

**Genetic indicators by lines of the Hutsul horse breed**

Line	GO-RA-LA	GROBY			GURGULA			OUSORA		
Offspring of stud stallions	85 Pedro	Total	84 Pegazik	Grobj XV-10 SK/Astor	Total	81 Orlyk 024	74 Merkur G E 2-8	Total	82 Ousor Vulkan	52 Varnik 255
n	9	36	33	3	28	17	11	23	18	5
Na	4,000	6,000	6,000	2,000	6,000	5,000	5,000	6,000	4,000	5,000
Ne	2,051	3,692	3,594	2,000	3,960	2,714	3,781	3,380	2,842	3,846
Ca	0,488	0,375	0,278	0,5	0,253	0,369	0,265	0,296	0,352	0,26
I	0,974	1,520	1,466	0,6	1,497	1,191	1,424	1,396	1,176	1,471
H	0,049	0,004	0,005	0	0,008	0,017	0,008	0,004	0,006	0
W	0,101	0,010	0,017	0	0,030	0,047	0,031	0,013	0,018	0
Ho	0,556	0,861	0,848	1,000	0,786	0,706	0,909	0,913	0,889	1,000
He	0,512	0,729	0,722	0,500	0,747	0,631	0,736	0,704	0,648	0,740
Def	-0,044	-0,132	-0,126	-0,5	-0,245	-0,075	-0,173	-0,29	-0,241	-0,26
uHe	0,542	0,739	0,733	0,600	0,761	0,651	0,771	0,720	0,667	0,822

The highest level of polymorphism in our line Gurgul (Ne – 3.960), the average level in the line Grobi (Ne – 3.692) and in the line Ousor (Ne – 3.380). The lowest in the line Goral (Ne – 2.051).

The highest coefficient of expected homozygosity (Ca) in the line Goral (stallion-stud stallioning 85 Pedro) (Ca – 0.488), the lowest in the line Gurgul (Ca – 0.253). And if by stallions-stud stallioning, then the highest in the line Grobi (stallion-stud stallioning Grobi XV-10 SK/Astore (Ca – 0.5)).

According to the indicator of homozygosity realization (W), it is the highest in the Goral line (W – 0.101) in the stud stallion 85 Pedro, the lowest in the Grobi line (W – 0.010).

The deficit of homozygotes in our country was the highest in the Ousor line (–0.29).

**Discussion.** Most cultural and historical arguments for conservation are not based on genetic information, but our study results can be used in the development of a breeding strategy for horse breeding in Ukraine. Various molecular genetic studies, as well as



immunogenetic studies, allow us to follow, and even further control, the movement of markers from generation to generation from the ancestor to the offspring. Such work is carried out in parallel with the latest DNA technologies, which complement each other.

Since there are only a few genetic studies of the endangered Hutsul horse, the aim of this work was to provide new information to horse associations about the population structure, genetic characteristics and genetic origin of the Hutsul horse, as well as its relationship among other horse breeds. We hope that our results will be useful for conservation plans and breeding strategies for the breed.

As a brief summary of our results, we conclude that the immunogenetic analysis of each of the four studied lines of the Hutsul breed has its own specific feature in the distribution of alleles of the D-system of the blood group.

### **Conclusions:**

1. All four lines of the Hutsul breed of horses have a unique, reliably different, immunogenetic profile.

2. In the genealogical lines of the Hutsul breed of horses, the transmission of alleles was inherited as follows:

a) in the Grobi line of the heir of the stud stallion 84 Pegazik, the Ddg allele was transmitted to his 14 offspring of stallions, and the Ddk allele to 19 offspring. In the heir of the Grobi XV-10 SK/Aster, the Dd allele was transmitted to 3 offspring, and the Dcgm allele was not transmitted;

b) in the line of the heir of the stud stallion 82 Ousora Vulkan, the Dde allele was transmitted to 6 offspring of the stallions, and the Ddg allele to 12 offspring. In the heir of 52 Varnik 255, the Ddg allele was transmitted to one offspring, and the Dcgm allele was transmitted to 4 offspring;

c) in the line of the heir of the stud stallion 81 Orlyk 024, the Dde allele was transmitted to 3 offspring, and the Dcgm allele to 14 offspring. In the heir of 74 Merkur Gu E 2-8, the Dde allele was transmitted to only 5 offspring;

d) in the line of the heir of the stud stallion 85 Pedro, the Ddk allele was transmitted to 8 offspring, and the Dde allele to one.

3. The highest frequency of the Ddk allele in the Goral line (stud stallion 85 Pedro) ( $q=0.667$ ). High frequency of the Ddk allele in the Grobi line from the heir of the Grobi stallion XV-10 SK/Astor ( $q=0.500$ ) and throughout this line ( $q=0.431$ ). The highest frequency of the Dcgm allele throughout the Gurgul line ( $q=0.357$ ). High frequency of the Dcgm allele in the Ousor (Poland) lines (stud stallion 52 Warnik 255) and Gurgul (stud stallion 81 Orlyk 024) lines ( $q=0.400-0.529$ ) respectively. The Ousor line from the heir of the 82 Ousor Vulkan is characterized by a high frequency of the Ddg allele ( $q=0.500$ ) and throughout this line ( $q=0.435$ ).

4. According to genetic indicators: the highest level of polymorphism in the Gurgul line ( $Ne - 3.960$ ), the lowest in the Goral line ( $Ne - 2.051$ ). The highest coefficient of expected homozygosity ( $Ca$ ) in the Goral line (stud stallion 85 Pedro) ( $Ca - 0.488$ ), the lowest in the Gurgul line ( $Ca - 0.253$ ). According to the breeding stallions, the highest in the Grobi line (stud stallion Grobi XV-10 SK/Astore ( $Ca - 0.5$ )). According to the homozygosity realization indicator, the highest in the Goral line ( $W - 0.101$ ) in the breeding stallion 85 Pedro, the lowest in the Grobi line ( $W - 0.010$ ). The deficit of homozygotes in our country was the highest in the Ousor line ( $-0.29$ ).

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## Ветеринарне благополуччя та якість продукції тваринництва

### *Veterinary welfare and quality of livestock products*

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#### **MICROSCOPIC STRUCTURE OF THE GIZZARD OF THE BUDGERIGAR (MELOPSITTACUS UNDULATUS) OF DIFFERENT AGES**

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*The features of the histological structure and morphometric indicators of the microstructures of the gizzard (ventriculus gastris s. pars muscularis) of budgerigars of 9 age groups were determined: 1-, 3-, 7-, 14-, 21-day-old, 1-, 2-, 6-month-old and 1-year-old. It was confirmed that the wall of the gizzard consists of four membranes: mucous, submucosa, muscular and serous. The mucous membrane is formed by two layers: the lamina propria and the epithelial layer, the surface of which is covered with cuticle. The lamina propria, formed by loose fibrous connective tissue, contains densely located simple tubular weakly branched glands throughout its depth that produce keratin. The lamina propria is composed of a deep layer containing secretory departments of glands with an expanded base and a superficial layer containing villi that function as excretory ducts, along which liquid secretion moves from the depths of the secretory departments to the surface of the mucous membrane and which has the form of columns that are oxyphilically stained. The epithelial layer of the mucous membrane is represented by a single-layered single-row prismatic epithelium, the cells of which have a weakly oxyphilic cytoplasm and a basally located oval-shaped nucleus. The muscularis mucosae is not found in the composition of the mucous membrane. The submucosa formed by densely arranged bundles of mainly collagen fibers, connects the mucous and muscular membranes. The muscular membrane consists of a single layer, which is built of contractile elements of unstriated muscle tissue and thin fibers of connective tissue. Age-related features of morphometric indicators of microstructures of the budgerigar gizzard were an increase in the thickness of the cuticle, mucous and muscular membranes with age and a decrease in the depth of the glandular portions of the tubular glands. The greatest thickness of the cuticle was in parrots 6 months to 1 year old, and the greatest depth of the glands that produce it was in chicks 1-21 days old.*

