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## Impact of climate change on cattle and ways of its mitigation

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The results of scientific research on climate change occurring on our planet at the present stage are presented in the review. The impact of heat stress on the well-being and productivity of large cattle is considered. Adaptation, as a process of adjustment in natural systems to global climate change, is presented. Among the major risks negatively affecting and continuing to impact the economic development of the livestock industry is climate change on our planet. In Ukraine, the problem of heat stress becomes urgent in the summer. Heat stress has a negative effect on the welfare, health, and productivity of animals. The responses to heat stress include decreased fodder consumption, searching for a shadow, greater sweat production and shortness of breath, higher consumption of water and frequency of drinking, longer standing time and shorter lying time. Heat stress has a direct effect on performance through the decrease in fodder consumption and milk synthesis. Heat stress causes a decrease in the reproductive function of animals. The consequences of the on the reproductive function of cattle depended on the magnitude and duration of its effect, the breed, and physical activity of animals. Many strategies for adapting to climate changes in livestock industry consider the short-term impact on animals during intense heat. However, in modern conditions of climate change, one should be governed by strategies leading to the long-term solution to the problem. One of these is the genetic adaptation of animals, involving the resistance to heat stress as a functional trait in the programs of animal breeding. Genetic diversity of animals will be important in further breeding work with cattle.

**Key words:** climate change, large cattle, heat stress, animal health, milk productivity, adaptation

## Introduction

Climate change is one of the threats facing humanity. It will have impact on the environment and economy. The average air temperature is the main index of climate change. In 1880–2020, the average ambient temperature over land and ocean increased by 1°C as compared to 1951–1980 [29]. In some parts of the world, warming has already exceeded 1.5°C as compared to the pre-industrial level. In many Arctic regions, the average temperature has already increased by

more than 3°C [74]. According to the simulated climate changes, the average temperature on the planet may be 2.6–4.8°C higher by 2100 as compared to the conditions in 2010 [11]. It is expected that when the planet becomes warmer, the changeability of the climate and weather will be greater. The changes in the incidence and gravity of extreme climatic phenomena and in the changeability of weather conditions will have considerable consequences for humans and natural systems. There is a forecast of higher incidence of heat stress, drought, and floods by the end of this century.



Climate change on the current stage, is one of the relevant risks, defining the global development of humanity. This process affects all the regions of the world and all the strata of the population. Even for rather moderate climatic zones, like Central Europe, the expected climatic conditions, especially in summer months, are deemed to include a higher incidence of heat and drought periods [28]. Based on the analysis of 20-year-long weather observations in July in Ukraine, namely in the western Forest-Steppe, O. Zhukorsky [81] not replaced specified the tendency of a temperature increase by 1.3°C and a higher number of tropic days. The author highlighted that the bioclimatic conditions for large full-grown cattle were characterized as comfortable, but there were more days not elaborated with the average rate of thermal stress. S. P. Ivaniuta et al. [35] note that in recent years, the frequency of days with a maximum temperature in summer above 35 and 40°C has almost doubled.

One of the envisaged consequences of climate change is the higher incidence and intensity of heat waves which are defined as several, usually from three to five, consecutive days when the maximal environmental conditions exceed some threshold [54, 44].

The geographic regions and economic branches are notable for different degrees of their susceptibility to climate change. In general, agriculture, forestry, aquaculture, and energy sectors may be the most susceptible to the manifestation of climatic changes, as they are anthropogenic and natural ecosystems [35]. T. F. Stocker et al. [70] noted that the changes in the climatic system create serious threats and challenges for the stable development of society, caused by higher risks for human health and activity, natural ecosystems, and economic sectors, and thus require detailed research and elaboration of the adaptation measures.

### **The impact of heat stress on livestock**

The environment plays a relevant role in maintaining the health, well-being, and performance of animals. The organism of an animal is impossible to imagine outside of the bounds of the environment and the interaction therewith. Every species of animal has its limits of comfortable ambient temperature. These limits are called a thermoneutral zone. The increase or decrease in the ambient temperature outside the bounds of the thermoneutral zone has a negative impact on the well-being and performance of animals [66]. Beyond the upper critical limit of the ambient temperature of the thermoneutral zone, the animal starts feeling heat stress. Heat stress is defined as a state when an animal cannot dissipate a sufficient volume of heat, regardless of its being produced or consumed by the organism, and to maintain heat balance of the body. It may cause physiological and behavioral reactions, which will lead to physiological disorders, resulting in a negative impact on the health, well-being, and performance of farm animals [52].

The ambient temperature, comfortable for large cattle, depends on the species-specific traits of animals. According to M. Fiedler et al. [20], the ambient temperature, comfortable for large cattle, is within 0...+15°C, and as per the FAO [50], in middle latitudes, these thresholds fluctuate from +4 to +24°C, in tropic latitudes — from +15 to +27°C.

Heat stress is more problematic and has a greater effect than cold stress [45]. Climate change elevates heat stress and decreases cold stress. Thus, heat stress dominates thermal stress [10].

The impact of hot weather on large cattle becomes ever more relevant due to climate change. The problem of the impact of climate change on livestock industry is as follows: how much do the animals depend on the thermal environment, and how can one mitigate the effect of higher ambient temperature on them? The current impact of the thermal environment is evaluated by the effect of climatic conditions on the health, well-being, and performance of animals [53].

To decrease climatic risks for livestock industry, one should understand in which way potential ecological stressors can affect the functioning of the animal organism and the implementation of its genetic potential [67].

### **The impact of heat stress on the well-being and health of animals**

The impact of climate change on the health of animals may be direct or indirect. Direct consequences are mainly conditioned by the changes in the environmental conditions, including air temperature, relative humidity, precipitation, drought, and floods. These environmental conditions cause diseases and death of animals related to air temperature. The indirect impact of climate change on health is conditioned by the microbial density and distribution of transmissible diseases, food and water shortage, or food-borne diseases [40].

Among different means that help maintain homeostasis, physiological adaptivity is considered to be one of the mechanisms of the primary response that helps animals survive. Respiratory and heart rates, rectal temperature, sweat production degree, and skin temperature are the physiological parameters that help maintain the heat balance and homeostasis under hyperthermia [1]. The first reaction of the animals to hot weather is an increase in the respiratory rate, rectal temperature, and heart rate, which has a direct impact on fodder consumption, decreases the rates of growth, the milk yield, and reproductive traits, and in extreme cases even leads to death. The imbalance between the metabolic heat production of the animals and its dissipation into the environment causes heat stress [16].

Usually, the observed responses to heat stress include decreased fodder consumption, searching for a shadow, greater sweat production and shortness of breath, higher consumption of water and frequency of drinking, longer standing time and shorter lying time, as well as lower frequency of defecation and urination [10].

The decreased fodder consumption is one of the reactions to high ambient temperature. Under higher heat stress, the ruminants lose appetite, have slower intestinal motility, and decreased rumination [80].

Neuroendocrine regulation is one of the decisive ways for animals to survive in a stressed state [2]. The hypothalamo-pituitary-adrenal system plays a relevant part in the thermoregulatory mechanisms of animals. The corticotrophic releasing factor, adrenocorticotrophic hormone, and glucocorticoids are primary products of the hypothalamo-pituitary-adrenal axis, which, in the long run, control the pathway of a response of animals to stress, regulating the energy distribution to maintain life activity in the process of hepatic gluconeogenesis [51]. During the heat tension period, there is an increase in the level of adrenalin and noradrenalin, which regulate the cardiovascular frequency during thermal stress and maintain blood supply to the organs [2].

Having conducted the study using the bulls of Angus and Volyn meat breeds, O. Zhukorsky [82] stated that a high heat burden for ten days led to heat stress. He observed a high rate of correlation between rectal temperature and air temperature, rectal temperature and prolactin, air temperature and prolactin. At the same time, the author noted breed-specific differences in the sensitivity to thermal stress, indicating that Angus bulls were more sensitive to heat stress than Volyn meat breed. The studies of A. Afsal et al. [2] with dairy cattle involving heat stress found a decrease in the prolactin concentration and an increase in the level of somatotrophic hormone, which had a negative effect on the performance.

The immune system of animals is the main defense of their organism, which protects them from environmental stress factors and other harmful effects [75]. Heat stress may have a negative impact on immune functions via the cellular and humoral immune response [4]. As a result, after the period of hot weather, the cattle may be more susceptible to diseases, for instance, there may be a more significant number of animals with mastitis, which will lead to economic losses [15]. N. Lacetera [40] stated that heat stress had a negative impact on the health of animals, causing metabolic disorders, oxidative stress, and immunity inhibition, which made the animals more susceptible to diseases.

Heat stress causes a decrease in the reproductive function of animals. The consequences of the heat stress effect on the reproductive function of cattle depended on the magnitude and duration of its effect, the breed, and physical activity of animals. The impact of heat stress due to hormonal imbalance caused a decrease in the quality of oocytes and sperm along with slower development of embryos and their survival. It occurs due to the decreased secretion of luteinizing hormone and estradiol, which leads to a shorter length and intensity of estrus expression, higher frequency of manifestations of quiet sexual excitement in farm animals. Oocytes, susceptible to heat stress, lose their ability to fertilization and development in the blastocyst stage. Poor secretion of progesterone

restricts the functions of the endometrium, and thus, the development of the embryo. On hot days, the temperature of the testes increases, so the fertility of breeding bulls decreases due to impaired spermatogenesis and sperm quality [38, 51].

J. W. Ross et al. [62], M. Cheng et al. [10] reported that heat stress affects the reproduction of both genders. In females, heat stress shortens the estral period and fertility, simultaneously increasing the frequency of embryo deaths. In males, there is a decrease in sperm quality, the volume of testes, and the amount of fertile sperm.

“The impact of heat stress on reproduction is complex and multifactorial and is compounded by growing challenges due to climate change. Both animal welfare and fertility are vulnerable parameters easily affected by heat stress. Heat stress leads to a marked decrease in the developmental competence of oocytes and the fertilizing capacity of spermatozoa, leading to a declining reproduction rate and losses for the cattle industry” [37].

M. M. Sharan, Yu. T. Salyha [68] note that advances in reproductive biotechnology are a powerful tool that can be used to improve production and address livestock challenges in the future.

The problem of heat stress is extremely urgent in the regions where the weather is characterized by high summer temperatures and humidity. This combination has a negative effect on the restorative ability of cows, the course of gestation, and the functional state of the newborn calves [83].

### **The impact of heat stress on the performance of animals**

Heat stress has a direct effect on performance through the decrease in fodder consumption and milk synthesis [16]. The metabolic production of heat, caused by heat stress, increases, and thus, milk production decreases [36]. M. Rhoads et al. [60] noted that under heat stress, dairy cows eat less fodder, which leads to their decreasing dairy performance by approximately 35%. According to U. Bernabucci et al. [7], the decrease in milk production caused by heat stress may amount to about 14% at the beginning of lactation and 35% in the middle of lactation.

The increase in air temperature becomes an urgent problem in summer. For instance, in Ukraine, R. M. Dibirov [17], N. Boltyk [8] found that due to high temperatures in July and August (+28...+30°C) as compared to June (+18...+20°C), the dairy performance of cows decreased by 7.4–16.0% in the northern zone, by 6.2–12.9% — in the central zone, and by 5.5–12.6% in the southern zone. T. O. Vasylenko et al. [77] stated that in August, under the average air temperature of 23.4°C, as compared to that of 14.7°C in May, the milk yield of cows decreased by 5.5%, the yield of fats — by 7.3%, and the yield of protein — by 5.7%. In the Mediterranean region, due to higher than comfortable

air temperature values in spring and summer, there is a reliable decrease in the daily milk yield of all cows regardless of their performance [23]. According to the data of R. V. Mylostyvyi et al. [51], V. Sejian et al. [66], in Switzerland, the Czech Republic, and Poland, in summer, dairy cows are under heat stress for 6–10 h a day, and in Spain, Italy, and south of France — from 13 to 18 h, thus losing 3.0–5.5 kg of milk. In Eastern Europe, the duration of the stress period is 30–60 days, which causes a drop in productivity of 10–35%.

Heat burden has a greater impact on highly productive cows [69, 79], which is reasonable because there is a positive correlation between higher milk yield, fodder consumption, and metabolic release of heat [36]. Similar conclusions were made by M. M. Rojas-Downing et al. [61], who found that highly productive dairy cows released more metabolic heat than cows of low productivity, and thus, they were more sensitive to heat stress.

During the dry season, heat stress affects the proliferation and development of the dairy glands which then leads to smaller milk yield. In dairy cattle, this situation leads to a considerable drop in milk production, especially in highly productive cows. The decrease in milk production due to heat stress may amount to almost 10–15% at the farms, using the cooling methods, and reach 40–50%, if no cooling is used [73].

M. A. North et al. [55], studying various predictive models of heat stress, indicate that they all lead to reduced milk production.

In addition to milk yield, a hot and humid environment also affects the milk composition. O. Ravagnolo et al. [59], T. Gorniak et al. [31] stated that heat stress on lactating cows led to a lower content of milk fat and protein. A negative correlation between the heat burden and milk fat and protein composition was reported by R. Bouraoui et al. [9], U. Bernabucci et al. [6], J. B. Garner et al. [22], J. M. L. Heck et al. [32], D. L. Hill et al. [33], C. Lambertz et al. [41], G. E. Pollott [56], M. A. Quist et al. [58]. Heat stress also changes the lipid profile of milk [43]. However, rather contradictory results were published regarding the impact of heat stress on the fat content in milk. For instance, F. C. Cowley et al. [14] did not find any changes in the portion of milk fat under heat stress but specified the tendency toward the decrease in the content of both protein and casein. According to the data of H. M. A. Gaafar et al. [21], the fat content in cows' milk under heat stress decreased by 3.79–3.49%.

Heat stress has a negative effect on the quality of products of animal production. A. Summer et al. [72] noted that a negative impact of heat stress on the milk composition (organic and inorganic components) led to its suitability for cheese production and product quality. These changes lead to considerable negative economic consequences for producers and consumers.

Due to lower metabolism rate and heat production, large meat cattle are usually considered to be less

susceptible to heat stress than dairy cattle. However, it also compensates for the increased body temperature using homeostatic mechanisms (shortness of breath, sweat production, and drooling) and behavioral changes, including a decrease in activity, greater consumption of water, and a decrease in fodder consumption. It leads to a slower rate of animal growth [72].

Heat stress has a negative effect on meat productivity. With a higher external heat burden, large cattle re-distribute the energy, usually designated for growth and maintaining homeostasis [36, 59], which leads to a decrease in the live bodyweight gain. There is a considerable variability in the average daily live bodyweight gain and fodder conversion in different studies, conducted in feedlots [24–26, 42, 71]. However, they reflect the general decrease in the growth rate of animals under the impact of heat burden. P. A. Gonzalez-Rivas et al. [30] reported that heat stress affected meat production for all the main commercial types of cattle. N. A. Elam et al. [19], A. Summer et al. [72] stated that ruminants, subjected to heat stress, were notable for poorer meat quality.

Generally, heat stress led to a decrease in milk and meat production in all types of cattle.

Whatever affects the production of fodder may also impact the feeding of animals, and thus, their performance. Therefore, a decrease in the harvest of fodder crops will lead to poorer availability of fodder and an increase in the cost of products. In cold zones, an increase in the average temperature may cause the prolongation of a vegetation period for fodder with a decrease in their quality [49].

It should be noted that many recent publications of the studies also demonstrated that the consequences of climate change differ depending on the region, duration, and distribution of heat stress. In addition, the effect on specific breeds and individual animals will vary greatly. Thus, one should define key factors for each geographic territory, which present specific interests, breed (genotype), and industrial system [28].

According to all the evaluated scenarios, it is forecasted that in this century, heat stress will become a serious problem for large livestock industry systems, resulting in a decrease in milk and meat production. Therefore, it is important to pay special attention to the potential magnitude and degree of adaptation measures that will be required in different places to counteract the consequences of ever-increasing heat stress for large cattle [76].

The data from the studies, conducted by different scientists, demonstrate a considerable decrease in the performance of animals, which leads to enormous economic losses during heat stress. It is evident that climate change will affect the performance of cattle in many regions, and many simulations define this effect to be harmful. The impact of climate change on the livestock industry will be a consequence of combined changes in ambient temperature, precipitation, incidence,

and magnitude of extreme weather phenomena. It will include both direct and indirect effects. Climate change enhances a general need for strategies for adapting to and mitigating consequences, which would cover available instruments of management, feeding, breeding, and health protection of animals. The envisaged changes will create the pressure of selecting with the consideration of the traits, relevant to biological suitability and production of specific products. The classified information, obtained regarding the evaluation of the impact of climate change on the performance and well-being of animals, may become very valuable for the elaboration of the relevant strategies of adapting to and mitigating the consequences to support the manufacture of animal products in the scenario of climate change.

### **The adaptation of livestock industry to climate change**

Adaptation to global climate change is a process of adjustment in natural or human systems in response to actual or expected climatic effects which would help decrease their negative consequences and utilize favorable possibilities. All animals can adapt to the thermal environment. The animals change their behavioral, physiological, and morphological characteristics or their combination in response to the thermal environment and thus adapt thereto [3, 27]. Therefore, animals can develop the mechanisms of survival that minimize the impact of thermal burden on the organism in general. The overcoming mechanisms, developed by animals in response to heat stress, are referred to as adaptation and adjustment.

The acclimation and adaptation ensure the resilience level of large cattle populations. Acclimation is a homeostatic process, governed by the endocrine system, which leads to cellular, metabolic, and systematic changes that allow animals to react to and cope with heat stresses [3]. Animals adjust to the environment they live in and to the external stress by acclimation to a specific stressor or several stressors [13, 27]. The adaptation is related to biological changes in subsequent generations via the support for genetic selection in the population through a permanent impact of the stressor, maintaining the survival of species [64].

Adaptation may be decisive for survival, but it often has a negative impact on the performance and profitability of the animal breeding systems. The ability to adapt in part depends on the plasticity of behavioral traits and in part — on morphological and physiological changes, which adjust animals to survival in a better way [48]. The adaptation is a coordinated phenotypic reaction to stressors, and the response will get weaker if the stressors are removed. If chronic stress lasts for several generations, the adaptation reaction

will become genetically “fixed”, and the animal will adjust to the environment [13]. The knowledge about genetic differences between adapted animals will promote breeding work under global climate changes.

The potential strategy of mitigating consequences should lie in breeding animals, resistant to climate change. Resilience is defined as the ability of the animal to get quickly restored after the disturbance effect or the ability to suffer the minimal effect of disturbance [12]. In the context of climate change, resilience will reflect the stable performance of animals regardless of a weather change.

The adjustment of an animal may be defined as an ability to survive and reproduce in a specific environment [57] or a degree to which an organism, population, or species may remain adjusted to a wide scope of environments using physiological or genetic means [5].

Flexibility is usually considered to be an evolutionary adjustment to environmental changes. Flexibility is a key mechanism, using which organisms can cope with climate change, since it allows them to react to changes throughout their lives. Flexibility is of great importance to large cattle since there is a large interval between generations, and evolutionary reactions via natural selection may not lead to rapid changes to mitigate the consequences of climate change. The species, which evolve in warm and stable tropic climate, have lower flexibility as compared to those in variable moderate environments since it is believed that the magnitude of the temperature variation is directly proportional to the flexibility ability. This is a great possibility to breed the populations of cattle, bred in European regions with various climatic conditions and variable temperatures. The methods of selection and experimental evolution demonstrated that flexibility is a trait that may develop during direct selection or as a correlated response to choosing specific traits. Therefore, it is reasonable to use the flexibility, accumulated in the breeds of large cattle, in the new selection goals [63].

The producers may adapt to climate change, adjusting the genetics of their animals to the changed environment or adapting the production environment, while preserving the genetic profile of animals. It is expected that farmers will first use the adaptation technologies, which can be quickly implemented and change the genetic profile of animals only when this process becomes inevitable [34]. J. M. Rust [65] stated that climate change affected both extensive and intensive systems of animal production.

The systems of intensive animal production have more possibilities to adapt via technological changes, and the latter can make them rather insusceptible to climate change and preserve highly productive breeds. To fight the consequences of short-time heat waves, one can use various technologies, including air-conditioning, shadowing, or raining, to decrease excessive heat burden [46]. Access to these technologies and



capital will define the ability of producers to protect their herds from the physiological stress of climate change. Large-scale implementation of these technologies will also depend on the availability and prices of energy and water. A question arises: how long can one support the industrial environment under these conditions [34]? Although the direct impact of climate change on animals may be insignificant, if the increase in temperature does not exceed 3°C [18], the forecasts demonstrate the need for further selection of breeds with efficient control of thermoregulation. It requires the inclusion of traits, related to thermal tolerance, into the selection indices, and a greater consideration of the interaction between a genotype and environment to identify animals, most adapted to specific conditions.

I. Hoffmann [34] stated that the adaptation to the climate change can be considered in two ways:

1. How can the genetic resources of animals cope with climate change and adapt to it, still ensuring food safety and earning in rural areas?

2. How can one preserve the value of genetic resources and minimize a potential loss of diversity in case of climate change?

Genetic diversity of animals within the breed is important for further improvement of cattle [39].

Genetic diversity of animals is decisive for food safety and the development of rural areas. It allows selecting the number of livestock or creating new breeds in response to the change in conditions, including climate change, new or renewed threats of diseases, new knowledge about human needs for food, and a change in market conditions or needs of the society. All these are considerably unforeseen. The factor that can be predicted is an increase in human need for food in the future. The most acute consequences will be in developing countries, where it is expected that demand will grow faster than production, and in the areas where climate change is forecast to have the greatest impact [34]. The importance of preserving the gene pool of local breeds is emphasized by M. M. McIntosh et al. [47], A. K. Wankar et al. [78].

A current tendency of climate change poses a threat to health, well-being, and performance of large cattle in the entire world. Heat stress makes animals more susceptible to diseases and is one of the reasons for performance losses. A real challenge is the mitigation of this effect and adaptation of the systems of animal breeding to the variable climate. The adaptation to extreme climatic conditions and the mitigation of their harmful effect will play a relevant role in the fight against heat stress for cattle. Many strategies for adapting to climate changes in livestock industry consider the short-term impact on animals during intense heat. However, in modern conditions of climate change, one should be governed by strategies leading to the long-term solution to the problem. One of these is the genetic adaptation of animals, involving the resistance to heat stress as a functional trait in the programs of animal breeding.

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## Вплив зміни клімату на організм великої рогатої худоби та способи його пом'якшення

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В огляді представлені результати наукових досліджень щодо зміни клімату, яка відбувається на нашій планеті на сучасному етапі. Розглянуто вплив теплового стресу на добробут здоров'я та продуктивність великої рогатої худоби. Представлено адаптацію як процес пристосування у природних системах до глобальної зміни клімату. До найбільших ризиків, які негативно впливають і надалі будуть впливати на економічний розвиток тваринницької галузі, належить зміна клімату, яка відбувається на нашій планеті. В Україні проблема теплового стресу актуальною стає у літній період. Тепловий стрес негативно впливає на добробут, здоров'я та продуктивність тварин. Реакції на тепловий стрес охоплюють зменшення споживання корму, пошук тіні, посилене потовиділення та задишку, збільшення споживання води та частоти пиття, збільшення часу стояння та зменшення часу лежання. Тепловий стрес безпосередньо впливає на продуктивність через зменшення споживання корму та, зрештою, синтезу молока. Тепловий стрес спричиняє зниження репродуктивної функції тварин. Наслідки впливу на репродуктивну функцію худоби залежали від величини й тривалості його дії, породи, а також фізичної активності тварин. Багато стратегій адаптації до кліматичних змін у скотарстві враховують короткочасний вплив на тварин під час інтенсивної спеки. Однак у сучасних умовах зміни клімату потрібно орієнтуватися на стратегії, які призводять до довгострокового вирішення проблеми. Однією із таких є генетична адаптація тварин, що передбачає залучення стійкості до теплового стресу як функціональної ознаки в програмах розведення тварин. Генетична різноманітність тварин матиме важливе значення у подальшій селекційній роботі з великою рогатою худобою.

**Ключові слова:** зміна клімату, велика рогата худоба, тепловий стрес, здоров'я тварин, молочна продуктивність, адаптація



## Dexamethasone marginally surpasses MSC-secretome in resolving acute liver failure in mice

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### Authors' Contributions:

**TD:** Data curation; Methodology; Investigation; Formal analysis.

**MH:** Project administration; Conceptualization; Formal analysis; Supervision; Writing — review and editing; Correspondence.

**EA:** Methodology; Investigation; Writing — original draft, review; Data collection.

### Declaration of Conflict of Interests:

None to declare.

### Ethical approval:

The protocol of the current study and the animal use were conducted following the ethical regulations. The study protocol was approved by the ethical committee of following the ethical regulations of animal care and use of the Faculty of Science, Tanta University Animal Care and Use Committee (IACUC-SCI-TU-0184).

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The anti-inflammatory roles of Mesenchymal stem cells (MSCs) and glucocorticoids are well-reported in both preclinical and clinical studies. However, it is not clear how far MSC-secretome offers sufficient protection against acute liver failure (ALF) compared to glucocorticoids. To answer this query, acute liver failure was induced in mice by a single toxic dose (400 mg/kg) of acetaminophen (APAP). Then mice were treated with Dexamethasone or transfused with MSC-secretome, which was derived from DEX-treated bone marrow mesenchymal stem cells. The results showed that 10 nM DEX has no impact on the viability or the mesenchymal characteristics of MSCs. While the transfusion of MSC-secretome provided a significant therapeutic effect against ALF, it was marginally less effective than DEX treatment. Hepatic markers (ALT, ALP, GGT, and bilirubin) were improved more significantly in DEX-treated mice than in MSC-secretome treated group. This improvement was accompanied by marked relief in the oxidative assessed in the liver as Nrf-2, MDA, and GSH. Additionally, the normal levels of angiogenic (VEGF), and inflammatory (TNF- $\alpha$ ) markers were effectively restored after DEX treatment. Also, both MSC-secretome and DEX resolved liver necrosis. In summary, these data suggest that dexamethasone demonstrates a better therapeutic effect than MSC-secretome in the treatment of ALF. Further studies are necessary to standardize MSC-secretome as an acellular therapeutic approach.

**Key words:** BM-MSCs, dexamethasone, acute liver failure, hepatoma cells, paracetamol, inflammation

## Introduction

Acute liver failure (ALF) is characterized by rapid and severe liver dysfunction and may lead to death if not properly treated. Pathologically, it is associated with some features including hepatic encephalopathy and coagulopathy, which can progress to necrosis of liver tissue. Also, the pathophysiology of ALF involves a systemic inflammatory response that may lead to complications in other organs like cerebral edema and renal failure [5]. Globally, ALF occurs in humans as a result of many etiologies including drug overdose, infection with hepatitis

viruses, and less frequently, autoimmune hepatitis [20]. Despite advances in critical care and liver transplantation, the mortality rate remains high, making early ALF management essential. Due to the association between liver inflammation and ALF, many authors involved the role of immunomodulatory therapies, such as corticosteroids [27] in ALF treatment. Dexamethasone, for example, showed a crucial role in managing different liver diseases due to its anti-inflammatory and immunosuppressive properties [28]. In and severe alcoholic hepatitis and autoimmune hepatitis, for instance, dexamethasone reduced liver inflammation, modulated the immune re-

sponse, prevented further liver necrosis, and improved patient outcomes. In a similar manner, treatment of liver transplant patients with dexamethasone is traditionally used to prevent acute graft rejection, contributing to patient survival. However, its use must be carefully monitored to avoid Dexamethasone-associated side effects, such as immunosuppression and subsequent inflammation. In these pathological conditions, the drug reduces the production of pro-inflammatory cytokines, limits the development of ALF-related cytokine storm, and limits further liver damage. In the past few decades, Mesenchymal stem cells (MSCs) transplantation was emerged as a promising therapeutic option for many disorders including acute liver failure (ALF). This was attributed to their regenerative potential and immunomodulatory properties [29]. These features were explained by the immunomodulation of the anti-inflammatory cytokines, released by the MSCs (secretome), which reduce inflammation and the progression of liver injury [11, 24]. Also, MSC differentiation into hepatocyte-like cells, promotion of the survival of endogenous liver cells [1], and modulation of angiogenesis and oxidative stress [7, 25] could be regarded as alternative mechanisms of MSCs-mediated ALF therapy. Additionally, the anti-fibrotic effect of MSCs was suggested due to their effect on the activity of hepatic stellate cells, which play a key role in liver fibrosis [21, 30]. The vast majority of these preclinical investigations were conducted using MSCs. Acellular regenerative approach, however, was recently adopted in which MSC-derived secretome and extracellular vesicles (EVs) replaced MSC-mediated therapy [3, 4, 14]. However, there is a lack of knowledge about the efficacy of MSC-secretome in the treatment of drug-induced liver injury (DILI) compared to Dexamethasone. To answer this query, this work was designed to compare the therapeutic efficacy of dexamethasone and MSC-secretome in the treatment of paracetamol-induced liver failure in mice.

## Materials and Methods

### Chemicals and reagents

Dexamethasone was purchased from *Amriya for Pharmaceutical Industries* (Alexandria, Egypt). Isolation and passaging of MSCs were carried out using cell culture media (DMEM) and supplements including L-glutamine, Fetal bovine serum (FBS), and Penicillin/streptomycin purchased from Lonza. Liver biomarker kits including alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and bilirubin were from *Spectrum Diagnostics* (Egypt).

### Mesenchymal stem cells isolation and collection of MSC-conditioned media

Bone marrow MSCs were isolated from an adult (250 g) male Sprague-Dawley rat, following the ethical regulations of animal care and use of the Faculty of Science, Tanta University Animal Care and Use Com-

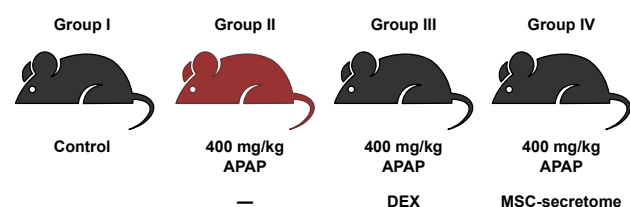
mittee (IACUC-SCI-TU-0184). Briefly, bone marrow from rat's bones (the tibia and femur) was collected in complete media containing 10% FBS and 1% penicillin/streptomycin. Cells were grown at 37°C, 95% air, and 5% CO<sub>2</sub>. BM-MSCs conditioned media was prepared by incubating MSCs in a serum-free media for 48 h, after which media were collected, centrifuged, to remove cell debris, and utilized in mice treatment.

### Annexin V/PI staining

The apoptosis assay was performed using an *Annexin-V FITC* kit (*Miltenyi Biotec*, CA, USA) following the manufacturer's guidelines. Briefly, cells were seeded in T25 flasks. After overnight incubation, cells were treated with 10 nM DEX for 24 h after which they were collected by Trypsin/EDTA, and centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended in PBS and incubated with 0.25 µg/ml Annexin V in 1X binding buffer for 15 min, followed by two washes with a Wash Buffer. Cells were resuspended again in a binding buffer PI and then subjected to flow cytometry analysis.