**Keywords:** budgerigar, gizzard, histological structure, age-related features, morphometric indicators.



## МІКРОСКОПІЧНА БУДОВА М'ЯЗОВОЇ ЧАСТИНИ ШЛУНКУ ХВИЛЯСТОГО ПАПУЖКИ (*MELOPSITTACUS UNDULATUS*) РІЗНОГО ВІКУ

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Визначали особливості гістологічної будови і морфометричні показники мікроструктур м'язової частини шлунку (шлуночка шлунку) хвилястого папужки 9 вікових груп: 1-, 3-, 7-, 14-, 21-добового, 1-, 2-, 6- місячного і 1-річного віку. Підтверджено, що стінка м'язової частини шлунка складається з чотирьох оболонок: слизової, підслизової, м'язової і серозної. Слизова оболонка утворена двома шарами: власною пластинкою і епітеліальним шаром, поверхня якого вкрита кутикулою. Власна пластинка, що утворена пухкою волокнистою сполучною тканиною, на всій глибині містить щільно розташовані прості трубчасті слабо розгалужені залози, які продукують кератин. У складі власної пластинки виділено глибокий шар, що містить секреторні відділи залоз із розширеною основою і поверхневий шар, що містить ворсинки, що виконують функцію вивідних протоків, по яким переміщається рідкий секрет з глибини секреторних відділів на поверхню слизової оболонки і який має форму стовпчиків, що оксифільно забарвлюються. Епітеліальний шар слизової оболонки представлений одношаровим однорядними призматичним епітелієм, клітини якого мають слабо оксифільну цитоплазму і базально розташоване овальної форми ядро. М'язової пластинки в складі слизової оболонки не виявлено. Підслизова основа, що утворена щільно розташованими пучками переважно колагенових волокон, сполучає між собою слизову і м'язову оболонку. М'язова оболонка складається з одного шару, що побудований з скоротливих елементів непосмугованої м'язової тканини і тонких волокон сполучної тканини. Віковими особливостями морфометричних показників мікроструктур м'язової частини шлунку хвилястих папужок було збільшення з віком товщини кутикули, слизової і м'язової оболонок і зменшення глибини секреторних відділів трубкоподібних залоз. Найбільша товщина кутикули була у папужок 6-місячного – 1-річного віку, а найбільша глибина залоз, що його продукують, у пташенят 1-21-добового віку.

**Ключові слова:** хвилястий папужка, шлуночок шлунку, гістологічна будова, вікові особливості, морфометричні показники.

**Introduction.** Parrots are kept in captivity for their color, vocal abilities, longevity, behavior, and also for the purpose of preserving them as a biological species (Larcombe S. D. et al., 2015). They are known for their complex cognitive and communicative abilities, are social animals and require many interactions. The most famous and widespread psittacines include budgerigars (*Melopsittacus undulatus*), which are considered the best wild birds to keep in captivity at home (Pekmezci D. et al., 2020). In recent years, amateur breeding of parrots has contributed to their appearance in the wild in countries where they were not previously found. Thus, in less than two years, 2,675 specimens of parrots of 49 species were found in the wild in Poland. Moreover, they were most often observed in regions characterized by a larger population, higher income and a share of people with higher education (Ważna A. et al., 2024). It is known that exotic birds kept by humans as companion animals can be carriers of zoonoses (Boseret G. et al., 2013; Yetismis G. et al., 2022). However, for



parrot owners, the main problem is their feeding. Most of them do not know how to feed their birds in a healthy way, and sometimes veterinarians and ethologists are faced with dramatic situations. It is believed that parrots in the wild in Australia, from which they have spread throughout the world, can balance their diets. According to Eggleston K. A. et al. (2019), in the wild in Australia, budgies feed mainly on seeds of grasses of the genus *Astrebla*, which are different from commercially available food. However, owners of companion birds often refuse to transfer them to complete pelleted food due to the difficulty of the transition (Cummings A. M. et al., 2022). At the same time, seed-based diets contain excess fat, low calcium to phosphorus ratios, are nutrient-deficient and monotonous. As a result, birds are fed a mixture of seeds, foods, and formulated diets (Brightsmith D. G., 2012). Balanced diets are needed, which are a key factor in maintaining the health of parrots (Orosz S. E., 2014).

Inconsistency of diet with natural needs leads to digestive diseases, which are common in parrots and often have similar clinical signs (Langlois I., 2003). A number of stomach diseases of both infectious and non-infectious etiology, including oncological ones, are common in parrots. Information on age-related features of the digestive system of both budgerigars and other parrot species is generally absent (Péron F. & Grosset C., 2014). As indicated by Eggleston K. A. et al. (2019), our knowledge of parrot nutrition is based on data from domestic chickens (*Gallus domesticus*), which became the basis for dietary recommendations for companion birds, which leads to a high risk of disease in them. The nutritional needs of young birds of different ages are even more unclear than for adult birds, and malnutrition among them is a common phenomenon. Currently, the issue of feeding, maintenance and medicine of old parrots is relevant (Lightfoot T. L., 2010; Reavill D. R. & Dorrestein G. M., 2010). There is no consensus among feed manufacturers regarding proper nutrition during parrot breeding (Cornejo J. et al., 2021). A basic understanding of the fine structure and physiology of the digestive organs is a necessary condition for the integration of pathophysiological and clinical signs associated with various disease processes, the compilation of feeding rations.

**The aim** of the study was to establish the features of the microscopic structure, determine the morphometric indicators of the microscopic structures of the muscular part of the stomach of the budgerigar (*Melopsittacus undulatus*) during the first year of the postnatal period of ontogenesis.

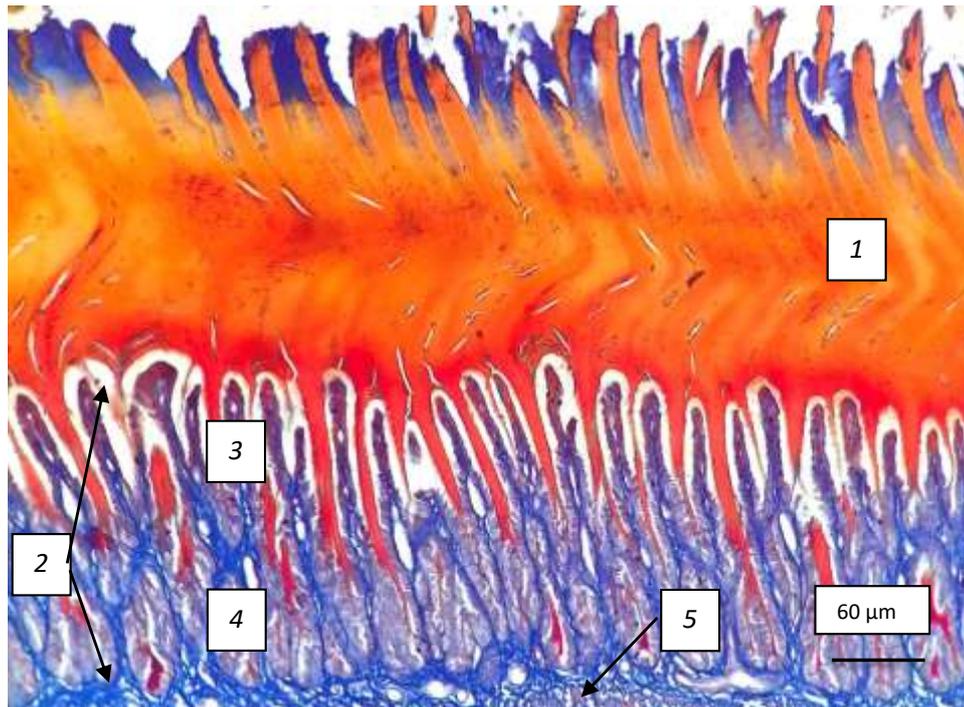
**Materials and methods of research.** The material for the preparation of histological preparations were samples of the gizzard of budgerigars of 9 groups: 1-, 3-, 7-, 14-, 21-day-old, 1-, 2-, 6-month-old and 1-year-old (n=5). The birds were kept and manipulated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The diet of the parrots was a grain mixture, which included yellow, white and red millet, canary flax and sunflower seeds, oats. The diet included sand, as well as dried vegetables and fruits. In addition, a boiled chicken egg was periodically introduced into it. Fresh water was constantly available. After hatching, the female fed the chicks with “crop milk”, and later with grain soaked in the wild.