### Induction of acute liver failure in mice and grouping

Male C57BL/6 mice (25–31 g) were purchased from The National Cancer Institute, Cairo University. Animals had free access to food and water and were housed in a 12 h light/dark cycle in standard conditions. All animal experimentation was in compliance with the guide for the care and use of laboratory animals, where the experimental design was approved by the Ethics Committee of the Faculty of Science, Tanta University (IACUC-SCI-TU-0184). Mice (n=24) were randomly assigned to 4 groups, six mice each (fig. 1). Group I was left untreated as a negative control group, whereas mice in groups II, III, and IV received a single intraperitoneal dose (400 mg/kg body weight) of APAP to develop ALF. Group II mice were slaughtered 24 h after acetaminophen (APAP) injection, whereas groups III and IV were treated once with 2 mg/kg DEX via i.p injection or transfused consecutively twice with 200 µl prefiltered BM-MSC-secretome via tail vein. Animals were slaughtered one week after treatment and both blood and liver tissues were collected for further investigations.



**Fig. 1.** Experimental design, animal grouping, and treatment protocols. Mice were divided into 4 groups. Group I (GpI) included healthy control mice. Mice in groups II, III, and IV were injected with a toxic dose of APAP to develop acute liver failure. Groups III and IV were treated with a single dose of DEX or two infusions with MSC-secretome one week apart. Mice were slaughtered one week after the last treatment

**Assessment of liver function**

Serum levels of ALT, ALP, GGT, and Bilirubin were measured using marker-specific kits, following the manufacturer’s guidelines. In parallel, liver tissue samples were homogenized in ice-cold phosphate buffer, pH 7.2, containing 1 mM EDTA-Na<sub>2</sub>. The homogenate was centrifuged at 15,000 g at 4°C for 15 min and its protein concentration was determined in the supernatant by the Bradford assay.

**Quantification of inflammatory, angiogenic, and oxidative stress markers in liver**

Tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), Nuclear factor-erythroid p45-related factor 2 (Nrf-2), and GSH were determined by ELISA kits, following the manufacturer protocols. Malondialdehyde (MDA) concentrations in liver homogenate were measured according to [19]. In this assay, 100 μl of liver homogenate sample was mixed with 300 μl perchloric acid (PCA) (0.1125 N) and thiobarbituric acid (TBA) (40 mM, 300 μl), and then placed in

a boiling water bath for 60 min. After cooling methanol (600 μl) and 20% TCA (200 μl) were added and mixed for 10 s. The samples were centrifuged at 10,000 rpm for 6 min, and the MDA concentration was quantified using a standard curve, which was obtained from hydrolyzed 1,1,3,3-tetramethoxypropane (TEP) dissolved in water in different concentrations.

**Histopathological analysis**

For histological assessments, portions of livers were excised, and fixed in 10% formalin. After dehydration and clearance, tissues were embedded in paraffin, sectioned in 5 μm thickness, stained with hematoxylin-eosin (H&E) following the standard protocol, and examined under a light microscope.

**Statistical analysis and software**

Statistical analysis was performed using *Graphpad Prism 5.0* software (*GraphPad Software Inc.*, San Diego, CA, USA). Data were expressed as mean ± standard deviation (SD). Mean values were compared using ANOVA test followed by Tukey test, where P<0.05 was considered statistically significant.

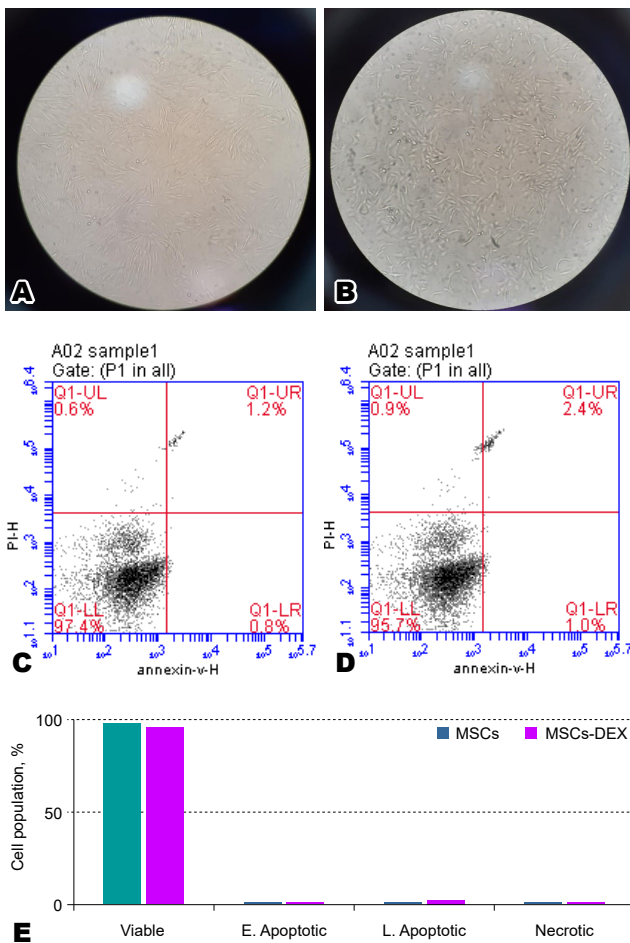
**Results**

Initially, we isolated BM-MSCs from rat’s bones. Adherent cells, maintained in standard culturing conditions, demonstrated a typical spindle, fibroblast-like shape (fig. 2). Incubation of cells in a complete media containing 10 nM DEX for 24 h did not affect cell morphology and viability. To ensure that, cells were dually stained with Annexin-V and PI, where both untreated and DEX-treated cells did not show significant apoptosis nor necrosis (fig. 2).

In parallel, we developed ALF in mice through the administration of a single toxic dose of APAP (400 mg/kg body weight). Signs of ALF were authenticated by monitoring the serum levels of liver function markers including ALT, ALP, GGT, and bilirubin, where all markers were significantly increased compared to the healthy group (P<0.001 with all serum markers) (fig. 3).

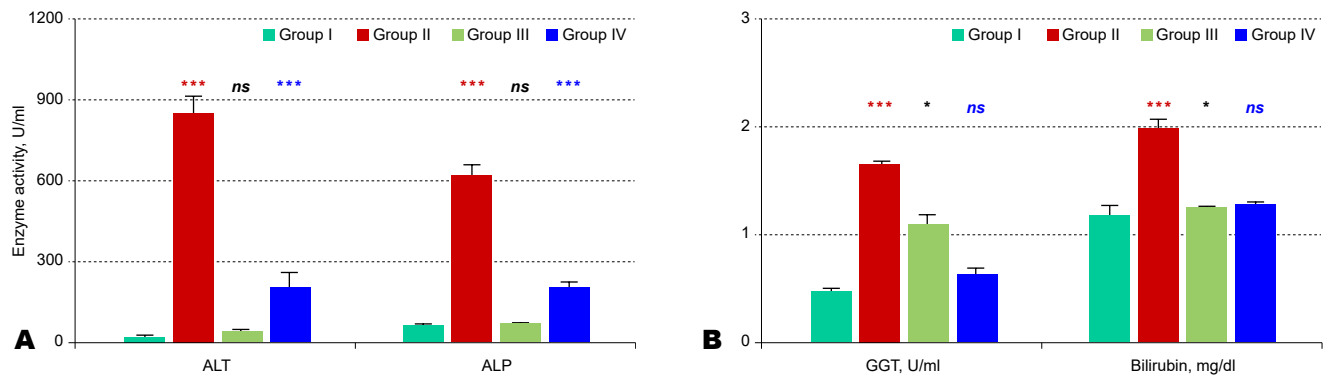
Cells were utilized to prepare MSC-secretome. Next, 24 h after the development of ALF, mice were treated with a single dose of 2 mg/ml DEX (in group III) or transfused twice with MSC-secretome. Mice treatments led to a significant decrease in the serum ALT, ALP, GGT, and bilirubin (fig. 3). The improvement in these markers was more pronounced in DEX-treated mice compared to the ALT and ALP. Since APAP development is associated with a significant increase in oxidative stress, we measured three oxidative stress markers. Treatments with both MSC-secretome and DEX were associated with the amelioration of the oxidative stress markers (Nrf-2, MDA, and GSH) in the liver (fig. 4).

Additionally, the high levels of the inflammatory marker (TNF-α) observed in the liver of ALF mice, significantly decreased in treated mice particular in DEX-treated mice.

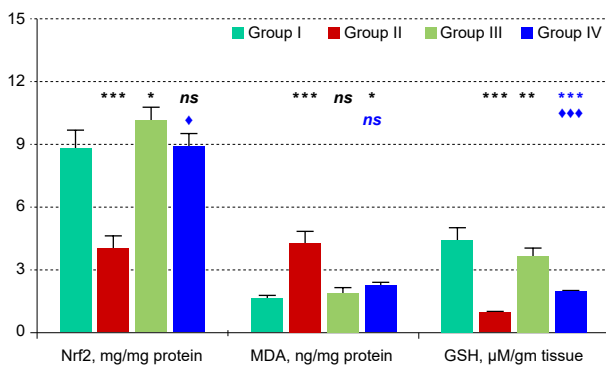


**Fig. 2.** Bone marrow MSCs isolation and viability assessments. MSCs were isolated from the rat’s bones (tibia and fibula) (A&B), maintained to the 3<sup>rd</sup> passage, and left untreated or treated with 10 nM DEX for 24 h. Annexin V/PI dual staining was utilized to access cell viability (C&D). Bar graph “E” demonstrates that DEX-treated MSCs maintained their viability similar to the untreated cells





**Fig. 3.** Effect of treatment of ALF mice with DEX and MSC-secretome on serum liver markers. Mice treatment with DEX or MSC-secretome ameliorated ALT, ALP, GGT, and bilirubin. Significant changes, compared to healthy mice (GpI), are indicated by (\*), where (\*) or (\*\*\*) refer to  $P < 0.05$  or  $P < 0.001$ , respectively; (♦) refers to a significant difference between the indicated groups versus the DEX-treated group (GpIII). Abbreviations: ALF — acute liver failure, ALT — alanine aminotransferase, ALP — Alkaline phosphatase, GGT — gamma-glutamyl transferase, and Bili — total bilirubin



**Fig. 4.** Oxidative stress markers in the liver. Mice in groups III and IV were treated with DEX or MSC-secretome, respectively. The results of Nfr-2, MDA, and GSH are shown as means ( $\pm$  SD) ( $n=6$ ) for each group ( $n=6$ ). Mice in groups II, III, and IV were injected with APAP to develop ALF. Statistical significances between treated groups are indicated as (\*) or (♦) to compare the indicated groups versus control or DEX-treated groups, respectively. Nrf-2: MDA: Malondialdehyde, GSH reduced glutathione

A similar improvement pattern was observed in the angiogenic marker (VEGF) (table 1). Furthermore, these biochemical changes were accompanied by significant resolving of the necrotic and inflammatory changes in the liver tissue of ALF mice (table 1) one week following treatments.

**Table 1.** Mean values of TNF- $\alpha$  and VEGF in liver

Groups	Group I (Control)	Group II (ALF)	Group III (DEX)	Group IV Secretome
TNF- $\alpha$	24.66 $\pm$ 2.51	87.66 $\pm$ 4.5 (***)	21.33 $\pm$ 2.51 (ns)	46.33 $\pm$ 5.1 (***)
VEGF	86.66 $\pm$ 4.04	147.33 $\pm$ 5.5 (**)	71 $\pm$ 6.55 (*)	100 $\pm$ 9.16 (***)
Histological score	0.0	0.5	0.0	0.578

*Note.* \*\*\* —  $P < 0.001$  — significant difference compared to healthy group (Gp1); \*\* —  $P < 0.01$  — significant difference compared to DEX-related group (GpIII)

## Discussion

This work suggests that dexamethasone (DEX) can cure paracetamol-induced liver failure more effectively than transfusion with MSC-secretome. We found that the liver function markers were improved in mice treated with DEX more efficiently compared to both untreated ALF mice and mice transfused twice with MSC-secretome. Also, the pro-inflammatory marker (TNF- $\alpha$ ), the angiogenic (VEGF), and the oxidative stress markers were ameliorated more efficiently in DEX-treated mice. Additionally, both treatment methods resolved hepatic necrosis. APAP-induced ALF is typically background with rapid liver cell injury and oxidative stress. In agreement with previous studies [13], ALF pathogenesis was associated with the development of significant hepatic inflammation (assessed by TNF- $\alpha$ ) and enhanced production of VEGF. The healing effect of MSCs against acute and chronic liver failure was repeatedly reported as cell-based regenerative therapy [21, 23]. Herein, we utilized acellular approach that may offer more advantages over MSC treatment. To avoid transfusion-associated inflammation, we treated MSCs, from which the secretome was collected, with 10 nM DEX for 24 h. This concentration did not induce any morphological or apoptotic changes in MSCs. Although both DEX and MSCs demonstrate anti-inflammatory effects [16, 22], they, however, adopt different mechanisms. The MSC-related anti-inflammatory role is attributed to the cytokines they release, such as IL10, and TGF- $\beta$ , which modulate T cells and macrophages to reduce inflammation and inhibit the production of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 [10]. Glucocorticoids, however, adopt different mechanisms as they activate glucocorticoid receptors (GR) leading to suppression of NF- $\kappa$ B and decreasing the production of TNF- $\alpha$  and IL1 $\beta$  [28]. Although both DEX and MSC-secretome affect the same immune cells, the former (DEX) may target a wide range of other non-immune cells as

**Table 2.** Factors affecting the variability in MSC-secretome composition

Modulatory factors	Secretome components	Description	References
Source	Growth and angiogenic Factors	BM-MSCs, AT-MSC, and UC-MSCs may secrete different levels of growth factors.	[6]
	Cytokines	The cytokine profile differ based on tissue origin.	[12]
	EVs (e.g., exosomes, microvesicles)	EVs-content vary depending on MSC source.	[26]
Culture Conditions and microenvironment	Hypoxic condition	Increase the secretion of angiogenic factors.	[2]
	Media and supplements	Serum-free conditions may alter the secretion of immunomodulatory cytokines.	[15]
	Cell passaging	Increased passaging may lead to reduced secretion of certain factors.	[8]
	Mechanical Stress	Mechanical stimulation may increase the secretion of MMPs.	[21]
	Preconditioning	Chemical preconditioning may modify the MSC secretome towards a specific lineage.	[22]

well. Beside its potent effect, DEX has the privilege as FDA-approved drug, where it is widely prescribed for inflammatory, rheumatoid arthritis, and autoimmune diseases [16]. As the liver is the main xenobiotic metabolizing organ, DEX is mainly converted via CYP3A4 and other CYPs into 6β-hydroxy-dexamethasone (6βOH-DEXA) and 6α-hydroxydexamethasone (6αOH-DEXA) [9]. Accordingly, it is anticipated that the therapeutic effect is implemented by DEX and its metabolites as well. Also, its overdose-related side complications and its involvement in glucose metabolism are well-identified. Although MSC-secretome presented a significant protection against ALF, it is still in the preclinical phase, and its promising therapeutic role is challenged by the relatively complex and costly preparations, unstandardized production, less-optimized delivery protocol, and ethical concerns.

Furthermore, it demonstrates significant variability in the bioactive molecules they include according to the source tissue of MSCs, the culture conditions, stability, and other aspects (table 2). More importantly, MSC-secretome therapy has some concerns about enhancing the proliferation and growth of endogenous cancer cells [26]. These concerns make it difficult to predict their therapeutic outcomes in different diseases.