Samples of the gizzard were taken from the same place in its middle part, fixed in 7-10% aqueous solution of neutral formalin. Histological preparations were prepared according to the classical histological technique. Paraffin sections were stained with hematoxylin and eosin, as well as Mallory. Histological preparations were studied and illustrations were made on a “Jenamed 2” microscope (Carl Seiss Jena, Germany). The size of the microscopic structures of the was determined using an ocular grid. The thickness of the muscular membrane of the was determined at the place of the greatest thickness of the thick muscles of the gizzard. The obtained digital indicators of the



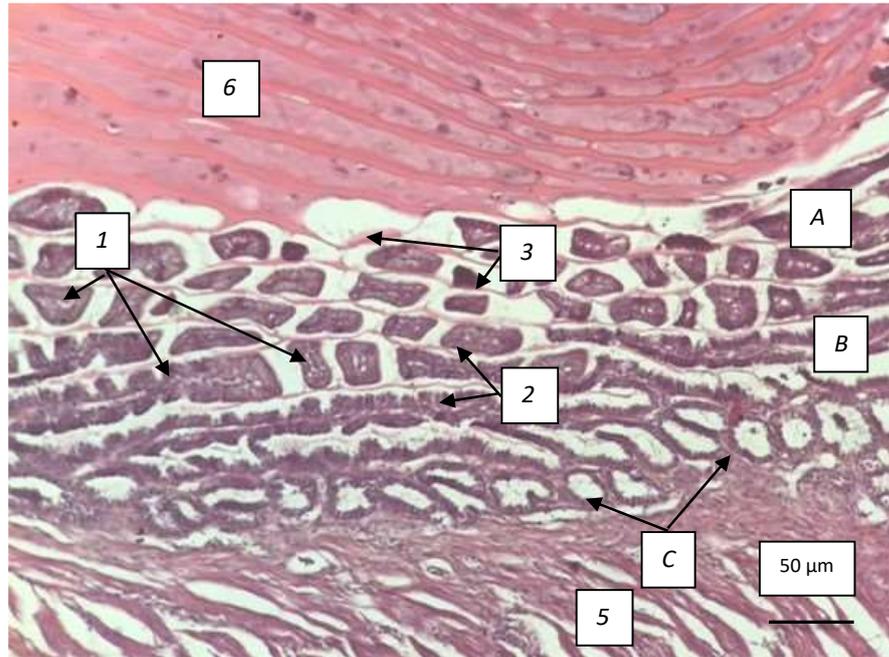
structures were processed using a one-factor analysis of variance (ANNOVA) using the *Biostat LE 7.3* program with the definition of the arithmetic mean – M and its standard deviation – Sd. The significance of the difference in the values of the indicators between age groups was determined according to the Tukey test, where the difference was considered significant at  $p < 0.05$ .

**Research results.** In the gizzard of the budgerigar, we identified four membranes: mucous, submucosa, muscular and serous. On the surface, the mucous membrane is covered with a cuticle. Two layers were identified in the composition of the mucous membrane: epithelial and lamina propria (Fig. 1). The muscularis mucosae in the composition of the mucous membrane was absent.

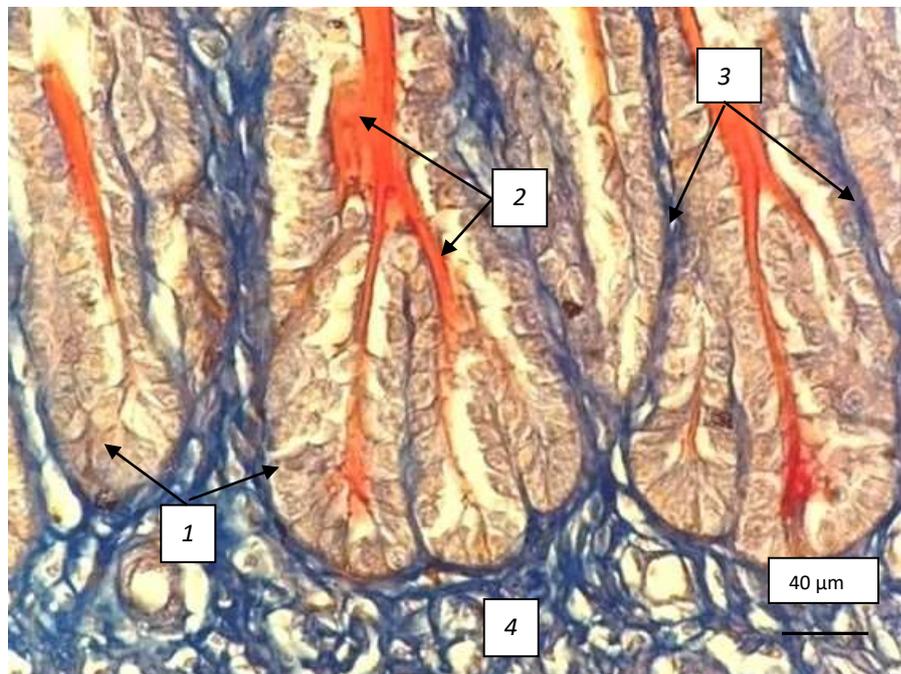


**Fig. 1. Gizzard of a budgerigar of 6 months of age. Histological preparation. 1 – cuticle; 2 – lamina propria of the mucous membrane; 3 – villi; 4 – glandular portions; 5 – submucosa; Mallory staining.**

Under the cuticle, in the composition of the lamina propria of the mucous membrane of the budgerigar's gizzard, a glandular layer was located. In the composition of this layer, its upper part, adjacent to the cuticle, contained villi (Fig. 2), and the lower part – glandular layer of branched tubular glands, consisting of two or three glandular portions (Fig. 3). In the composition of the glandular portions in the form of thin strands, and in the space between the villi in the form of wide homogeneous strands, there was a secret from which the cuticle was formed. The lower part of the glandular portions was expanded.



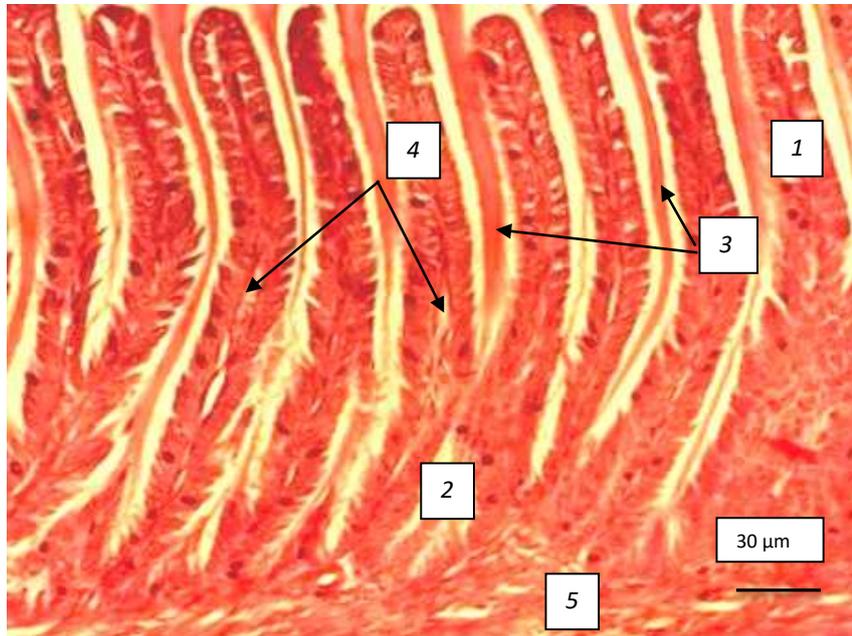
**Fig. 2. Glands of the lamina propria of the mucosa of the gizzard of a 21-day-old budgerigar in transverse and longitudinal sections. Histological preparation. A – transverse section of villi; B – longitudinal section of villi; C – transverse section of glandular portions; 1 – core of villi; 2 – epitheliocytes of villi; 3 – glandular secretion; 4 – submucosa; 5 – muscular membrane; 6 – cuticle; stained with hematoxylin and eosin.**



**Fig. 3. Gizzard of a 2-month-old budgerigar. Histological preparation. 1 – glandular portions; 2 – glandular secretion; 3 – loose fibrous connective tissue of the lamina propria; 4 – submucosa; Mallory staining.**



In 1-3-day-old budgies, despite the presence of secretion between the strands of cells that formed the glands, there was no clear division into glandular portions and excretory ducts in the glandular layer (Fig. 4), which indicates active processes of morphogenesis.



**Fig. 4. Glands of the lamina propria of the the gizzard mucous membrane of a budgerigar of 3 days of age. Histological preparation. 1 – villus; 2 – glandular portions; 3 – glandular secretion; 4 – loose fibrous connective tissue of the core of the villi; 4 – submucosa; staining with hematoxylin and eosin.**

The thin serous membrane of the budgerigar gizzard consisted of a thin connective tissue plate and mesothelium. Sometimes ganglia of the nervous plexus were found under the serous membrane.

The core of the villi and the space between the secretory sections of the glands were built from loose fibrous connective tissue, in which its cells and fibers, as well as small blood vessels, were found. The core of the villi and the space between the secretory sections of the glands were built from loose fibrous connective tissue, in which its cells and fibers, as well as small blood vessels, were found. Epitheliocytes of both the villi and the glandular portions had a prismatic shape, basophilic cytoplasm and a round-shaped nucleus with clearly visible grains and pockets of heterochromatin and one or two nucleoli.

Between the mucous and muscular membrane of the budgerigar's gizzard, in the form of a wide layer, consisting mainly of a dense plexus of connective tissue fibers, was the submucosa. In its composition, both blood capillaries, arterioles and venules were found. Macroscopically, on the transverse section of the gizzard, on its opposite sides, the thick muscles of the stomach – *m. crassus cranioventralis* and *m. crassus caudodorsalis* – were clearly distinguished by their convex shape, which were connected to each other by two thin connective tissue plates of intermuscular tendons – *centrum tendineum*. Microscopically, the muscular membrane, which was formed by the thick muscles of the stomach, consisted of one layer, which had a circular orientation of bundles of smooth muscle tissue, which were separated from each other by thin connective tissue fibers.



According to the results of morphometric studies, the smallest value of the cuticle thickness of the gizzard was established in chicks of 1 day of age (Table 1). With age, it gradually increased and reached the largest value – 470.6±40.0 µm at 1 year of age. During the first month, it increased by 25.7%, during the second – by 22.1%. During the first month, during the first week, it increased by 118.3%, during the second – by 6.4%, during the third – decreased by 6.5%, during the fourth – increased by 3.9%.

*Table 1*

**Morphometric parameters of the cuticle, mucosa and submucosa of the gizzard of budgerigars 1-day-old - 1-year-old (µm, M±Sd, n=5)**

Age	Mucosa thickness	Cuticle thickness	Submucosa thickness	Muscular membrane thickness
1 day	271,6±28,7 <sup>bc</sup>	140,2±11,2 <sup>a</sup>	18,6±1,1	3171,2±324,7 <sup>a</sup>
3 days	357,2±23,3 <sup>c</sup>	174,6±19,2 <sup>ab</sup>	25,2±0,8	4031,3±297,6 <sup>b</sup>
7 days	287,8±26,8 <sup>bc</sup>	306,0±26,7 <sup>b</sup>	28,2±1,1	4337,2±316,3 <sup>bc</sup>
14 days	265,2±18,3 <sup>b</sup>	325,6±37,3 <sup>bc</sup>	29,4±1,1	5326,4±402,3 <sup>cd</sup>
21 days	275,0±25,9 <sup>bc</sup>	304,4±27,8 <sup>bc</sup>	28,6±1,1	5626,4±381,7 <sup>cd</sup>
1 month	217,4±13,1 <sup>a</sup>	316,4±29,2 <sup>bc</sup>	29,2±1,8	6251,1±406,5 <sup>d</sup>
2 months	219,8±23,5 <sup>ab</sup>	386,2±27,0 <sup>c</sup>	26,4±0,9	6591,6±424,7 <sup>d</sup>
6 months	227,6±25,1 <sup>ab</sup>	455,8±31,1 <sup>d</sup>	34,9±3,7	5046,8±322,2 <sup>c</sup>
1 year	236,1±19,1 <sup>ab</sup>	470,6±40,0 <sup>d</sup>	37,7±2,0	5384,1±340,8 <sup>cd</sup>

The smallest value of the height of the villi in the glandular layer of the gizzard mucosa – 96.0±15.4 µm was found in 1-month-old parrots, the largest – 152.4±11.7 µm in 3-day-old birds (Table 2). At the same time, the largest values of the depth of the glandular portion were found in 1-day-old parrots – one month old, the smallest – in parrots aged 2 months to 1 year. Moreover, the maximum value of this indicator – 204.8±14.2 µm was found in 3-day-old birds, the minimum – 95.6±9.3 µm in 6-month-old parrots.

In general, the highest values of the thickness of the gizzard mucous, which consisted mainly of glands, were characteristic of birds 1-21 days old. The maximum value of this indicator – 357.2±23.3 µm was established in 3-day-old chicks. In older birds, the thickness of the glandular layer of the mucous was smaller and amounted to 217.4±13.1 – 236.1±19.1 µm.

The thickness of the muscular membrane was the smallest in 1-day-old chicks, and then it increased until 2 months of age. In parrots 6 months and 1 year of age, its thickness was smaller. Moreover, during the first month it increased by 97.1%, and during the second – by 5.4%. During the first month, during the first week, it increased by 36.8%, during the second month, by 22.8%, during the third month, by 5.6%, and during the fourth month, by 11.1%.

Compared with 1-day-old birds, at 1-year-old birds, the thickness of the cuticle of the gizzard was 235.7% greater, the mucous membrane was 13.1% less, the submucosa was 102.7% greater, and the muscular membrane was 69.8% greater.

The thickness of the submucosa of the gizzard wall was the smallest in 1-day-old budgerigars and was 18.8 µm, the largest in 6-month-old and 1-year-old birds and was 35.3 and 37.5 µm, respectively. In the period from 3 days to 2 months of age, it was 25.0-29.4 µm.



Table 2

**Morphometric parameters of the glandular layer of the gizzard of budgerigars  
1 day old - 1 year old ( $\mu\text{m}$ ,  $M \pm Sd$ ,  $n=5$ )**

Age	Height of villi	Depth of glandular portion	Width of glandular portion
1 day	108,4 $\pm$ 19,0 <sup>ab</sup>	163,2 $\pm$ 19,9 <sup>cd</sup>	35,5 $\pm$ 2,9 <sup>ab</sup>
3 days	152,4 $\pm$ 11,7 <sup>b</sup>	204,8 $\pm$ 14,2 <sup>d</sup>	32,5 $\pm$ 3,3 <sup>ab</sup>
7 days	103,2 $\pm$ 17,9 <sup>ab</sup>	184,6 $\pm$ 24,4 <sup>cd</sup>	35,3 $\pm$ 3,9 <sup>ab</sup>
14 days	101,2 $\pm$ 16,6 <sup>ab</sup>	164,0 $\pm$ 18,2 <sup>cd</sup>	36,8 $\pm$ 2,9 <sup>b</sup>
21 days	119,8 $\pm$ 12,2 <sup>ab</sup>	155,2 $\pm$ 13,9 <sup>c</sup>	42,6 $\pm$ 2,2 <sup>c</sup>
1 month	96,0 $\pm$ 15,4 <sup>a</sup>	121,4 $\pm$ 9,6 <sup>b</sup>	37,0 $\pm$ 3,8 <sup>bc</sup>
2 months	121,8 $\pm$ 17,7 <sup>ab</sup>	98,0 $\pm$ 13,8 <sup>ab</sup>	40,6 $\pm$ 4,7 <sup>bc</sup>
6 months	132,0 $\pm$ 11,8 <sup>b</sup>	95,6 $\pm$ 9,3 <sup>a</sup>	32,4 $\pm$ 3,8 <sup>ab</sup>
1 year	128,3 $\pm$ 16,1 <sup>ab</sup>	107,8 $\pm$ 9,0 <sup>ab</sup>	30,3 $\pm$ 3,9 <sup>a</sup>

The largest values of the width of the glandular portion of the glands – 32.5 $\pm$ 3.3 – 42.6 $\pm$ 2.2  $\mu\text{m}$  were determined in parrots aged from 1 day to 2 months, the smallest – 30.3 $\pm$ 3.9 – 32.4 $\pm$ 3.8  $\mu\text{m}$  – in older birds.

Discussion. Knowledge of the anatomy and physiology of the gastrointestinal tract is necessary to determine the ability of a species of bird to use a particular type of feed, which can be used to create a nutritionally adequate diet. Understanding the factors that influence the choice of an appropriate diet allows poultry farmers, veterinarians and nutritionists to successfully keep and breed these birds (Koutsos E. A. et al., 2001).

In the gizzard of the budgerigars, we identified four membranes: mucous, submucosa, muscular, and serous, which is consistent with the data of Al-Saffar F. J. & Al-Samawy E. R. M. (2014), Beheiry R. R. (2018) regarding the structure of this organ in birds. The cuticle covering the mucous membrane from the surface is known to be a product of the activity of its glands (Bartels T. et al., 1998; Rossi J. R. et al., 2005). As indicated by Abumandour M. M. A. (2014), its thickness depends on the type of bird and food. Two layers were identified in the composition of the mucous: epithelial and lamina propria. We did not detect a muscularis mucosae. These facts are consistent with the information of Matsumoto F. S. et al. (2009); Beheiry R. R. (2018), Ahmed Y. A. et al. (2011) regarding its absence in budgerigars, domestic turkeys, quails and contradicts the data of Feder F. H. (1969), according to which it can be clearly seen in the wall of the gizzard of budgerigars, since it is separated from the muscularis mucosae by a layer of connective tissue. Al-Samawy E. R. M. et al. (2021) in the wall of the gizzard of the African grey parrot (*Psittacus erithacus*) observed the muscularis mucosae as a thinner layer of circularly arranged bundles of cells separating the lamina propria from the submucosa.