Conclusively, this work suggests that treatment of ALF with DEX or MSC-secretome resolved hepatic necrosis and improved the liver function. However, DEX demonstrated better outcomes as indicated by the effective amelioration in liver function, inflammatory, and angiogenic markers in addition to the restoration of hepatic architecture. Although MSC-secretome demonstrated good healing effects, it is challenged by many obstacles that are required to be addressed through further research. Standardization of preparation and delivery protocols is essential to ensure their safe and effective application.

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## Дексаметазон незначно перевершує МСК-секретом у вирішенні гострої печінкової недостатності у мишей

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Протизапальну роль мезенхімальних стовбурових клітин (МСК) і глюкокортикоїдів добре описано як у доклінічних, так і в клінічних дослідженнях. Однак неясно, наскільки МСК-секретом забезпечує достатній захист від гострої печінкової недостатності (ГПН) порівняно з глюкокортикоїдами. Для вивчення цього питання у мишей спровокували гостру печінкову недостатність одноразовою токсичною дозою (400 мг/кг) ацетамінофену (АРАР). Потім мишей лікували дексаметазоном або переливали МСК-секретом, отриманий з оброблених DEX мезенхімальних стовбурових клітин кісткового мозку. Результати показали, що 10 нМ дексаметазону не впливають на життєздатність або мезенхімальні характеристики МСК. Хоча переливання МСК-секретом забезпечувало значний терапевтичний ефект проти ГПН, воно було дещо менш ефективним, ніж лікування дексаметазоном. Печінкові маркери (АлАТ, ЛФ, ГГТ і білірубін) вираженіше покращилися у мишей, які отримували дексаметазон, ніж у групі, яка отримувала МСК-секретом. Це покращення супроводжувалося помітним полегшенням окислення, оціненого в печінці як Nrf-2, MDA та GSH. Крім того, нормальні рівні ангіогенних (VEGF) і запальних (TNF- $\alpha$ ) маркерів були ефективно відновлені після лікування дексаметазоном. Крім того, як МСК-секретом, так і дексаметазон усувають некроз печінки. Ці дані свідчать про те, що дексаметазон демонструє кращий терапевтичний ефект, ніж МСК-секретом, у лікуванні ГПН. Необхідні подальші дослідження для стандартизації МСК-секретом як безклітинного терапевтичного підходу.

**Ключові слова:** КМ-МСК, дексаметазон, гостра печінкова недостатність, клітини гепатоми, парацетамол, запалення





## Organisation of dairy cattle herd reproduction

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**GM:** Conceptualization; Project administration; Validation; Writing — original draft, review & editing.  
**PV:** Methodology; Investigation; Data curation.  
**TM:** Formal analysis; Visualization.

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A fairly high hereditary potential of cows of modern Ukrainian breeds in terms of the main traits of milk production is discussed. It was found that higher milk yield, content and amount of fat in milk are inherent in cows of the Ukrainian Black Speckled Dairy breed, which in the context of the four evaluated lactations were better, except for the third (where no clear leader was found for the main traits). In modern high-yielding herds of Ukrainian cattle, the duration of lactation, regardless of the genotype with or without Holstein bloodlines, exceeds the optimal value (305 days), which is associated with later insemination of cows after calving and an extended service period. Therefore, when assessing the efficiency of dairy cows, it is advisable to take into account the number of milk days and adjust their milk production and reproductive capacity accordingly. And the effect of Holsteinisation on the lengthening of the lactation period occurs only in the herd of the Ukrainian Black Speckled Dairy breed. The milk yield reflex of the cows of the studied breeds is within the limits of the accepted optimal indicators, which indicates their good adaptability and adaptation to the technology of machine milking, and the live weight of animals of the three studied breeds at the end of the growing period is within the breed standards. However, it is higher in the Red and Black Speckled Dairy breeds, which indicates their better ability to high growth intensity under appropriate growing conditions and, as previous studies have shown, to better milk production. The degree of development of the main body structure measurements of cows is within the standards and corresponds to the norms of the dairy cow type, and no clear advantage in favour of a certain group of cows was found for the main measurements. The height at the withers, depth and width of the chest are better developed in Ukrainian Black Speckled Dairy cattle, and the oblique length of the body and the girth of the metacarpal — in the Ukrainian Red Dairy breed, with a larger girth of the chest in the Ukrainian Red Speckled Dairy breed. The analysis of the reproductive function of cows gives grounds to assert that among all the studied breeds there is a significant deterioration, which leads, regardless of breed affiliation, to an increase in the duration of service period (128–132 days) and the period between calvings (406–423 days), and this negatively affects the calf yield per year and, as a result, significantly increases the insemination index (6.40–6.59). The analysis of correlations between the main selection traits of mothers and their daughters established high predictions for their inheritance (0.48–1.06), which will significantly increase the efficiency of selection for milk yield and milk fat in these herds of modern breeds.

**Key words:** reproductive function, sexual desire, service period, insemination index, dry period, artificial insemination, milk production, breed



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## Introduction

The intensification of dairy farming is inextricably linked to the mechanisation and automation of labour-intensive processes, the creation of a solid feed base, the acquisition of highly productive cattle, and the use of technology that takes into account the biological characteristics of animals. In this regard, there is a need to evaluate and select animals [27]. Studying the productive, technological and reproductive characteristics of animals of the Ukrainian Black Speckled Dairy, Ukrainian Red Speckled Dairy and Ukrainian Red Dairy breeds of the country for the purpose of its further improvement and rational use is of great scientific and practical importance for the successful conduct of breeding work and dairy business [32].

In modern conditions of cultural cattle breeding, artificial selection plays a major role. When determining the effectiveness of selection, the following main indicators are usually taken into account: the degree of inheritance, selection differential, intergenerational interval, reliability of identifying the best animals, the number of traits for which selection is carried out, the degree of genetic and phenotypic variability of traits and the correlative relationships of traits among themselves. The degree of inheritance of a trait to a certain extent determines the rate of genetic improvement of the population in which this trait is selected. Almost all economically useful traits of dairy and beef cattle are quantitative and have a degree of inheritance sufficient for effective selection, which makes it possible to predict them [5]. However, selection for milk yield should not lead to a deterioration in other traits of milk production. At the same time, it is necessary to control the fat and protein content in milk and other traits at a level that ensures the production of high quality milk and the minimum requirements for technological properties that determine the suitability of cows for use on mechanised farms [17].

Therefore, the aim of our work is to evaluate and predict the organisation of reproduction, selection of cows according to the main economically useful traits among the livestock of modern Ukrainian breeds and to model the efficiency of selection at different intensities, which is very relevant from both a practical and scientific point of view.

## Literature Review

Breeding is an important factor in the intensification of dairy farming. Modern methods of breeding involve the creation of highly productive animals that are well adapted to the least costly production technologies, taking into account objective assessment of their breeding qualities, application of population genetics and automated information systems for managing the breeding process. In particular important is the scientifically based selection of breeds, the direction and pace of increasing their genetic potential [24, 41].

F. Eisner believes that breeding work in cattle breeding is aimed at increasing dairy and meat productivity, improving product quality and reducing its cost of production. The main elements of breeding work are selection, selection, and breeding methods, reproduction techniques, targeted rearing of young animals, zootechnical and breeding and breeding records. In improving the breeding and productive qualities of animals, selection is crucial [11, 12]. That is why dairy cows are evaluated and selected for milk productivity, body type, live weight, intensity of milk production, origin; bulls — for body type, live weight, growth intensity, origin; young animals — for body type, origin [2, 8, 13].

Boning is an organisational measure for selecting animals on farms. According to its results, animals are divided into the following groups: the breeding nucleus, cows of the production cows to be culled and gelded, and a group of repair heifers, young animals for breeding sale, animals for fattening [23, 25].

After boning, breeding farms carry out individual selection, i.e. each cow is matched with a bull sire, taking into account its belonging to a particular line and family. In non-breeding farms, the group selection is used. For based on the genealogy of the herd, 2–3 bulls are selected for the breeding stock and assigned to them for two years [2]. Based on the boning materials, a herd recruitment plan is developed with breeding animals, determine the number of young animals that need to be raised for own needs and for sale to other farms.

On commercial farms, cows, heifers and calves older than 6 months of age. Cows are assessed for their origin, milk production, live weight, appearance, constitution, reproductive capacity and health. The entire herd is divided into three groups: breeding, production for milk production and production for meat production [14].

Every year, in order to improve the health and composition of the herd, we cull animals to the fattening group with subsequent sale for meat. This contributes to the growth of the intensity of reproduction and turnover of livestock, and enables a faster increase in of production. At the same time, the farm replenishes its cow herd with the help of its own production, i.e. growing animals and then transferring them to the appropriate groups.

However, there is a culling of young animals, which leads to a reduction in the number of cows (due to unsatisfactory development of the animal health service, animal care, and in modern conditions often due to lack of funds for the purchase of medicines and biological products) [9, 10].

In agricultural formations producing livestock products, it is necessary to carry out constant reproduction of the herd in order to ensure the number of livestock and rhythmic production of livestock products. This means organising timely replacement of retired animals due to aging, disease or other reasons.

According to the definition of A. S. Vsevykh [38], herd reproduction is the regular replacement of retired animals of the same purpose with younger and more

highly productive ones. There are simple, extended and narrowed reproduction of the herd. It is divided into two parts — adult livestock and young animals of different ages. In this case, the adult breeding stock is classified as to fixed assets, and young stock to current assets [6].

The organisation of herd reproduction is determined by a number of natural (biological) and organisational and economic conditions. Biological conditions include the age of the first mating of sows, the period of their pregnancy, the cyclicity of the sow's heat and the time of its onset after childbirth, fertility and early maturity of animals, their life expectancy and economic use. Organisational and economic conditions include: customer orders for the production and sale of products, terms of their sale, transfer of animals through intra- and inter-farm cooperation, elimination of the stock of the breeding stock and increase of its fertility, timely culling of low-breed and low-productive animals, improvement of breeding qualities, provision of capital facilities, material, technical and labour resources.

In agrarian formations, herd reproduction is carried out in two main ways: by raising their own livestock and young animals and by purchasing them from other farms. A significant part of agrarian formations grows replacement young stock, creating a specialised farm for this purpose, while smallholder farms may not have such a farm [3].

In recent pre-war years, herd reproduction has become quite widespread, which is carried out on the basis of inter-farm cooperation, when specialised farms for rearing heifers or first-born cows, reproductive or other farms, reproductive or other farms. There are also breeding farms to provide agricultural units that producing livestock products with high-quality breeding stock. Hence, deepening specialisation and further development of inter-farm cooperation have an impact on the organisation of livestock reproduction, which leads to the creation of commercial farms for the cultivation of repairing young stock [18, 26].

D. T. Vinnichuk, P. M. Merezko point out that the transfer of livestock to an industrial basis increases the requirements for the reproduction of livestock and herd completion of animals raised by complexes and farms. In these conditions, there is an intensive use of animals and shortening of their service life, and the need for repair of young animals. Since industrial-type farms and complexes have a much higher culling of adult animals with low productivity or unsuitable for industrial technology, and production on such farms and complexes is characterised by fluidity and rhythmicity, then every certain period of time the corresponding number of animals should be culled or sold. Therefore, the same number of them must be introduced into the herd [37].

Therefore, the level of renewal and culling of breeding stock. Given the high costs of breeding them it is in principle beneficial for each farm to use queens for as long as possible. However, this is only true if old queens retain high productivity and reproductive capacity not lower than the average in the herd. Therefore, it is justified, from both

a zootechnical and economic point of view, a level of culling and renewal of the herd, which helps to increase its productivity and improve the quality of the herd.

The main requirements for the correct organisation of herd reproduction are: timely mating of repairing young stock, elimination of milkiness and increase of fertility of queens; complete preservation of offspring; improvement of breeding qualities of animals; timely culling of low-productive and unsuitable for breeding animals [1, 26].

The rational structure of the herd can be established by taking into account certain organisational economic and biological factors. The main ones are the production direction of animal husbandry; the age of young animals sold for meat; the term of productive use of adult animals and the percentage of their annual culling; the number of calvings of sows per year and their fertility per calving; livestock growth rates. The decisive influence on the formation of the herd structure is the production direction of the livestock sector. A corresponding herd structure is characterised for each direction [41].

In the process of herd reproduction, quantitative changes occur in its composition and structure in connection with the receipt and rearing of offspring, the transfer of young animals from younger groups to the older ones, and the sale of young animals from the use part of adult animals. There are also qualitative changes in the composition of the livestock by breed, age and productivity due to the implementation of the breeding plan and the selection of the best animals, culling of low-productive, old and sick animals, and the purchase of breeding animals.

It is necessary to study the structure of the herd, identify the changes that have occurred in it and give them economic assessment, i.e. to show how appropriate changes in the structure of the herd are in terms of increasing the volume of production, rational use of labour and feed resources and maximising profits. To do this, the actual level of the above indicators are compared with the calculated level that would have occurred under all actual conditions, but with the planned (basic) structure of the herd [34]. It is also necessary to study the breed composition for each group of animals, to determine the proportion of each breed in the total livestock to establish changes in the breed composition of the herd compared to the plan and data of previous years. When determining the economic efficiency of different breeds of animals, the following should be taken into account productivity, feed and labour costs per 1 animal and 1 tonne of production, costs and profit per 1 animal and 1 tonne of production. Analysis of the breed composition of the herd and calculation of the impact of this factor on the results of economic activity is carried out for each group and type of animals with the subsequent generalisation of the analysis results [40].

Another method of selection and breeding work when planning the structure of the herd is the timely introduction of first-born cows into the herd. It has been established that the selection of first-born cows by their own

productivity is about twice as effective as selection by origin. Therefore, for reproduction of the herd, 78–82% of the received heifers are used in order to 100 cows to raise at least 34–35 first-born heifers. Breeding work with the herd is based on the principles of inter-farm specialisation. For the rational use of the obtained the firstborn cows, it is better to test them in control barns or control groups. To organise them, the oldest groups of cows should be fully or half disbanded groups of cows into milkers and put heifers in the vacant places [20, 32].

The question of whether to use the first-born heifer to repair the main herd should be decided before she is inseminated (during the first 2–3 months of lactation). Up to 30% of firstborns are subject to rejection and culling, this ensures the introduction of the most highly productive animals into the main herd. When using the first-born evaluation system based on their own productivity, there should be an increase in the repair herd of heifers — 85–90 average annual animals for every 100 cows. The service life of a highly productive cow should be at least 6 lactations, and cows with record milk production — up to 8 lactations. The most optimal age structure of the dairy herd can be as follows: first-born — 21–22%, the second calving — 18–19, the third — 16–17, the fourth — 14–15, the fifth and older — 27–32%. It is desirable to carry out targeted recruitment of the best herds with the most productive first-born cows [28].

Thus, selection methods in planning the structure of the animal herd should be aimed at creating dairy herds that would have high productivity, good health strong constitution, suitable for machine milking, adapted to the conditions of and the accepted technology of milk production, and to pass on their qualities to their offspring in a sustainable manner.

Therefore, the goal of the research was: to determine the effectiveness of selection of dairy cows at different intensities. To achieve this goal, the following tasks were set: to assess the hereditary potential of female cattle ancestors; to conduct a comparative analysis of the milk production of cows of different breeds — Ukrainian Red Dairy (URD), Ukrainian Black Speckled Dairy (UBSD) and Ukrainian Red Speckled Dairy (URSD); to study the peculiarities of growth and development of females and their relationship with milk production; to evaluate the reproductive traits of cows; to investigate the level of inheritance of the main traits of animal selection; to model the selection effect and evaluate its implementation in cattle herds.

## Materials and Methods

The research was carried out on the basis of “Kolos 2011” LLC located in the Matrosivka village, Ochakiv district, Mykolaiv region in the period 2020–2024. Object of research: analysis of the dynamics of selection traits under the influence of different selection pressures. Subject of research: the degree of inheritance of traits at different selection intensity in dairy cows.