The lamina propria of budgerigar gizzard contained a glandular layer under the cuticle, which is consistent with the data of Rossi et al. (2005); Ahmed et al. (2011). The glandular layer is a tubular gland contained in the loose connective fibrous tissue of the lamina propria. It consists of an outer layer formed by villi that act as excretory ducts and an inner layer formed by the glandular portions of the glands. It should be noted that some researchers indicate that the outer layer of the lamina propria of the mucous membrane contains crypts, i.e., the immersion of the epithelial layer into the lamina propria (Zaher M. et al., 2012; Qureshi A. S. et al., 2017), or represents its folds (Rodrigues M. N. et al., 2012). However, as can be seen from Fig. 2, these are indeed villi, which is clearly visible on their longitudinal and transverse sections. According to the results of our studies, the



submucosa of the budgerigar's gizzard had the appearance of a wide layer formed by a dense plexus of connective tissue fibers. Given that this layer is constructed from a dense plexus of predominantly collagen fibers that had different directions, and, accordingly, a relatively small content of ground substance and cells, the connective tissue of the submucosa of budgerigar's gizzard can probably be classified as dense irregular. These data are consistent with the information of other researchers: in the yellow-blue macaw and the African gray parrot (*Psittacus erithacus*), it is constructed from dense connective tissue (Rodrigues M. N. et al., 2012; Al-Samawy E. R. M. et al., 2021), in the domestic turkey – from connective tissue formed mainly from collagen fibers (Beheiry R. R., 2018).

We have identified one layer in the muscular membrane of the budgerigar gizzard, which is consistent with similar data for the yellow-and-blue macaw (Rodrigues M. N. et al., 2012), the domestic turkey (Beheiry R. R., 2018), and the common quail (Zaher M. et al., 2012). At the same time, according to Al-Saffar F. J. & Al-Samawy E. R. M. (2015), in the mallard (*Anas platyrhynchos*), the gizzard muscular membrane consists of three layers – a thin inner, a longitudinal outer and a very thick intermediate one, according to Al-Samawy E. R. M. et al. (2021) in the African grey parrot it consists of two thin layers, and in the common francolin (*Francolinus francolinus*) – of three.

According to the results of determining the size of the microscopic structures of the budgerigar gizzard, two stages of cuticle thickening were established: during the first week – by 118.6% and in the period from 2 months to 1 year of age – by 54.6%. Probably, the period from 1 day to 1 month of age, when the cuticle thickness was  $140.2 \pm 11.2 - 325.6 \pm 37.3 \mu\text{m}$  and the period after 1 month of age and older, when it was  $386.2 \pm 27.0 - 470.6 \pm 40.0 \mu\text{m}$  reflect the time periods of feeding with different food. After hatching and up to 1 month of age, the parrots are fed with the mother's "milk" and soaked grain in her will, older birds are fed with whole grain food. Accordingly, different types of food correspond to different cuticle thicknesses. The high value of the thickness of the mucous and the depth of the glandular portions in 3-day-old chicks –  $357.2 \pm 23.3$  and  $204.8 \pm 14.2 \mu\text{m}$ , respectively, probably indicates a significant intensity of the process of synthesis of cuticle substances. Unlike the cuticle, the absolute and relative thickness of which increased with age, the thickness of the glandular layer of the mucous membrane, where it was formed, decreased. Moreover, within the glandular layer, the depth of the glandular portions decreased to a greater extent. Thus, this indicator in parrots 1-21 days old was  $155.2 \pm 13.9 - 204.8 \pm 14.2 \mu\text{m}$ , and in 2 months. – 1 year old –  $95.6 \pm 9.3 - 107.8 \pm 9.0 \mu\text{m}$ . Also smaller in 6-month-old parrots – 1-year-old age was also the width of such glandular portions of the stomach. It should be noted that up to 1 month of age, the index of the depth of the secretory departments had a greater value than the height of the villi, and after 1 month of age, the index of the height of the villi was greater, and the depth of the glandular portions was smaller. Unlike the glandular layer, the thickness of the submucosa gradually increased with the age of the birds and, compared with 1-day-old parrots, in 1-year-olds it was 102.7% greater. Compared with the mucous, the thickness of the submucosa in parrots of different ages was 5.8-9.6 times less. At the same time, according to Rodrigues M. N. et al. (2012), in the yellow-blue macaw it was thicker than the lamina propria. The thickness of the muscular membrane increased until 2 months of age, and was smaller in older birds thereafter.

Thus, the most intensive change in the parameters of the microscopic structures of the budgerigar gizzard occurred during the first weeks of the postembryonic period of ontogenesis, which is consistent with the data of Baltz A. P. & Clark A. B. (2001), Kubiak M. (2020) regarding the very short duration of the breeding season of budgerigars in Australia. A number of researchers have reported on the intensive nature of the growth of



the digestive organs, which outpaces the growth rate of the whole body in young domestic birds (Gille U. et al., 1999, Makhotina D. S. et al., 2020). It should be noted that changes in various morphometric indicators of the gizzard occurred asynchronously.

### **Conclusions.**

1. The microscopic structure of the gizzard of budgerigars corresponds to the general patterns of its structure in other bird species. Its wall consists of four membranes: mucous, submucosa, muscular and serous. The mucous membrane consists of an epithelial layer and a lamina propria, which contains simple, weakly branched tubular glands, the secretion of which forms a cuticle.

2. The submucosa is located between the mucous and muscular membranes and is built of tightly intertwined bundles of mainly collagen fibers.

3. The muscular membrane of the budgerigar gizzard consists of a single layer, built of smooth muscle tissue. Its thickness increased up to 2 months of age, in older parrots it was smaller.

4. The histological structure of the wall of the gizzard of budgerigars 1 day old – 1 year old is characterized by significant changes in the parameters of its microstructures. Against the background of an increase in the thickness of the cuticle with age, the depth of the glandular portions of the glands that form it decreases.

The prospect of further research is to determine the microscopic structure of other digestive organs of budgerigars.

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## SANITATION OF AIR IN LIVESTOCK FACILITIES

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*The health of productive animals is directly dependent on their housing conditions and utilization. The environment of livestock biocenoses must comply with current standards and be safe from a sanitary standpoint. Achieving this is only possible with the implementation of highly efficient disinfection technologies in production. A pressing issue today remains the development of innovative devices and methods for air decontamination in livestock facilities. Device construction was based on the results of patent research and a comparative analysis of existing analogs and prototypes. The effectiveness of disinfectants was assessed in accordance with applicable regulatory documents. As a result of the work carried out, a device was developed for air purification in livestock facilities. It features two-stage biological-droplet filters, sedimentation chambers, water supply regulation valves, water recirculation pipeline systems, and systems for water supply and drainage. A device for air sanitation in livestock facilities was also developed, which provides simultaneous air ionization and ultraviolet irradiation. This device includes a source of ultraviolet radiation and a water drainage and purification system for processed water. Additionally, methods for deodorization, aerosol disinfection, and fumigation of the air environment in livestock facilities were created. These methods involve the use of innovative disinfectants from various chemical groups under appropriate application regimes. They meet modern requirements for livestock management and are effective, environmentally safe, economical, and easy to use. The prospect of further research lies in improving technological approaches to decontaminating livestock environments.*

**Keywords:** facilities, air, device, disinfection, disinfectant.



## **САНАЦІЯ ПОВІТРЯ ТВАРИННИЦЬКИХ ПРИМІЩЕНЬ**

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*Здоров'я продуктивних тварин має пряму залежність від умов утримання та використання. Середовище тваринницьких біоценозів повинно відповідати діючим нормам та бути безпечним в санітарному відношенні. Це все можливе лише при впровадженні у виробництво високоефективних технологій дезінфекції. Актуальним питанням на сьогодні залишається розробка інноваційних пристроїв та способів знезараження повітря тваринницьких приміщень. Конструювання пристроїв проводили за результатами патентного пошуку та порівняльного аналізу існуючих аналогів та прототипів. Оцінку ефективності застосування деззасобів проводили відповідно до чинних нормативних документів. За результатами проведеної роботи розроблено пристрій для очищення повітря тваринницьких приміщень з двоступеневими біологічно-крапельного типу фільтрами, відстійниками, вентилями регулювання подачі води, системами трубопроводів рециркуляції води та системами трубопроводів водопостачання та відведення. Розроблено пристрій для санації повітря тваринницьких приміщень, який забезпечує проведення одночасно аероіонізації та ультрафіолетового опромінення повітря та містить джерело ультрафіолетового опромінення та систему водовідведення і очищення відпрацьованої води. Також розроблені способи дезодорації, аерозольної дезінфекції та фумігації повітряного середовища тваринницьких приміщень. Дані способи передбачають застосування інноваційних знезаражуючих засобів з різних хімічних груп за відповідних режимів застосування. Вони відповідають сучасним вимогам щодо організації тваринництва, є ефективними, екологічно безпечними, економічними та простими при застосуванні. Перспектива подальших досліджень полягає у вдосконаленні технологічних підходів знезараження об'єктів тваринництва.*

**Ключові слова:** приміщення, повітря, пристрій, дезінфекція, дезінфектант.

**Introduction.** An important aspect of ensuring high animal productivity and obtaining high-quality animal-derived products is maintaining sanitary and hygienic conditions for the livestock (Paliy A. P. et al., 2018; Dallago G. M. et al., 2021). Housing a large number of animals within a limited area creates epizootic risks for the spread of pathogenic and opportunistic microflora among susceptible livestock. Therefore, one of the main practices in any livestock farm is the implementation of comprehensive veterinary and sanitary measures based on the use of modern disinfection technologies (Shkromada O. et al., 2019; Ventura G. et al., 2021; Moje N. et al., 2023).



To eliminate microbiota, various methods are used, involving both chemical (Addie D. D. et al., 2015; Paliy A. et al., 2024; Tyski S. et al., 2024) and physical (Memarzadeh F., 2021; Sun Y., et al., 2023) agents. Commonly used chemical compounds in production include chlorine-based, aldehyde-based, oxygen-based preparations, and others (Paliy A. P., 2018; Curran E. T. et al., 2019; Artasensi A. et al., 2021). Despite their high bactericidal properties, chemical compounds are characterized by several negative effects, such as toxicity and carcinogenicity (Frentzel H. et al., 2013). Moreover, not all currently available antimicrobial agents are effective in livestock production, and their application regimes are often ineffective in dense livestock biotopes (Kim S. et al., 2020). Reports indicate the development of resistance in microbiota to the bactericidal effects of certain disinfectants, making them less effective and economically inefficient (Souza C. et al., 2020; Li Q. et al., 2021).

It is considered that the most environmentally safe disinfection methods are physical ones, including UV radiation, high temperature, and others (Reed N. G., 2010; Memarzadeh F., 2021). However, the implementation of these methods in practical livestock farming is limited due to their high cost, complexity in operation and maintenance, and the lack of a wide range of options on the domestic market. Moreover, there is a significant lack of scientific research dedicated to the effects of physical disinfection methods on the microbiota of livestock premises and the technological schemes for their application on an industrial scale.

Considering the above, it can be concluded that the improvement and scientific-experimental substantiation of disinfection technologies in livestock farming is a relevant task for modern agricultural science.

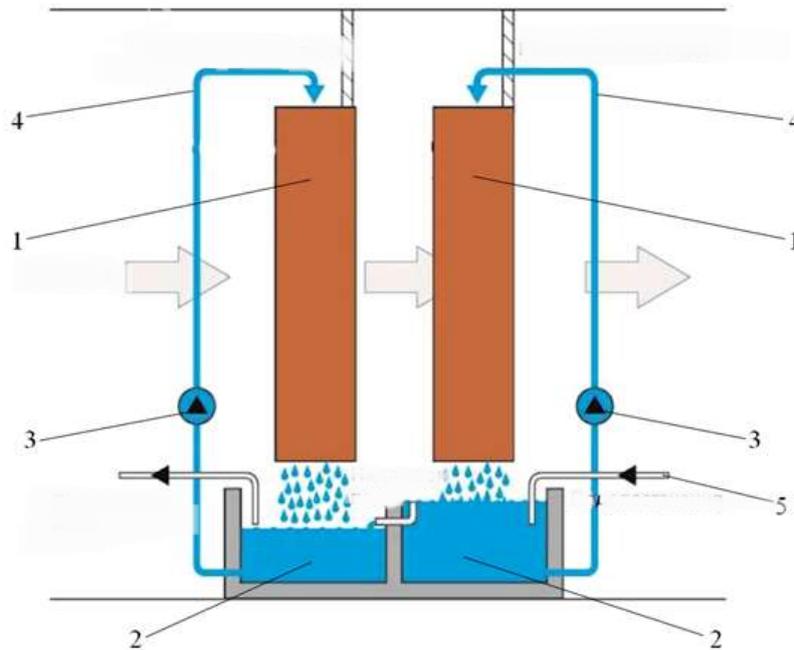
**The aim** of our research was to develop innovative devices and methods for disinfecting the air in livestock facilities.

**Materials and methods.** The development of the devices and methods was carried out based on the results of a patent search and a comparative analysis of existing analogs and prototypes. The work was conducted at the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine." The effectiveness of disinfectants was evaluated in accordance with current regulatory documents (Kovalenko V. L. et al., 2014).

**Results.** Most devices designed to maintain the necessary microclimate parameters in livestock facilities are quite bulky, complex to use, unreliable in operation, as well as energy-consuming and metal-intensive.

The first utility model focuses on the development of a device for air purification in livestock facilities. It includes two-stage biological-droplet type filters that are washed with water, sedimentation tanks, water supply control valves, a water recirculation pipeline system, and a water supply and drainage pipeline system. This design allows for effective air purification, provides functional flexibility, ensures optimal investment efficiency, reduces operational costs, significantly lowers energy consumption during use, and minimizes the need for manual cleaning and maintenance (Fig. 1).

The air purification device for livestock facilities consists of two-stage biological-droplet type filters (1) washed with water, sedimentation tanks (2), water supply control valves (3), a water recirculation pipeline system (4), and a water supply and drainage pipeline system (5). The device operates as follows: water is supplied to the device through the water supply and drainage pipeline system (5) and accumulates in the sedimentation tanks (2). Once sufficient water is accumulated, it circulates through the recirculation pipeline system (4).



**Fig. 1. Device for air purification in livestock facilities**

Air from the livestock facility is drawn through the two-stage biological-droplet type filters (1), which are washed with water. This process removes ammonia, dust, and odors from the air. A biofilm consisting of various microorganisms naturally present in the air forms on the filters (1). Some microorganisms convert ammonia, while others feed on substances like sludge and dust. The water, after washing the filters (1), flows into the sedimentation tanks (2) and is then recirculated through the pipeline system (4). This water continues to circulate through the filters (1) as long as they can absorb ammonia. Once the water is saturated, it is drained into the sludge collector via the water supply control valves (3) and the drainage system (5), and fresh water is supplied to the system. This development is protected by the Ukrainian utility model patent No. 145194.

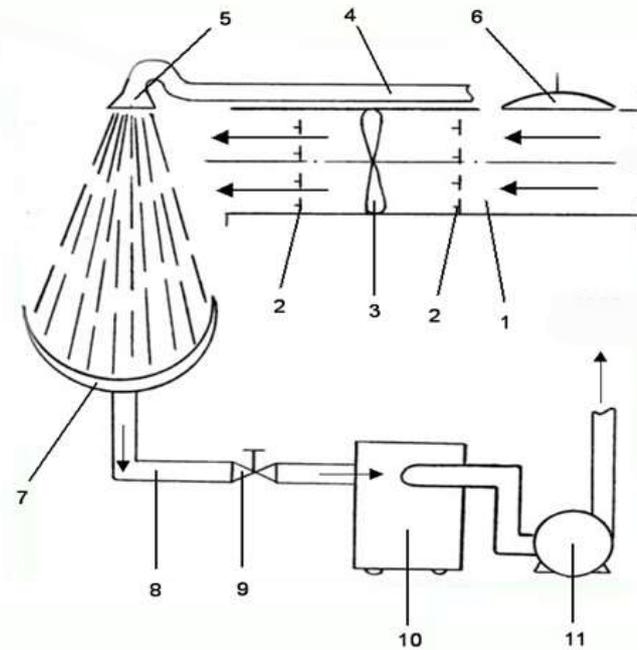
The next utility model aims to improve the efficiency of air purification in livestock facilities and reduce energy consumption during the device's operation. This goal is achieved by equipping the device with a source of ultraviolet radiation and a wastewater drainage and treatment system. These enhancements significantly increase the efficiency of air purification while substantially lowering energy consumption during use (Fig. 2).

The air sanitation and ionization device for livestock facilities consists of an air duct (1) containing corona electrodes (2) and a fan (3), a water pipeline (4) installed on the air duct with longitudinal mobility and ending in a shower nozzle (5), a high-voltage power supply unit (not shown), an ultraviolet radiation source (6), a water collection tray (7), a flexible hose (8) with a tap (9) for draining water into a sedimentation tank (10), and a water pump (11). The device operates as follows: water from the pipeline (4) flows into the shower nozzle (5), creating an artificial waterfall.

Air is drawn into the air duct (1) by the operating fan (3), where it is irradiated with ultraviolet rays from the source (6) at the inlet, effectively destroying microorganisms. Simultaneously, the corona electrodes (2) impart a negative charge to the air particles, some of which settle on the water droplets from the artificial waterfall. Coulomb forces arise between the negatively charged particles and the water, causing suspended air particles to settle on the water, thereby compensating for the lack of aeroions in the facility. The water, in turn, flows into the collection tray (7) and is drained



via the flexible hose (8) with the tap (9) into the sedimentation tank (10), where mechanical impurities settle to the bottom. The cleaned water is then pumped by the water pump (11) back to the shower nozzle (5), forming a closed-loop system. This innovation is protected by Ukrainian utility model patent No. 71809.