The study analysed the selection and breeding work on the farm, breeding and reproduction of the herd, veterinary and sanitary conditions, mechanisation of production processes, organisation and remuneration of labour. The materials used in this study were production activities, zootechnical, production and accounting records kept on the farm. The study of the main selection traits was based on the methods generally accepted in zootechnology [7, 22, 40].

The formula [4] was used to determine the level of inheritance of selection traits:

$$h^2 = 2 \times r \quad (1),$$

where  $h^2$  is inheritance rate;

$r$  is a correlation between mother and daughter traits.

The following formulas were used to model the selection effect [3, 11, 12]:

$$SE = Sd \times h^2 \quad (2),$$

where  $SE$  is a selection effect;

$Sd$  is a selection differential.

$$Sd = X_{bc} - X_{hr} \quad (3),$$

where  $X_{bc}$  is the average productivity of animals of the breeding nucleus;

$X_{hr}$  is the average productivity of the herd.

$$X_{bc} = X_{hr} + \delta \times i \quad (4),$$

where  $i$  is an intensity of selection (15, 30, 45%).

$i = 1,5486, u = 1,04; i = 1,1617, u = 0,52; i = 0,8791, u = 0,13$  respectively.

$$X_u = X_{hr} + u \times \delta \quad (5),$$

where  $X_u$  is a breeding limit.

The indicators of the investigated traits were determined according to generally accepted algorithms of variation statistics in animal husbandry. The average values of the females of the herd were taken as the control group in the experiment without determining the breed.

At the final stage of the research, conclusions and proposals were drawn up, including suggested measures.

## Results and Discussion

The genetic potential is a set of genetic information carriers that determine the ability of animals to produce products under certain conditions of feeding, housing, use, etc. This indicates the relevance of research aimed at a comprehensive solution to the problem of



**Table 1.** Milk yield of female ancestors of cows (for 305 days, kg) for higher lactation

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
Mothers						
URD	30	5405±313.0	1713	31.7	-203±368	0.55
UBSD	30	5656±425.0	2328	41.2	48±467	0.10
URSD	30	5762±254.6	1394	24.2	154±320	0.48
On average	90	5608±193.8	1838	32.8	×	×
Mothers of mothers						
URD	30	4578±160.3	878	19.2	-1143±248	4.6***
UBSD	30	7072±363.0	1988	28.1	1351±410	3.29**
URSD	30	5511±255.3	1398	25.4	-210±318.2	0.65
On average	90	5721±189.9	1802	31.5	×	×
Mothers of fathers						
URD	30	8712±260.1	1424	16.4	-865±343	2.52*
UBSD	30	10180±309.0	1693	16.6	603±381	1.58
URSD	30	9840±505.8	2770	28.1	263±553	0.47
On average	90	9577±223.4	2120	22.1	×	×

**Table 2.** Fat content in milk (%) of female progenitors of cows for higher lactation

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
Mothers						
URD	30	3.76±0.032	0.17	4.73	-0.01±0.035	0.28
UBSD	30	3.75±0.026	0.14	3.73	-0.02±0.030	0.67
URSD	30	3.80±0.015	0.08	2.22	0.03±0.02	1.5
On average	90	3.77±0.015	0.14	3.70	×	×
Mothers of mothers						
URD	30	3.72±0.030	0.17	4.44	-0.04±0.037	1.08
UBSD	30	3.83±0.058	0.32	8.24	0.07±0.062	1.13
URSD	30	3.73±0.023	0.13	3.48	-0.03±0.032	0.93
On average	90	3.76±0.023	0.22	5.90	×	×
Mothers of fathers						
URD	30	4.31±0.060	0.33	7.69	0.10±0.075	1.33
UBSD	30	4.36±0.102	0.56	12.81	0.15±0.111	1.35
URSD	30	3.97±0.049	0.27	6.78	-0.24±0.047	5.10***
On average	90	4.21±0.046	0.44	10.37	×	×

**Table 3.** Amount of milk fat (kg) of female progenitors of cows during higher lactation

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
Mothers						
URD	30	204±12.3	67.2	32.9	-7±14.3	0.48
UBSD	30	212±16.2	88.9	41.9	1±17.8	0.05
URSD	30	218±9.3	50.8	23.2	7±11.9	0.59
On average	90	211±7.4	70.2	33.2	×	×
Mothers of mothers						
URD	30	170±6.2	33.8	19.8	-46±9.7	4.74***
UBSD	30	271±14.5	79.4	29.3	55±16.3	3.37**
URSD	30	206±9.8	53.5	25.9	-10±12.3	0.81
On average	90	216±7.5	71.5	33.1	×	×
Mothers of fathers						
URD	30	376±13.4	73.2	19.4	-31±17.9	1.73
UBSD	30	450±23.3	127.9	28.4	43±26.2	1.64
URSD	30	393±21.9	120.1	30.5	-14±24.9	0.56
On average	90	407±11.9	113.0	27.8	×	×

accelerating the pace of genetic progress in dairy cattle breeding through theoretical substantiation and practical implementation of methodological principles for evaluating and selecting animals by a set of traits and creating an improved breeding system on this basis [16, 21]. Therefore, the genetic potential of cows of modern Ukrainian breeds created with the involvement of the world's best gene pool was studied and their influence on the degree of its implementation in dairy cattle herds was established. Thus, having assessed the hereditary potential of cows of the experimental stock in terms of milk yield, it should be noted that mothers have a milk yield of at least 5000 kg. Mothers of URSD cows have a higher value — 5762 kg (table 1).

The difference with the control group is 154 kg. At the same time, Ukrainian red dairy ancestors are characterised by the lowest milk yields. The difference in favour of the control values is 203 kg of milk. The mothers of the mothers are characterised by slightly lower productivity indicators with a fairly wide range of variation from the control group — from 210 to 1351 kg. In this generation of ancestors, the highest values of milk yield are inherent in the UBSM cattle — 7072 kg ( $P>0.99$ ) while its lowest values are in the URSD breed — 4578 kg. They are significantly inferior to the control data by 1143 kg ( $P>0.999$ ). The highest values of the hereditary potential for milk yield are characterised by mothers of fathers whose level of trait development reaches more than 10000 kg of milk. The female ancestors of the URD breed have lower milk yields — 8712 kg, which are 865 kg less than the control animals with a probability of  $P>0.95$ . The mothers of the fathers of the UBSM breed have the highest productivity indicators — 10180 kg, which exceed the control values by 603 kg. Among the last two generations of female ancestors, another Ukrainian dairy breed (Red Speckled) occupies an intermediate place in terms of milk yield development.

When assessing the hereditary potential for fat content in milk, we observe a fairly high level of its manifestation. Mothers of the URSD breed have a milk fat content of 3.80%, which is the highest advantage over the control group of animals — 0.03%. The fat content of mothers of the other two breeds (URD and UBSM) does not differ significantly from each other — 3.76 and 3.75%, respectively (table 2).

The trend of fat content in milk among mothers has changed somewhat. Thus, female progenitors of the UBSM breed are characterised by higher values — 3.83%, which is 0.07% higher than the control indicator. The fat content of the URD and URSD breeds also does not differ significantly (3.72 and 3.73%). Mothers of fathers of the experimental groups of cows have the highest fat content in milk — 3.97–4.36%. The largest fluctuation from the control data was observed in the mothers of fathers of URSD breed by 0.24% in favour of the former with a reliability of  $P>0.999$ . The other two experimental groups of female ancestors, on the contrary, exceed the control group by 0.10 and 0.15%, although the difference is not significant.

The female ancestors are also characterised by a fairly high hereditary potential in terms of the amount of milk fat (table 3). For mothers, the highest amount of milk fat is inherent in URSD cows — 218 kg, which is 7 kg more than the control data. A similar trend is observed among the mothers of the URD breed. but they are already inferior to the control indicators. Among the mothers, a higher level of development of this trait is already characteristic of the UBSD breed — 271 kg which is significantly ( $P>0.99$ ) higher than the control value by 55 kg. Similarly to the previous group of ancestors, the lowest level of development of the trait is inherent in the ancestors of the URD breed — only 170 kg of milk fat which is a difference of 46 in favour of control animals ( $P>0.999$ ). A similar trend is observed among the mothers of the fathers. Thus, representatives of the URD breed are characterised by lower values of milk fat — 376 kg compared to the other two breeds — UBSD (450 kg) and URSD (393 kg) but this difference is not significant.

Characterising the milk yield of cows of different breeds during the first lactation, we note that it is quite high for first-calf cows — from 4974 to 6210 kg of milk. and the maximum is observed in UBSD cows — 6210 kg; they exceed the control group by 743 kg with a significant difference ( $P>0.999$ ). The lowest milk yield is characteristic of URSD cows (4974 kg). Their difference with the control group in favour of the latter is 493 kg. The trend of milk yield for the second lactation has changed and the cows of URD cattle are characterised by lower milk yields — only 5964 kg. They are 383 kg lower than the control data (table 4).

The other two experimental groups of cows (URSD and UBSD) exceeded the control group by 258 and 641 ( $P>0.95$ ) kg of milk, respectively. The data of the third lactation confirm that again no clear advantage in milk yield was found. Thus, the maximum milk yield was 7124 kg, with an advantage over the control group of 676 kg of milk. The other two experimental groups of cows are inferior to the control data. The minimum advantage is observed in UBSD cattle — only 31 kg. The analysis of higher lactation showed that the URSD cattle again had the lowest milk yield, which reached a level of only 6978 kg. The maximum milk yield of the UBSD breed is 7593 kg, which is 301 kg higher than the control values. The other experimental group of cows occupies an intermediate place, but exceeds the indicator of the control group.

Our comparative analysis of the fat content in milk suggests that during the first lactation, it varies from the control group by  $\pm 0.01\%$ . UBSD cows have the highest fat content in milk (3.81%). Two other Ukrainian breeds have the same level of development of this trait within 3.79% (table 5). Indicators of the second lactation in terms of fat content in milk, compared to the first slightly, decreased (3.73–3.80%). But its maximum manifestation is also inherent in UBSD cows — 3.80% with an advantage over control animals by 0.04%. On the contrary, URSD cattle have the lowest fat content in milk — 3.73%. The fat content in milk during the third lactation changed its direction again. Thus, UBSD cows have the lowest fat content (3.79%), which

**Table 4.** Milk yield in cows of different breeds for 305 days of lactation, kg

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X}\pm S_x$	$\sigma$	$C_v$	$d\pm S_d$	$t_d$
First lactation						
URD	30	5216 $\pm$ 258.2	1415	27.1	-251 $\pm$ 290.6	0.86
UBSD	30	6210 $\pm$ 153.4	841	13.5	743 $\pm$ 203.3	3.65**
URSD	30	4974 $\pm$ 210.8	1150	23.1	-493 $\pm$ 249.5	1.97
On average	90	5467 $\pm$ 133.4	1266	23.1	x	x
Second lactation						
URD	30	5964 $\pm$ 264.1	1444	24.2	-383 $\pm$ 309.5	1.24
UBSD	30	6988 $\pm$ 257.5	1410	20.2	641 $\pm$ 303.9	2.11*
URSD	30	6089 $\pm$ 288.4	1575	25.9	-258 $\pm$ 330.5	0.78
On average	90	6347 $\pm$ 161.4	1532	24.1	x	x
Third lactation						
URD	30	7124 $\pm$ 352.6	1928	27.1	676 $\pm$ 432.3	1.56
UBSD	30	6417 $\pm$ 479.5	2626	40.9	-31 $\pm$ 540.8	0.06
URSD	30	5802 $\pm$ 439.1	2403	41.4	-646 $\pm$ 505.4	1.28
On average	90	6448 $\pm$ 250.2	2374	36.8	x	x
The highest lactation						
URD	30	7304 $\pm$ 305.4	1673	22.9	12 $\pm$ 258.2	0.05
UBSD	30	7593 $\pm$ 257.8	1412	18.6	301 $\pm$ 153.4	1.96
URSD	30	6978 $\pm$ 269.5	1475	21.1	-314 $\pm$ 210.8	1.49
On average	90	7292 $\pm$ 161.1	1528	20.9	x	x

**Table 5.** Fat content in milk (%) of cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X}\pm S_x$	$\sigma$	$C_v$	$d\pm S_d$	$t_d$
First lactation						
URD	30	3.79 $\pm$ 0.023	0.13	0.35	-0.01 $\pm$ 0.026	0.38
UBSD	30	3.81 $\pm$ 0.019	0.10	2.73	0.01 $\pm$ 0.023	0.43
URSD	30	3.79 $\pm$ 0.025	0.14	3.60	-0.01 $\pm$ 0.028	0.36
On average	90	3.80 $\pm$ 0.013	0.12	3.22	x	x
Second lactation						
URD	30	3.76 $\pm$ 0.024	0.13	3.51	0	0
UBSD	30	3.80 $\pm$ 0.021	0.12	3.08	0.04 $\pm$ 0.028	1.43
URSD	30	3.73 $\pm$ 0.026	0.14	3.87	-0.03 $\pm$ 0.029	1.03
On average	90	3.76 $\pm$ 0.014	0.13	3.55	x	x
Third lactation						
URD	30	3.81 $\pm$ 0.022	0.12	3.18	-0.01 $\pm$ 0.025	0.40
UBSD	30	3.79 $\pm$ 0.024	0.13	3.52	-0.03 $\pm$ 0.027	1.11
URSD	30	3.85 $\pm$ 0.024	0.13	3.39	0.03 $\pm$ 0.027	1.11
On average	90	3.82 $\pm$ 0.013	0.13	3.38	x	x
The highest lactation						
URD	30	3.79 $\pm$ 0.022	0.12	3.15	0.01 $\pm$ 0.026	0.38
UBSD	30	3.77 $\pm$ 0.021	0.12	3.09	-0.01 $\pm$ 0.025	0.40
URSD	30	3.77 $\pm$ 0.031	0.17	4.46	-0.01 $\pm$ 0.034	0.29
On average	90	3.78 $\pm$ 0.014	0.13	3.59	x	x

is 0.03% less than the control data. At the same time, the URSD cattle of the same age showed the maximum fat content — 3.85% and their advantage over the control values is 0.03%. The comparative characterisation of this trait for higher lactation again does not give unambiguous results. The two Ukrainian speckled dairy breeds, Black and Red, have the same fat content of 3.77%, which is the lowest compared to URD cattle (3.79%).