**Fig. 2. Device for air sanitation of livestock facilities**

The next stage of our research involved assessing the effectiveness of chemical disinfectants for air sanitation in livestock facilities. For this purpose, two complex disinfectants were used during aerosol disinfection and fumigation.

In the first case, the task was to develop a method of aerosol disinfection that includes mechanical cleaning of livestock facilities, their sealing, maintaining the appropriate microclimate (temperature not lower than 12 °C, relative humidity not less than 60%), disinfection with a preparation, and bacteriological quality control of the performed disinfection. The disinfectant used contains glutaraldehyde, glyoxal, formaldehyde, quaternary ammonium compounds (QACs), triamine, fog-forming components, and water, with an exposure time of 1 hour.

Thus, before disinfection, the facility is mechanically cleaned of manure, feed residues, debris, etc. Equipment is cleaned of technical and organic contaminants. Floors, ceilings, and walls are washed with water under pressure. Doors, windows, manure channel outlets, and natural and forced ventilation hatches are sealed. The facility's temperature is maintained at no lower than 12 °C, and the relative humidity at no less than 60%. Aerosol disinfection is then carried out using the following formulations:

Method 1: Glutaraldehyde – 0.78%, glyoxal – 0.24%, formaldehyde – 0.66%, QACs – 0.36%, triamine – 0.09%, fog-forming components – 0.60%, water – 97.27%.

Method 2: Glutaraldehyde – 1.04%, glyoxal – 0.32%, formaldehyde – 0.88%, QACs – 0.48%, triamine – 0.12%, fog-forming components – 0.80%, water – 96.36%.

Method 3: Glutaraldehyde – 1.30%, glyoxal – 0.40%, formaldehyde – 1.10%, QACs – 0.60%, triamine – 0.15%, fog-forming components – 1.0%, water – 95.45%.

Method 4: Glutaraldehyde – 1.56%, glyoxal – 0.48%, formaldehyde – 1.32%, QACs – 0.72%, triamine – 0.18%, fog-forming components – 1.20%, water – 94.54%.

The exposure time was 1 hour.



After disinfection, samples are collected using sterile cotton-gauze swabs soaked in sterile distilled water. Swabs are taken from 10 different areas of the facility, each measuring 10×10 cm, using a metal stencil to define the sampling area. Samples are washed in the same test tube by repeated immersion and squeezing of the swab. The swabs are then removed, and the liquid is centrifuged for 20–30 minutes at 1500 rpm. The supernatant is decanted, and sterile distilled water is added to the sediment, which is shaken and centrifuged again. After the second centrifugation, the supernatant is decanted, and 0.5 cm<sup>3</sup> of the centrifugate is inoculated onto nutrient media: meat-peptone broth (MPB) and meat-peptone agar (MPA). The cultures are incubated in a thermostat for 24 hours at 37.0±0.5 °C. Growth is monitored after 12 and 24 hours. The grown culture is examined under a microscope. The disinfection quality is deemed satisfactory if no microbial colony growth is observed in all tested samples.

The efficiency of the proposed method is presented in Table 1.

*Table 1*

**Aerosol disinfection method**

Preparation's composition	Concentration, %	Microbial growth	
		Prior to disinfection	After disinfection
glutaraldehyde	0.78	+	+
glyoxal aldehyde	0.24		
formaldehyde	0.66		
QAC	0.36		
triamine	0.09		
fog-forming components	0.60		
water	97.27		
glutaraldehyde	1.04	+	+
glyoxal aldehyde	0.32		
formaldehyde	0.88		
QAC	0.48		
triamine	0.12		
fog-forming components	0.80		
water	96.36		
glutaraldehyde	1.30	+	-
glyoxal aldehyde	0.40		
formaldehyde	1.10		
QAC	0.60		
triamine	0.15		
fog-forming components	1.0		
water	95.45		
glutaraldehyde	1.56	+	-
glyoxal aldehyde	0.48		
formaldehyde	1.32		
QAC	0.72		
triamine	0.18		
fog-forming components	1.20		
water	94.54		

*Note: «+» - micribial growth; «-» - no growth.*



From the data in Table 1, it is evident that swabs taken from livestock facilities before disinfection revealed the presence of catalase-positive and oxidase-negative staphylococci, *Escherichia coli*, mono- and diplococci. When using a preparation containing glutaraldehyde (0.78–1.04%), glyoxal (0.24–0.32%), formaldehyde (0.66–0.88%), quaternary ammonium compounds (QACs, 0.36–0.48%), triamine (0.09–0.12%), fog-forming components (0.60–0.80%), and water (97.27–96.36%), complete elimination of microorganisms was not achieved, as evidenced by their growth on nutrient media.

In contrast, swabs taken after the application of a preparation containing glutaraldehyde (1.30–1.56%), glyoxal (0.40–0.48%), formaldehyde (1.10–1.32%), QACs (0.60–0.72%), triamine (0.15–0.18%), fog-forming components (1.0–1.2%), and water (95.45–94.54%) showed no microbial growth in any case.

The results of the conducted studies indicate that the proposed aerosol disinfection method meets modern requirements for livestock facility management. It is effective, environmentally safe, economical, and easy to apply. This development is protected by the Ukrainian patent for a utility model № 96618.

The second utility model aimed to develop a fumigation disinfection method, which includes the mechanical cleaning of livestock premises, their sealing, maintaining the appropriate microclimate (temperature 12–35 °C), and disinfection using a preparation containing peracetic acid, acetic acid, hydrogen peroxide, and water, with an exposure time of 30 minutes and a consumption rate of at least 20 ml/m<sup>3</sup>.

Prior to disinfection, the premises are mechanically cleaned of manure, feed residues, debris, etc. Equipment is cleaned of technical and organic contaminants. The floor, ceiling, and walls are washed with pressurized water. Doors, windows, manure channel openings, and natural and forced ventilation hatches are tightly sealed. The temperature inside the premises is maintained at 12–35 °C. Disinfection is then carried out by fumigation with the following formulations:

Method 1: peracetic acid – 0.75 %; acetic acid – 0.95 %; hydrogen peroxide – 1.05 %; water – 97.25 %.

Method 2: peracetic acid – 1.20 %; acetic acid – 1.52 %; hydrogen peroxide – 1.68 %; water – 95.60 %.

Method 3: peracetic acid – 1.50 %; acetic acid – 1.90 %; hydrogen peroxide – 2.10 %; water – 94.50 %.

Method 4: peracetic acid – 1.80 %; acetic acid – 2.28 %; hydrogen peroxide – 2.52 %; water – 93.40 %.

The efficiency of the proposed method is presented in Table 2.

The data from Table 2 show that using a preparation containing peracetic acid (0.75–1.20%), acetic acid (0.95–1.52%), hydrogen peroxide (1.05–1.68%), and water (97.25–95.60%) does not fully disinfect the treated veterinary surveillance objects.

In contrast, swabs taken after applying a preparation with higher concentrations of peracetic acid (1.50–1.80%), acetic acid (1.90–2.28%), hydrogen peroxide (2.10–2.52%), and water (94.50–93.40%) showed no microbial growth in any case, indicating complete disinfection of the treated objects.

The results of the conducted studies demonstrate that the proposed fumigation disinfection method meets modern livestock management requirements. It is effective, environmentally safe, technological, economical, and simple to use. This development is protected by the Ukrainian patent for utility model No. 104050.



Table 2

**Fumigation disinfection method**

Preparation's composition	Concentration, %	Microbial growth	
		Prior to disinfection	After disinfection
peracetic acid	0.75	+	+
acetic acid	0.95		
hydrogen peroxide	1.05		
water	97.25		
peracetic acid	1.20	+	+
acetic acid	1.52		
hydrogen peroxide	1.68		
water	95.60		
peracetic acid	1.50	+	-
acetic acid	1.90		
hydrogen peroxide	2.10		
water	94.50		
peracetic acid	1.80	+	-
acetic acid	2.28		
hydrogen peroxide	2.52		
water	93.40		

Note: «+» - microbial growth; «-» - no growth.

The foundation of this utility model aims to develop a method for deodorizing livestock facilities, which includes mechanical cleaning of livestock premises and deodorizing them using a preparation that serves as a disinfectant. The preparation contains benzalkonium chloride, eucalyptus oil, fir oil, thyme oil, and water, with an exposure time of 15 minutes at a dosage of 1–2 cm<sup>3</sup> per 1 m<sup>3</sup> of the room's volume.