We also evaluated the amount of milk fat in cows of the experimental groups (table 6). A higher amount of milk fat during the first lactation was observed in UBSD cows (237 kg), which is 29 kg higher than the control data with a significant difference ( $P>0.99$ ). The other two groups of animals have no significant difference between them and are characterised by the amount of milk fat in the range of 190–198 kg. A similar trend is observed in the second

**Table 6.** Amount of milk fat (kg) in cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X}\pm S_x$	$\sigma$	$C_v$	$d\pm S_d$	$t_d$
First lactation						
URD	30	198±10.5	57.4	28.9	-10±11.8	0.85
UBSD	30	237±6.0	33.1	13.9	29±8.1	3.58**
URSD	30	190±8.7	47.6	25.1	-18±10.2	1.76
On average	90	208±5.4	50.9	24.5	x	x
Second lactation						
URD	30	224±9.6	52.6	23.5	-15±11.3	1.33
UBSD	30	265±9.7	52.9	19.9	26±11.4	2.28*
URSD	30	227±10.4	57.3	25.3	-12±12.0	1.0
On average	90	239±6.0	57.0	23.9	x	x
Third lactation						
URD	30	271±13.2	72.2	26.7	26±16.2	1.60
UBSD	30	242±17.9	98.2	40.5	-3±20.2	0.15
URSD	30	222±16.5	90.4	40.7	-23±18.9	1.22
On average	90	245±9.4	88.9	36.3	x	x
The highest lactation						
URD	30	277±11.6	63.5	22.9	2±13.1	0.15
UBSD	30	287±9.7	53.4	18.6	12±11.4	1.05
URSD	30	263±10.4	56.9	21.7	-12±12.0	1.00
On average	90	275±6.1	58.3	21.1	x	x

**Table 7.** Dynamics of lactation duration (days) in cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X}\pm S_x$	$\sigma$	$C_v$	$d\pm S_d$	$t_d$
First lactation						
URD	30	330±11.0	60.3	18.3	-4±12.9	0.31
UBSD	30	348±11.4	62.4	17.9	14±13.3	1.05
URSD	30	325±12.8	69.9	21.5	-9±14.5	0.62
On average	90	334±6.8	64.4	19.2	x	x
Second lactation						
URD	30	342±15.7	85.9	25.1	3±17.5	0.17
UBSD	30	349±13.7	74.9	21.4	10±15.7	0.63
URSD	30	326±10.4	56.9	17.4	-13±12.9	1.00
On average	90	339±7.7	73.3	21.6	x	x
Third lactation						
URD	30	354±18.2	99.5	28.1	40±21.3	1.88
UBSD	30	315±19.5	107.3	33.9	1±22.5	0.04
URSD	30	274±18.3	100.5	36.6	-40±21.4	1.87
On average	90	314±11.2	106.4	33.8	x	x
The highest lactation						
URD	30	360±13.6	74.4	20.6	10±15.3	0.65
UBSD	30	357±13.5	73.8	20.7	7±15.2	0.46
URSD	30	334±7.9	43.1	12.9	-16±10.5	1.52
On average	90	350±6.9	65.7	18.7	x	x

lactation. UBSD cows have a higher manifestation of this trait — 265 kg with a probable advantage over the control data of 26 kg ( $P>0.95$ ). The other two experimental groups of cows do not have a significant difference between them and are inferior to the control indicator by 15 and 12 kg of milk fat. The degree of manifestation of the amount of milk fat in the third lactation has changed slightly and the best cows were URD breed — 271 kg. The other two breeds have lower values of milk fat and are inferior to the control data. Analysing the highest lactation by the level of development of this trait, we note again the natural tendency of the UBSD cows to be superior — 287 kg. At the same time, the minimum amount of milk fat is inherent in URD cows (263 kg).

Cows of Ukrainian Black Speckled and Red Speckled dairy breeds are characterised by high genetic potential in terms of milk production and reproductive capacity. However, in herds that currently have a high proportion of heredity for the Holstein breed, there is a deterioration in the main indicators of reproductive capacity.

One of the most significant indicators that affects the level of productivity and reproductive capacity of cows is the duration of lactation or the number of milk days. In particular, the number of lactation days has a 21.1% influence on milk yield, and the amount of milk fat — 19.7% [36]. It has been established that the most economically profitable animals are those that lactate for 305 days and give birth to one calf per year. To ensure such a long lactation period, it is necessary to inseminate cows after calving in the third heat. This makes it possible to increase the service period to 60 days and the lactation period during pregnancy to 245 days. Even a slight improvement in reproductive performance leads to an increase in milk production in cows, therefore, it is necessary to strive to maintain the optimal periodicity of pregnancy in cows, which in turn will contribute to the growth of productivity in the herd [25]. Therefore, the research was aimed at conducting a comparative analysis of the duration of lactation in cows bred with and without the Holstein breed. Thus, the dynamics of the duration of the first lactation, based on the data in table 7, slightly exceeds the generally accepted norms — 305 days. Speaking in terms of breeds, it should be noted that URSD cows have the lowest number of milk days (325 days). At the same time, representatives of the URD breed do not have Holstein blood in their genotype and do not differ in its lowest manifestation — 330 days. And accordingly, the longest lactation is characteristic of UBSD cows (348 days). A similar trend is observed in the context of the second lactation, where the largest number of milk days is observed in UBSD cattle — 349 days, and the smallest (326 days) — in cows of another Ukrainian breed, in the creation of which Holsteins took part. The data of the third lactation in terms of its duration are somewhat different from the above trends. Namely, the cows bred by Holstein sires have the most optimal lactation duration of 274 and 315 days, which is almost within the zootechnical standards. At the same time, URD cows have the longest lactation period — 354 days, which is significantly higher than the accepted norms and is not



economically viable. The analysis of higher lactation also suggests that milk production in this farm during this period is economically unprofitable, as the number of milking days is almost one year.

In production conditions, animals are forced to adapt to new conditions with the stress of their physiological systems, which subsequently leads to poor health and the development of stress. It is this condition that negatively affects productivity and product quality, causing large losses to economic activity [7]. In cattle breeding, some animals have the ability to quickly adapt to the latest technologies and conditions, others are slower or not capable of such adaptation at all. In the industry, up to 30% of highly productive cows are culled annually for this reason, thereby causing losses to the farm, both the failure to obtain a significant amount of milk from them and the failure to select young animals of breeding value [40]. Machine milking of lactating animals is now almost completely mechanised, but not all farms have a high level of cow productivity. That is why, in order to obtain high milk production in this way, knowledge of the biological basis of lactation function and the ability to use it in dairy farming practice is necessary [42]. Milking is a complex biotechnological process where, with the help of a machine, human influence is directed to the living organism of cows, and the value of productivity per lactation depends on the fullness of interaction between them [3]. Therefore, the purpose of our research was to trace the degree of adaptation of high-yielding cows to the technology of machine milking, depending on their breed. Thus, it should be noted that in the context of the studied lactations, the intensity of milk production of all breeds without exception is in the optimal range of 1.83–2.01 kg/min. (table 8). The maximum intensity of milk production in UBSD cows is also noteworthy, which in the context of all studied lactations was 1.96–2.02 kg/min. Other first-born cows had the intensity of milk production at the level of 1.83–1.87 kg/min, which is 0.05–0.01 kg/min lower than that of UBSD cows. Regarding the intensity of milk production in experimental cows with two calvings, it was practically at the same level in Ukrainian Red Speckled and Red dairy animals — 1.81 and 1.85 kg/min. And as already noted, the best intensity of milk production is inherent in UBSD cows — 2.01 kg/min. The data on milk production of cows at an older age after the third calving do not differ significantly.

According to the best practices, intensive growth and development of repair heifers largely determines the desired body type of adult animals and, as a result, allows to maximise the hereditary potential of the subsequent milk production of cows [7, 10]. From the production point of view, the early maturity of repair heifers reduces the unproductive period of growing from birth to calving. From the selection point of view, it accelerates the process of evaluating bulls by the quality of offspring and promotes intensive reproduction of the herd, which ultimately significantly determines the level of profitability of dairy farming [22, 40]. In addition, it was found that the value of live weight of heifers at the end of the growing

**Table 8.** Dynamics of milk production rate (kg/min) in cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
First lactation						
URD	30	1.83±0.015	0.08	4.44	-0.05±0.019	2.63'
UBSD	30	1.96±0.031	0.17	8.63	0.08±0.034	2.35'
URSD	30	1.87±0.011	0.06	3.35	-0.01±0.017	0.59
On average	90	1.88±0.013	0.13	6.69	x	x
Second lactation						
URD	30	1.81±0.015	0.08	4.62	-0.08±0.022	3.64**
UBSD	30	2.01±0.035	0.19	9.56	0.12±0.038	3.16**
URSD	30	1.85±0.009	0.05	2.61	-0.04±0.018	2.22'
On average	90	1.89±0.016	0.15	7.98	x	x
Third lactation						
URD	30	1.85±0.010	0.05	3.02	-0.05±0.019	2.63'
UBSD	30	2.01±0.037	0.20	10.18	0.11±0.040	2.75**
URSD	30	1.84±0.017	0.09	5.25	-0.06±0.023	2.60'
On average	90	1.90±0.016	0.15	8.16	x	x
The highest lactation						
URD	30	1.85±0.011	0.06	3.23	-0.06±0.019	3.15**
UBSD	30	2.02±0.037	0.20	10.03	0.11±0.039	2.82**
URSD	30	1.86±0.010	0.05	2.80	-0.05±0.018	2.77**
On average	90	1.91±0.015	0.15	7.74	x	x

period and the beginning of the mating period is positively correlated with the subsequent milk production for the first and other lactations [35, 39].

According to M. Zubets and co-authors [43], an integral part of the advanced selection of dairy cattle is the evaluation of breeding animals at an early age and at different stages of their individual development. In this case, the main method of morphological studies of animal growth involves recording live weight. The results of these observations are indicators of animal growth and development, which characterise the intensity of metabolic processes occurring in the body. Therefore, taking into account the relevance of this issue, we studied the features of growth and development of heifers and their ability to high growth intensity under appropriate growing conditions, and how the latter affects their productivity. Thus, having carried out (table 9) an assessment of live weight at birth, it should be noted that cows in the genotype of which there is the presence of Holstein blood are distinguished by a higher live weight at birth — UBSD (30.9 kg), URSD (33.8 kg).

Moreover, the latter significantly exceed the control values by 2.7 kg ( $P > 0.99$ ), compared to URD cattle (only 28.4 kg), which are inferior to the control group at the third level of probability ( $P > 0.999$ ). The dynamics of live weight at three months has changed somewhat. there is an unusual variability in the growth and development of cows originating from the Holstein breed — heifers of the URD group, on the contrary, they are characterised by the highest live weight, which reaches 88.9 kg, compared to two other Ukrainian Black Speckled and Red Speckled breeds — 82.9 and 85.6 kg, respectively. By the way, UBSD cattle, unlike the previous period, has the lowest live weight. A similar trend is observed in the following age

periods at the age of six and nine months: URD heifers exceed their URSD and UBSD peers in terms of live weight development, and the latter generally have the lowest live weight in the above two age periods. At twelve months of age, there were also changes in the growth and development of heifers. Although URD cattle remained the leader in terms of live weight — 228.6 kg among the animals of the other two Ukrainian breeds, there was a rotation. That is, the UBSD cattle of the same age grew and gained weight slightly better than their Red Speckled counterparts — 209.3 and 207.4 kg, respectively. A similar trend is observed at the age of fifteen months — the advantage remains in favour of the URD breed with 265.1 kg. However, the UBSD cattle of the same age as

the URSD cattle used feed better and the difference in weight between them and the representatives of the first group decreased to 10 kg. The level of live weight development at the age of eighteen months has a similar manifestation to the previous age period. At the end of the growing period, at the age of twenty-four months, the live weight of URD and UBSD cows almost levelled off and amounted to 326.1 and 325.7 kg, respectively. At the same time, the URSD cattle of the same age were significantly inferior to them in terms of live weight development (only 287.9 kg,  $P>0.99$ ), that is within 30 kg less than the above-mentioned breeds.

In the process of ontogenesis, hereditary transmission and variability of maternal traits are carried out as a result of genotype and environmental conditions. During growth and development, an animal acquires not only breed and species characteristics, but also peculiar features of constitution, appearance and productivity [25, 41]. Therefore, the study of individual animal development is of great scientific and practical importance, as it allows for the selection and cultivation of the most valuable individuals — the fathers of the next generations.

In all countries of the world of intensive livestock production, the assessment of the appearance and constitution of animals is used. In the context of modern housing technologies that require standardisation of animals by key indicators, a comprehensive assessment of dairy cattle is required, in which body type assessment and selection is becoming increasingly important. Evaluation of body type is included as a component of all breeding programmes when improving existing and creating new types and breeds [41]. For the successful operation of animals in industrial technology, dairy cows must be distinguished by the appropriate exterior type: strong body structure, developed body, strong hooves and correct limb position, appropriate morphological and functional properties of the udder. Animals with well-developed traits are usually characterised by higher productivity and a longer service life [42].

Since breeds and intrabreed types of dairy cattle in Ukraine have certain differences in appearance due to the use of a multi-breed maternal basis in the process of their creation and different options for selection and selection even within individual breeding herds, the study of cows of modern URD, URSD and UBSD breeds to determine the main traits of the exterior and their influence on milk production are considered relevant from both scientific and practical points of view, which was the purpose of our research. Thus, the assessment of the height at the withers (table 10) shows that its highest value is observed among UBSD cows — 134 cm and their superiority over the control data by 2 cm with a significant difference ( $P>0.99$ ). URD cattle, on the contrary, are inferior to all experimental groups in this respect (131 cm) and URSD breed peers have similar data on withers to control animals — 132 cm.

Oblique length has slightly different characteristics. Thus, URD cows have the highest development of

**Table 9.** Dynamics of live weight (kg) of heifers, heifers and cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X}\pm S_x$	$\sigma$	$C_v$	$d\pm S_d$	$t_d$
At birth						
URD	30	28.4±0.25	2.86	10.1	-2.7±0.54	5.0***
UBSD	30	30.9±0.83	4.55	14.7	-0.2±0.96	0.21
URSD	30	33.8±0.83	4.53	13.4	2.7±0.96	2.81**
On average	90	31.1±0.48	4.58	14.8	x	x
3 months						
URD	30	88.9±2.05	11.23	12.6	3.1±2.47	1.25
UBSD	30	82.9±2.74	15.03	18.1	-2.9±3.07	0.94
URSD	30	85.6±2.25	12.33	14.4	-0.2±2.64	0.07
On average	90	85.8±1.38	13.05	15.2	x	x
6 months						
URD	30	150.1±4.55	24.90	16.6	13.6±5.20	2.61*
UBSD	30	126.5±4.30	23.54	18.6	-10.0±4.98	2.0
URSD	30	132.8±2.97	16.27	12.25	-3.7±3.89	0.95
On average	90	136.5±2.52	23.88	17.5	x	x
9 months						
URD	30	182.3±3.97	21.73	11.9	9.9±4.96	1.99
UBSD	30	164.2±6.19	33.91	20.6	-8.2±6.86	1.19
URSD	30	170.8±4.64	25.43	14.9	-1.6±5.51	0.29
On average	90	172.4±2.97	28.20	16.3	x	x
12 months						
URD	30	228.6±5.95	32.61	14.3	13.5±7.18	1.88
UBSD	30	209.3±7.80	42.71	20.4	-5.7±8.78	0.65
URSD	30	207.4±6.54	35.83	17.3	-8.0±7.68	1.04
On average	90	215.1±4.02	38.10	17.7	x	x
15 months						
URD	30	265.1±6.86	37.60	14.2	13.8±8.34	1.65
UBSD	30	254.4±10.11	55.36	21.8	3.1±11.17	0.28
URSD	30	234.5±6.45	35.34	15.1	-16.8±8.01	2.10*
On average	90	251.3±4.75	45.05	17.9	x	x
18 months						
URD	30	299.9±8.16	44.72	14.9	16.6±9.85	1.68
UBSD	30	289.8±11.95	65.44	22.6	6.5±13.16	0.49
URSD	30	260.3±6.46	35.39	13.6	-23±8.50	2.70*
On average	90	283.3±5.52	52.35	18.5	x	x
24 months						
URD	30	326.1±8.47	46.39	14.2	12.9±10.32	1.25
UBSD	30	325.7±12.98	71.07	21.8	12.5±14.26	0.88
URSD	30	287.9±6.92	37.89	13.2	-25.3±9.09	2.78**
On average	90	313.2±5.90	56.03	17.9	x	x

160 cm and significantly exceed the control group by 2 cm ( $P>0.95$ ). And representatives of two other Ukrainian breeds, Black Speckled and Red Speckled, have the same degree of oblique body length development — 157 cm, which is probably due to their common origin.