Before deodorization, mechanical cleaning of the premises is carried out to remove manure, feed residues, debris, and other waste. Following this, the deodorization of the premises is performed using the preparation with the following compositions:

Method 1: benzalkonium chloride 0.008%, eucalyptus oil 0.0018%, fir oil 0.0018%, thyme oil 0.0018%, water 99.9866%.

Method 2: benzalkonium chloride 0.024%, eucalyptus oil 0.0054%, fir oil 0.0054%, thyme oil 0.0054%, water 99.9598%.

Method 3: Benzalkonium chloride 0.04%, eucalyptus oil 0.009%, fir oil 0.009%, thyme oil 0.009%, water 99.933%.

Method 4: Benzalkonium chloride 0.056%, eucalyptus oil 0.0126%, fir oil 0.0126%, thyme oil 0.0126%, water 99.9062%.

The results of the method's effectiveness are presented in Table 3.

The data in Table 3 show that when using the preparation containing benzalkonium chloride at 0.008–0.024%, eucalyptus oil at 0.0018–0.0054%, fir oil at 0.0018–0.0054%, thyme oil at 0.0018–0.0054%, and water at 99.9866–99.9598%, the unpleasant odor in the premises was not completely eliminated.

However, it was found that when using the preparation containing benzalkonium chloride at 0.04–0.056%, eucalyptus oil at 0.009–0.0126%, fir oil at 0.009–0.0126%, thyme oil at 0.009–0.0126%, and water at 99.933–99.9062% with an exposure time of 15 minutes at a dosage of 1–2 cm<sup>3</sup> per 1 m<sup>3</sup> of room volume, unpleasant odors were absent for 24 hours.



Table 3

**Deodorizing method in livestock farming**

Preparation's composition	Concentration, %	Unpleasant odor	
		Prior to deodorizing	after deodorizing
benzalkonium chloride	0.008 %	+	+
eucalyptus oil	0.0018 %		
fir oil	0.0018 %		
thyme oil	0.0018 %		
water	99.9866 %		
benzalkonium chloride	0.024 %	+	+
eucalyptus oil	0.0054 %		
fir oil	0.0054 %		
thyme oil	0.0054 %		
water	99.9598 %		
benzalkonium chloride	0.04 %	+	-
eucalyptus oil	0.009 %		
fir oil	0.009 %		
thyme oil	0.009 %		
water	99.933 %		
benzalkonium chloride	0.056 %	+	-
eucalyptus oil	0.0126 %		
fir oil	0.0126 %		
thyme oil	0.0126 %		
water	99.9062 %		

Note: «+» - presence of unpleasant odor; «-» - odor is absent.

The results of the conducted studies indicate that the proposed method of deodorization in livestock facilities meets modern production organization requirements, is effective, environmentally safe, economical, and simple. This development is protected by Ukrainian Utility Model Patent No. 95338.

**Discussion.** The role of air pollution in the development of respiratory diseases, including acute respiratory infections and chronic obstructive pulmonary diseases, is well-documented. Therefore, the use of air purifiers and filters is one of the proposed strategies to improve indoor air quality (Vijayan V. K. et al., 2015). Filtration of recirculated air results in the lowest overall dust concentration (0.12 mg/m<sup>3</sup>), and lung health is found to be the best in animals housed in facilities equipped with recirculated air filtration modules (Wenke C. et al., 2018). To address issues associated with high dust concentrations, ventilation systems with air filters and humidity control have been introduced. However, their widespread adoption is hindered by high costs, significant energy consumption, and labor-intensive maintenance (Hou R. et al., 2024). Our proposed technical solutions offer high efficiency and functional flexibility, ensuring optimal capital investment efficiency and reduced operational costs.

The effectiveness of disinfection technologies based on ultraviolet (UV) radiation is promising, but it depends on numerous environmental, physical, and technical factors. UV disinfection should not be used in isolation but should be considered as a supplement to protocol-driven standard operating procedures for cleaning and disinfection (Memarzadeh F., 2021). Therefore, we consider combining physical and chemical



methods for air sanitation in livestock facilities, which, in turn, will minimize the risks of uncontrolled spread of airborne pollutants.

A wide range of antimicrobial agents is used in livestock production; however, not all are environmentally safe and highly effective (Campagna M. V. et al., 2016). In our research, we employed modern chemical disinfectants that demonstrated high efficiency under industrial conditions, allowing us to develop technological protocols for their application. According to other researchers, aldehyde-based aerosol disinfectants have also shown high effectiveness in poultry houses (Jiang L. et al., 2018). The use of peracetic and acetic acids in spray methods for intermediate disinfection is recommended (Scheib S. et al., 2023).

Odor emissions significantly affect human and animal health as well as the environment (Cao T. et al., 2023). For deodorizing livestock facilities, we propose using eucalyptus, fir, and thyme oils in appropriate concentrations, which have proven effective in combating unpleasant odors. Other scientists suggest using deodorizing microbial strains (Ma H. et al., 2021). The spraying of plant extracts is also recommended by other researchers (Wang Y. C. et al., 2021).

It has been reported that washing followed by disinfection is not always highly effective. Therefore, the use of technological breaks by relocating animals in facilities is proposed as an alternative (Connor J. T. O. et al., 2017).

Thus, despite the progress achieved in the development and implementation of disinfection technologies, there remains a need to explore more environmentally friendly and versatile antimicrobial agents, as well as to develop technical solutions for sanitizing livestock facilities.

#### **Conclusions:**

1. The proposed device for air purification in livestock facilities is economical, simple, and convenient to use. It ensures complete air environment purification, is reliable in operation, requires minimal manual cleaning and maintenance, and offers functional flexibility, which guarantees optimal investment efficiency and reduced operational costs.

2. The proposed device for air sanitation in livestock facilities enables simultaneous air ionization and ultraviolet radiation treatment to eliminate pathogenic and opportunistic microorganisms. It is reliable, simple to use, economical, and does not require significant material or energy expenditures.

3. Developed methods for deodorization, aerosol disinfection, and fumigation of the air environment in livestock facilities involve the use of innovative disinfecting agents.

4. The prospect of further research lies in improving technological approaches to the disinfection of livestock facilities.

**Acknowledgement.** This article is dedicated to the cherished memory of a renowned scientist, a talented organizer of educational and scientific processes, doctor of agricultural sciences, professor Andrii Paliy.

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### ***Ветеринарне благополуччя та якість продукції тваринництва***

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## ПРАВИЛА ОФОРМЛЕННЯ ТА ПРИЙОМУ СТАТЕЙ ДО «НАУКОВО-ТЕХНІЧНОГО БЮЛЕТЕНЮ ІНСТИТУТУ ТВАРИННИЦТВА НААН»

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<b>Індекс УДК</b>	виключка за лівим краєм без абзацного відступу, 12 pt
<b>Назва статті</b>	великими літерами, виключка по центру. 14 pt, напівжирним шрифтом
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<b>Анотація</b>	Курсивом, 12 pt, не менше 1800 знаків з пробілами.



	<p>В анотації лаконічно та інформативно описуються дані щодо 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.</p>
<b>Ключові слова</b>	<p>Курсивом, 12 pt, 5-7 слів або словосполучень.</p>
<b>Вступ</b>	<p>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 <b>мета дослідження</b>. 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.</p>
<b>Матеріали та методи досліджень</b>	<p>Зазначається об'єкт дослідження та експериментальна база. Викладена методологія повинна дати можливість повністю відтворити Ваші дослідження.</p>
<b>Результати досліджень</b>	<p>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. <b>Заголовок таблиці</b> – 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: <i>Рис. 1. Назва.</i>) – 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.</p>



<b>Обговорення</b>	У цьому розділі необхідно зіставити результати власних досліджень з результатами робіт інших авторів, яких цитували у вступній частині, що найбільш близькі до теми дослідження.
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<b>Подяки</b>	Розділ не є обов'язковим, у ньому висловлюється подяка організаціям за посильну технічну допомогу; ідеї; фінансову підтримку, завдяки якому дослідження стало можливим.
<b>Бібліографічний список</b>	<p>Джерела розташовувати в алфавітному порядку, без нумерації.</p> <p>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).</p> <p>За потреби посилань на патенти, стандарти, технічну документацію, їх робити як зноски внизу сторінки, але НЕ включати в список літератури.</p> <p>Слід уникати посилань на свої роботи - не більше 10 %.</p> <p>Вказуються прізвище та ініціали всіх (!) авторів. Ініціали пишуться після прізвища.</p> <p>Список літератури оформлюється лише латиницею. Англійські посилання оформлюються згідно з зарубіжними бібліографічними стандартами, стиль APA <a href="https://apastyle.apa.org/">https://apastyle.apa.org/</a></p> <p>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.</p>

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**НАЦІОНАЛЬНА АКАДЕМІЯ АГРАРНИХ НАУК УКРАЇНИ  
ІНСТИТУТ ТВАРИННИЦТВА**

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