The degree of development of the chest, which is characterised by the depth of the chest and its width, is better in UBSD cattle — 73 and 46 cm, respectively. Another dairy breed, the URSD, is also characterised by good chest development: 72 and 45 cm, respectively. Slightly smaller measurements are inherent in URD cattle — 71 and 44 cm, respectively. The first two breeds, regarding the research, are potentially capable of producing more milk because their rib cage is somewhat better developed compared to URD cattle. A somewhat different trend is observed in the development of the chest girth, where a better degree of development is observed in URSD cows (206 cm), while its values are worse in UBSD cows (203 cm). Its differences with the control group are within  $\pm 1$ –2 cm.

No significant difference in the level of development of the metacarpal girth was found; its value in cows of the experimental groups is in the range of 19.5–19.9 cm with fluctuations from the control values at the level of 0.1–0.3 cm.

The degree of development of the width of the hind-quarters in maklaks among cows of all experimental breeds has the same value — 51 cm and no absolute difference between them was found.

In the context of intensification and specialisation of dairy farming on an industrial basis, high productivity and regular reproduction of animals determine the profitability of breeding farms. The high intensity of animal selection, which is the basis for the genetic progress of the herd, places high demands on the reproductive function of animals [1]. Increasing the level of reproductive function in cattle breeding has always been problematic and is currently of great practical and scientific interest, especially to highly productive animals and animals of new genotypes, since reproductive disorders, primarily in cattle, reduce the period of economic use, reduce the level of milk production, and at the same time the profitability of the industry as a whole [7].

The level of reproductive capacity of cows is significantly influenced by the proportion of heredity for the improving breed. With the increase of conditional bloodlines in the Holstein breed, the reproductive capacity of cows improves [15]. But there are also opposing statements [29, 41]. Therefore, we set out to investigate the level of reproductive capacity of cows that were created using the best world gene pool under the conditions of a given farm.

When making a comparative assessment of the duration of the service period, it should be noted that among all the studied groups it is significantly extended — 128–132 days, which will have a negative impact on the profitability of milk production. The lowest number of days from calving to fertile insemination is spent by URSD dairy cows — 128 days, and the highest — by the peers of the other group — UBSD — 132 days (table 11). The variation from the control group is  $\pm 2$  days.

**Table 10.** Linear measurements (cm) of first-born cows of various breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
Height at the withers						
URD	30	131 $\pm$ 0.5	2.59	1.9	-1 $\pm$ 0.58	1.72
UBSD	30	134 $\pm$ 0.5	3.05	2.3	2 $\pm$ 0.58	3.44**
URSD	30	132 $\pm$ 0.5	2.93	2.2	0	0
On average	90	132 $\pm$ 0.3	3.02	2.3	x	x
Oblique body length						
URD	30	160 $\pm$ 0.7	4.16	2.6	2 $\pm$ 0.92	2.17*
UBSD	30	157 $\pm$ 1.0	5.65	3.6	-1 $\pm$ 1.17	0.85
URSD	30	157 $\pm$ 1.4	7.99	5.1	-1 $\pm$ 1.52	0.66
On average	90	158 $\pm$ 0.6	6.28	4.0	x	x
Chest depth						
URD	30	71 $\pm$ 0.7	3.92	5.5	-1 $\pm$ 0.81	1.23
UBSD	30	73 $\pm$ 0.6	3.21	4.4	1 $\pm$ 0.72	1.39
URSD	30	72 $\pm$ 0.7	3.83	5.3	0	0
On average	90	72 $\pm$ 0.4	3.69	5.1	x	x
Chest width						
URD	30	44 $\pm$ 0.7	3.62	8.2	-1 $\pm$ 0.81	1.23
UBSD	30	46 $\pm$ 0.5	3.06	6.6	1 $\pm$ 0.64	1.56
URSD	30	45 $\pm$ 0.8	4.26	9.5	0	0
On average	90	45 $\pm$ 0.4	3.72	8.3	x	x
Chest circumference						
URD	30	205 $\pm$ 1.9	10.42	5.1	1 $\pm$ 2.15	0.46
UBSD	30	203 $\pm$ 1.6	8.79	4.3	-1 $\pm$ 1.89	0.53
URSD	30	206 $\pm$ 1.7	9.56	4.6	2 $\pm$ 1.97	
On average	90	204 $\pm$ 1.0	9.59	4.7	x	x
Metacarpal girth						
URD	30	19.9 $\pm$ 0.31	1.69	8.5	0.3 $\pm$ 0.34	0.88
UBSD	30	19.6 $\pm$ 0.24	1.30	6.7	0	0
URSD	30	19.5 $\pm$ 0.28	1.52	7.8	-0.1 $\pm$ 0.32	0.31
On average	90	19.6 $\pm$ 0.16	1.51	7.7	x	x
Width in macklacks						
URD	30	51 $\pm$ 0.5	2.77	5.4	0	0
UBSD	30	51 $\pm$ 0.5	2.57	5.0	0	0
URSD	30	51 $\pm$ 0.4	2.42	4.7	0	0
On average	90	51 $\pm$ 0.3	2.57	5.0	x	x

The analysis of the duration of the dry period in the context of the studied livestock did not reveal any significant differences from physiological and zootechnical standards. Thus, in URD and UBSD cows it is 63, 62 days, respectively, and only in URSD there is a slight increase to 72 days. The differences with the control group are  $\pm 2$ –7 days.

The evaluation of the cow insemination index gives us negative results. Because the insemination index is the number of inseminations spent on one insemination. Thus, insemination results are considered optimal if the index is 1.5: good — 1.6–1.8; satisfactory — 1.9–2.0; poor — 2.1 and more. Based on our calculations, the insemination index in this farm is very poor — 6.40–6.59. Its lowest value is observed in URSD cows (6.40), which is far from even a satisfactory state of reproductive function. The value of the insemination index in the URD breed peers is even higher — 6.52 and its maximum value is noted in UBSD cattle (6.59).



**Table 11.** Characteristics of the reproductive function in cows of different breeds

Breed	n	The level of development of the trait and its variability and probability				
		$\bar{X} \pm S_x$	$\sigma$	$C_v$	$d \pm S_d$	$t_d$
Duration of the service period (for higher lactation), days						
URD	30	130±15.9	87.4	67.3	0	0
UBSD	30	132±13.1	71.6	54.2	2±15.3	0.13
URSD	30	128±12.7	69.5	54.4	-2±15.0	0.13
On average	90	130±8.0	75.7	58.2	×	×
Length of dry period (for higher lactation), days						
URD	30	63±2.5	13.9	21.9	-2±3.60	0.54
UBSD	30	62±5.5	30.1	48.0	-3±6.12	0.49
URSD	30	72±5.3	29.0	40.5	7±5.95	1.17
On average	90	65±2.7	25.5	38.7	×	×
Insemination index (for higher lactation)						
URD	30	6.52±0.798	4.37	67.0	0.01±0.89	0.01
UBSD	30	6.59±0.654	3.58	54.3	0.08±0.77	0.10
URSD	30	6.40±0.635	3.48	54.4	-0.11±0.75	0.15
On average	90	6.51±0.399	3.79	58.2	×	×
Calving period (for higher lactation), days						
URD	30	423±13.7	74.9	17.7	7±15.4	0.45
UBSD	30	420±14.3	78.6	18.7	4±15.9	0.25
URSD	30	406±8.3	45.2	11.1	-10±10.9	0.92
On average	90	416±7.1	67.6	16.2	×	×

**Table 12.** Inheritance of the main selection traits

Breed	n	Yield		Amount of milk fat	
		$r_p \pm S_r$	$h^2$	$r_p \pm S_r$	$h^2$
URD	30	0.45±0.14**	0.90	0.53±0.13***	1.06
UBSD	30	0.02±0.18	0.04	-0.03±0.18	0.06
URSD	30	0.24±0.17	0.48	0.29±0.17	0.58
On average	90	0.29±0.09**	0.58	0.32±0.09**	0.64

Since we have established a significant lengthening of the period from calving to fertile insemination (up to 132 days) and, accordingly, a significant increase in the insemination index, the lengthening of the period between calvings (from 406 to 423 days) is directly proportional to the increase in the overall performance of the industry, since this state of reproductive function does not provide for the production of one calf per year from a cow. A more detailed analysis of the inter-calving period revealed that the cows with the shortest service period and insemination index, respectively, also had the shortest period between calvings — 406 days. Among the other two breeds, no such natural trend was found. Thus, cows with the highest values of service-period and insemination index: UBSD, respectively, had a mediocre inter-calving period of 420 days compared to 423 days for their URD peers.

The experience of many countries with highly developed dairy farming and scientific forecasts of breeding scientists indicate that breeding work with the breed should be carried out on the principles of large-scale selection, which includes the intensive and centralised use of bulls-improvers using in-depth knowledge of the main methods of assessing the breeding qualities of animals, population genetics, patterns of variability and heritability

of economically useful traits in populations and herds. Such an approach to breeding work will make it possible to increase the genetic progress in the population up to 60 kg of milk per cow per year [19]. For more than 40 years, the system of selection and breeding work in dairy cattle breeding has been based on the principles of large-scale selection: centralised evaluation, selection and intensive use of highly valuable sires on a breed-wide scale, creation of a semen bank for proven bulls, use of computers, methods of population genetics and other achievements of science and technology. Methods of computer modelling of selection and genetic processes in dairy cattle populations and genetic and economic optimisation of large-scale selection programmes have been developed [21, 30]. In the domestic and foreign literature, sufficient data have been obtained to date to show that all quantitative traits of livestock productivity obey the law of distribution of individuals, according to which about two-thirds of individuals in each population are characterised by indicators corresponding to the average value of the trait in this population. In the remaining individuals, this trait may be greater than the average value or less [28, 39]. The degree of inheritance of a trait to a certain extent determines the rate of genetic improvement of the population in which selection for this trait is carried out. Almost all economically useful traits of dairy and beef cattle are quantitative and have a sufficient degree of inheritance for effective selection, with the exception of fertility [26, 39]. Therefore, it is quite relevant to study the inheritance of the main selection traits in the context of new modern Ukrainian breeds in the conditions of the breeding farm. We estimated the following correlation coefficient between the main selection traits — milk yield and amount of milk fat between mothers and daughters (table 12).

Thus, low and medium positive correlations are observed between the milk yield of mothers and their daughters, up to 0.45. Moreover, the highest relative variability between the above traits is observed among URD cows —  $r_p=0.45$  at the second level of reliability ( $P>0.99$ ), which, accordingly, makes the maximum heritability coefficient —  $h^2=0.90$ . Average values of relative variability are observed in URSD cows —  $r_p=0.24$  and  $h^2=0.48$ . And not high positive correlations are noted in UBSD cattle — 0.02 and 0.04, respectively. The assessment of the inheritance of milk fat has slightly different trends. The mother-daughter generation of the URD breed is characterised by a very high relative variability — 0.53%, which gives a very high inheritance coefficient  $h^2=1.06$ . The level of correlation of the amount of milk fat among URSD cattle is almost at the level of the previous trait — 0.29 and, accordingly, high  $h^2=0.58$ . But the correlation between the amount of milk fat of mothers and daughters among UBSD breeds changed its direction and became negative  $r_p = -0.03$  with a low inheritance coefficient  $h^2=0.06$ .

The productivity of cows in most agricultural enterprises in terms of milk production is at an arbitrarily low level. One of the reasons for low milk yields is poor breeding practices. There is virtually no division of the herd into

a breeding core, a production group and a reject. In such conditions, heifers are obtained without a purposeful purpose, where the best cows in terms of productivity are kept for reproduction [28, 39]. It has been established that there are individual animals with high productive potential in the cow herd. It is important to get descendants from them to reproduce the herd. The intensity of herd reproduction depends on the number of cows that are allocated to the breeding nucleus. Thus, an increase in the number of cows in the breeding nucleus leads to a decrease in the average milk yield in its group, and a decrease leads to an increase in the milk yield in the herd [4, 31, 33]. The number of first-calf cows raised depends on the culling of cows, therefore, herd reproduction and culling are closely linked. It is necessary to take into account the possibility of expanding the capacity of milk production enterprises [22]. The first stage of breeding work is to assess the productivity of cows, study the conditions of housing and feeding, identify cows with high genetic potential and preserve such cows to obtain replacement heifers, heifers and first-born cows. At the same time, it is important to establish the optimal number of cows in the breeding nucleus and its impact on increasing milk production [26, 28]. Therefore, the purpose of further research was to establish the effect of selecting cows for the first lactation in the breeding core of the herd, their breeding limit and productivity at different selection intensities. So, at a selection intensity of 15%, that is, the selection pressure is 85% or only five cows are included in the breeding nucleus, and their milk yield will increase compared to the average value by up to 22%. Consequently, the milk yield of these cows will be 7406 kg for the URD breed, while the average data for the herd is 5216 kg accordingly, the selection differential will be 2190 kg and the selection effect will be 1971 kg. Accordingly, the breeding limit or minimum milk yield of

cows that will be selected for the breeding group will be 6686 kg (table 13). Among the UBSD cows, the average milk yield in the herd is 6210 kg. The productivity of the breeding core cows is 7512 kg, respectively  $Sd=1302$  kg and  $SE=52$  kg. Such a low selection effect is due to the low inheritance coefficient  $h^2=0.04$ . Due to the highest productivity of cows in the breeding group of this breed, the highest selection limit is also noted  $\bar{X}_u=7084$  kg. Although cattle of the URSD breed have the lowest breeding values ( $\bar{X}_{bc}=6755$  kg,  $Sd=1781$  kg,  $\bar{X}_u=6170$  kg), they have a higher breeding effect than the previous group due to a higher level of heritability —  $SE=855$  kg. At a selection intensity of 30%, the milk yield of the breeding core cows will increase to 19% among the studied breeds. The breeding differential with the increase of cows in the breeding nucleus significantly decreases. The selection of 30% of the best cows resulted in a selection differential of 1643 kg (URD), 977 kg (UBSD) and 1336 kg (URSD) of milk. At a selection intensity of 45%, the selection differential is:  $Sd=1243$  kg,  $Sd=739$  kg,  $Sd=1011$  kg of milk, respectively. These data indicate the potential for increasing milk yields from a smaller number of cows in the breeding core.

The effect of breeding (selection) depends on the transmission of hereditary information to its offspring. Studies have shown that the coefficient of heritability in herds of URD, UBSD and URSD breeds, respectively, was  $h^2=0.9$ ,  $h^2=0.04$  and  $h^2=0.48$ , therefore, of the above possible increase in milk yields with the intensity of selection of 30% will actually be manifested in the descendants of only 1479 kg, 39 kg and 641 kg and at a selection intensity of 45%, the selection effect will be 1119 kg 30 kg and 485 kg, respectively. As a result, depending on the intensity of selection, the selection limit will decrease (5991 kg, 6647 kg, 5572 kg and 5400 kg, 6319 kg, 5123 kg of milk yield, respectively).

**Table 13.** Modelling the effect of selection in cows of different breeds by milk yield, kg

Selection parameters	n	Breed		
		URD	UBSD	URSD
Intensity of selection – 15%				
$\bar{X}_{hr}$	30	5216	6210	4974
$\bar{X}_{bc}$	5	7406	7512	6755
$Sd$		2190	1302	1781
$SE$	5	1971	52	855
$\bar{X}_u$	5	6686	7084	6170
Intensity of selection – 30%				
$\bar{X}_{hr}$	30	5216	6210	4974
$\bar{X}_{bc}$	9	6859	7187	6310
$Sd$		1643	977	1336
$SE$	9	1479	39	641
$\bar{X}_u$	9	5951	6647	5572
Intensity of selection – 45%				
$\bar{X}_{hr}$	30	5216	6210	4974
$\bar{X}_{bc}$	13	6459	6949	5985
$Sd$		1243	739	1011
$SE$	13	1119	30	485
$\bar{X}_u$	13	5400	6319	5123

**Table 14.** Modelling the effect of selection in cows of different breeds by the amount of of milk fat, kg

Selection parameters	n	Breed		
		URD	UBSD	URSD
Intensity of selection – 15%				
$\bar{X}_{hr}$	30	198	237	190
$\bar{X}_{bc}$	5	272	288	264
$Sd$		74	51	74
$SE$	5	78	3.1	43
$\bar{X}_u$	5	247	271	239
Intensity of selection – 30%				
$\bar{X}_{hr}$	30	198	237	190
$\bar{X}_{bc}$	9	253	275	245
$Sd$		55	38	55
$SE$	9	58	2.3	32
$\bar{X}_u$	9	223	254	215
Intensity of selection – 45%				
$\bar{X}_{hr}$	30	198	237	190
$\bar{X}_{bc}$	13	240	266	232
$Sd$		42	29	42
$SE$	13	44	1.74	24
$\bar{X}_u$	13	204	241	196

We also modelled the effect of selection on the amount of milk fat under the same conditions of intensive selection. Thus, at an intensity of selection of 15%, the productivity of cows in the breeding group will significantly increase — 272 kg (URD), 288 kg (UBSD) and 264 kg (URSD) of the breed (table 14). At a selection pressure of 70% or  $i=0.30\%$ , respectively, the productivity of these animals will slightly decrease compared to the previous group to 253, 275, 245 kg, respectively, and at  $p=55\%$  or  $i=45\%$  to 240, 266, 232 kg, respectively. There is also a direct dependence of the selection differential on the selection pressure: 15%, so the theoretical increase in productivity will be at the level of 74, 51, 74 kg, respectively; at 30 — 55, 38, 55 kg and at 45% — 42, 49 and 42 kg, respectively. The actual increase in productivity is directly related to the inheritance coefficient  $h^2=1.06$  (URD),  $h^2=0.06$  (UBSD) and  $h^2=0.58$  (URSD). The level of increase in actual productivity or the effect of selection was not unambiguous — 25, 2.3 and 32 kg ( $i=0.30\%$ ) and 44, 1.74, 24 kg ( $i=0.45\%$ ), respectively. Thus, a decrease in the number of cows in the breeding nucleus leads to a greater increase in milk yield and milk fat. Thus, with 15% of cows in the breeding nucleus, milk yields will increase to 42%, with 30% — to 31%, and with 45% — to 29%. The increase in the amount of milk fat, depending on the intensity of selection, is up to 39, 29 and 22%. In our opinion, from an economic point of view, it is more expedient to use moderate selection with its intensity of 30% or culling cows from the herd of 70%, which will increase productivity by 31 and 29%.

As a result of the research, a rather high hereditary potential of cows of modern Ukrainian breeds was established for the main signs of milk production.

Analysing the signs of dairy productivity represented by milk yield, content and amount of fat in milk, it should be noted that the highest indicators are characterised by cows of the Ukrainian Speckled dairy breed, which in the context of four lactations (6210–7593 kg of milk, 237–287 kg of milk fat) were better, except for the third one, where a clear leader in the main signs was not found.

In modern high-yielding herds, the duration of lactation (325–360 days), regardless of genotype (with or without Holstein bloodline), exceeds the optimal value (305 days), which is associated with later insemination of cows after calving and extended service period.

The milk yield reflex in cows of the studied breeds is within the limits of the accepted optimal values (1.81–2.02 kg/min), which indicates their good adaptability and adaptation to the technology of machine milking.

It was found that the live weight of animals of the three studied breeds at the end of the growing period is within the breed standards (287.9–326.1 kg), but the higher weight is distinguished by the peers of Ukrainian red (326.1 kg) and black speckled (325.7 kg) dairy breeds.

It was proved that there was no clear advantage in favour of a certain group of cows by the main measurements. Thus, the height at the withers (134 cm), depth (73 cm) and width of the chest (46 cm) are better devel-

oped in Ukrainian Black Speckled cattle, and the oblique length of the body (160 cm) and the girth of the metacarpal (19.9 cm) — in the red dairy breed, with a larger girth of the chest (206 cm) in the peers of the Red Speckled Dairy breed.

The analysis of the reproductive function of cows gives grounds to assert that among all studied breeds there is a significant deterioration of its function, this leads, regardless of genotype, to an increase in the duration of service period (128–132 days) and the period between calvings (406–423 days), and this negatively affects the yield of calves per year and, as a result, significantly increases the insemination index (6.40–6.59).

The analysis of correlations between the main selection traits of mothers and their daughters established high predictions regarding their inheritance (0.48–1.06), this will significantly increase the efficiency of selection for milk yield and milk fat in these herds of modern breeds.

Reducing the number of cows in the breeding nucleus leads to a greater increase in milk yield and milk fat. For example, with 15% of cows in the breeding nucleus, milk yields will increase to 42%, with 30% — to 31%, and with 45% — to 29%. The increase in the amount of milk fat, depending on the intensity of selection, is up to 39, 29 and 22%.

Based on our research, we recommend “Kolos 2011” LLC:

1. To conduct more precise selection and breeding work with cattle of the Ukrainian Red Speckled dairy breed by: using the parameters of relative variability and heritability in the assessment of trait development; applying cow selection to the breeding core with an intensity of 30%; continuing the practice of directed heifer rearing with constant monitoring of their live weight.

2. Since the level of reproductive function in the farm is problematic, which reduces the period of economic use of cows, reduces the level of their milk production, and at the same time the profitability of the industry as a whole, we recommend that the veterinarian and chief zootechnician develop both therapeutic and preventive measures to improve it, and introduce biotechnological methods of organising animal reproduction.

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## Організація відтворення стада худоби молочного напрямку продуктивності

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Ми можемо говорити про достатньо високий спадковий потенціал корів сучасних українських порід за основними ознаками молочної продуктивності. Встановлено, що вищий надій, вміст та кількість жиру у молоці властиві коровам української чорно-рябої молочної породи, які у розрізі чотирьох оцінених лактацій виявлялися кращим, окрім лише третьої (де чіткого лідера за основними ознаками не виявлено). У сучасних високопродуктивних стадах худоби українських порід тривалість лактації, незалежно від генотипу з голштинською часткою кровності чи без неї, перевищує оптимальне значення (305 днів), що пов'язано із більш пізніми строками осіменіння корів після отелення та подовженою тривалістю сервіс-періоду. Тому, оцінюючи ефективність використання молочних корів, доцільно враховувати кількість дійних днів і відповідно до цього проводити корегування їх молочної продуктивності і відтворної здатності. А вплив голштинізації на подовження тривалості лактаційного періоду проявляється лише у стаді української чорно-рябої молочної породи. Рефлекс молоковіддачі у корів досліджених порід коливається в межах прийнятих оптимальних показників, що вказує на їх добру пристосованість та адаптацію до технології машинного доїння. Жива маса тварин трьох досліджених порід на кінець періоду вирощування залишається в межах стандартів порід, але вона вища в ровесниць червоної та чорно-рябої молочних порід, що вказує на їхню кращу здатність за відповідних умов вирощування до високої інтенсивності росту і, як показали попередні дослідження, до кращої молочної продуктивності. Ступінь розвитку основних промірів будови тіла корів перебуває в межах стандартів і відповідає нормам молочного типу корів, а чіткої переваги на користь певної групи корів за основними промірами не виявлено. Висота в холці, глибина та ширина грудей краще розвинені у представниць української чорно-рябої молочної худоби, а коса довжина тулуба та обхват п'ястка — в української червоної молочної породи, при більшому обхваті грудей у ровесниць української червоно-рябої молочної породи. Проведений аналіз відтворювальної функції корів дає підставу стверджувати, що серед усіх досліджених порід простежується значне її погіршення, що призводить, незалежно від породної належності, до подовження тривалості сервіс-періоду (128–132 дні) та періоду між отеленнями (406–423 дні); це негативно впливає на вихід телят за рік та як наслідок — значно підвищує індекс осіменіння (6,40–6,59). Аналіз кореляційних зв'язків між основними ознаками селекції матерів та їх дочок встановив високі прогнози щодо їх успадкування (0,48–1,06), що значно підвищить ефективність селекції за надоєм та кількістю молочного жиру в цих стадах сучасних порід.

**Ключові слова:** репродуктивна функція, статевая охота, сервіс-період, індекс осіменіння, сухостійний період, штучне осіменіння, молочна продуктивність, порода



## The optimization of insemination methods and techniques in sows

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Artificial insemination of sows in modern farms producing high-quality pork is justified both economically and technologically. The studies were implemented on a herd population of 3,300 sows of the high prolificacy hybrid (YL) of Danish selection — PIC. For artificial insemination of sows was used next methods: cervical insemination (Conventional Artificial Insemination), Intrauterine Artificial Insemination. Analyzing the fertility of sows, prolificacy and the number of live-born piglets, it was found that with the same conditions, feeding, timing and methods of weaning piglets, as well as obtaining, evaluating, processing and using the semen of boars, for all experimental and control groups, the obtained results allow to optimize the methods of artificial insemination of sows, by reducing of semen doses volume and amount, and increasing reproductive indicators, maximizing the potential of physiologically mature sows of the 4<sup>th</sup>–6<sup>th</sup> parity.

**Key words:** sow, fertility, artificial insemination, semen dose, optimization



## Introduction

The use of artificial insemination in pig farming dates back to the 30s of the last century, however, the industrial application of this method became widespread in the 80s [2]. Currently, most pig farms have switched from the practice of natural mating of animals to artificial insemination, which is due to both the economic feasibility and technological advantages of this method [16]. In the past three to four decades, high-quality diluents for semen have emerged, enabling the attainment of the desired concentrations without loss of sperm spermatozoa viability. Moreover, these medications allowed for the prolonged preservation of the diluted semen when compared to natural conditions, which is to say, when compared to the reproductive tract of a sow [12]. The use of semen material from one boar producer to fertilize several sows using the same ejaculate has led to radical changes in pig reproduction technology.

One boar ejaculate can contain up to 10 billion sperm cells. This indicator may vary depending on

the breed of boars [9]. For successful fertilization by the classical method (cervical insemination), a diluted ejaculate in the volume of 80–100 milliliters containing up to 40 million sperm per milliliter is used [2, 5, 16]. This means that boars of highly productive breeds are able to produce 25 or more doses of sperm from one ejaculate, suitable for artificial insemination. In comparison with the natural method of reproduction, where no more than 45 sows per boar per year are recommended [8], artificial insemination is a more cost-effective method, since it significantly reduces the need to maintain a large number of breeding boars. When performing artificial insemination, the proportion of boars in the livestock should not exceed 0.5% [4].

The use of the method of artificial insemination of sows has demonstrated its practical feasibility and effectiveness. The procedure is not complicated, does not require significant time costs and is easily mastered by employees of pig farms. From the point of view of efficiency, artificial insemination demonstrates a higher level of fertilization, exceeding 90%, which is

much higher compared to natural mating [6, 7]. All this contributed to the further development of methods of artificial insemination of sows, among which three main approaches can be distinguished:

- a) Conventional Artificial Insemination (CAI),
- b) Intrauterine Artificial Insemination (IAI),
- c) Deep Intrauterine Artificial Insemination (DIAI).

#### *Conventional Artificial Insemination*

It is performed by inserting a plastic catheter with a soft tip into the cervix of the sow. A semen dose is used in a volume of 80 to 100 ml, containing 30 million spermatozoa sperm per milliliter. The catheter is not removed immediately, but after 5–7 min. Preliminary stimulation of the sow by the artificial insemination operator, establishment of contact with the boar (nose to nose) and achievement of the immobility reflex are mandatory conditions [7, 13].

The advantages of this method are as follows: it simulates the natural mating process, is easy to use, and has high efficiency. The disadvantages include the fact that it requires more time and a larger volume of sperm.

#### *Intrauterine Artificial Insemination*

The process performed by inserting a plastic catheter equipped with a soft tip into the cervix of a sow, followed by the insertion of a flexible cannula into it, which allows to reach the uterine cavity. A semen dose is used in a volume of 30 to 60 ml, containing 30 million sperm per milliliter. The specificity of this method lies in the fact that the process of stimulating sows and determining of heat is processed by artificial insemination operators in advance, at least half an hour before the start of the mating procedure. At the time of catheter insertion, the sow should be relaxed, since with reflex immobility of the sow, it is difficult or practically impossible to insert the cannula into the uterine cavity. After inserting a catheter with a cannula into the uterine cavity, the contents of dose are artificially pressed in, and the catheter is removed immediately after the procedure [7, 13].

The advantages of this method are: lower volume of semen dose; the ability to vary the concentration of sperm; fewer operators; high performance.

The disadvantages of this method include the complexity of its application for gilts and sows after the first farrowing, as well as the need for more highly qualified personnel. In addition, there is an increased risk of infection.

#### *Deep Intrauterine Artificial Insemination*

The process is carried out by inserting a plastic catheter with a soft tip into the cervical region of the sow. Then a long flexible cannula is inserted into the catheter, which allows you to reach the horns of the animal's uterus. A special feature of the method is the small volume of semen dose, up to 10 ml [10, 11, 13]. The

stimulation of sows and the detection of estrus, similar to that of post-cervical insemination, is carried out by artificial insemination technicians well in advance, ideally at least half an hour prior to the procedure. After the insertion of a catheter with a cannula, the contents of the sperm are forcibly injected into the uterus. The catheter is removed immediately after the procedure is completed.

This method has the highest efficiency, but at the same time involves considerable labor and requires the operator to have skills and experience in manipulating the catheter. In commercial pig farms, this method is usually not used because it is not practical. However, this method finds application for research purposes, and, like any other, has its advantages and disadvantages. The advantages of this method include: an extremely small amount of seed dose and high efficiency. However, this method also has a number of disadvantages: it is impractical in farms with large livestock, requires careful training of personnel, as well as expensive consumables such as a special catheter. In addition, there is a high risk of infection.

The choice of the method of artificial insemination of sows, as well as the determination of the volume and concentration of semen dose, is due to a number of factors, among which the key ones are the size of the farm, the number of sows in it, the cycle of production, the level of technological equipment, the qualification of personnel and the availability of semen material.

The research conducted in this area is aimed at determining the most effective combination of artificial insemination methods in combination with the volume and concentration of semen dose. This will improve the effectiveness of insemination and optimize the economic efficiency of using sows.

## **Materials and Methods**

The research was carried out at the *Porco Bello* SRL pig breeding complex, located in the central zone of the Republic of Moldova (Cimisheni village, Criuleni district). The complex is a full-cycle farm for the breeding and growing of pigs. The size of the sow herd population is 3300 animals. The production potential of the complex is 100,000 piglets per year. The herd of sows at the complex is represented by a hybrid of the first generation (F1), obtained as a result of crossing sows of the Landrace breed and boars of the Yorkshire breed. This hybrid proved to be at its best in production conditions based on technology with a weekly production cycle adopted at the complex. As part of the scientific and production experiment, the entire sow population of the complex was involved, starting with the first and ending with the tenth farrowing for the period from January 1, 2020 to December 31, 2022. The conditions of keeping, diet,

as well as the timing and method of weaning piglets from sows were identical for all experimental groups of sows. As a control, the results obtained with the classical (cervical) method of insemination were used.

The main source of information on all the studied parameters of sows in the control and experimental groups was the database of a specialized herd management program used at the complex — *AgroVision (AgroSoft)*.

The object of the study was the methods of artificial insemination of sows, which are clearly shown in fig. 1. In particular, classical (1) and post-cervical (2) insemination were considered, as well as various volumes of seed material used in these methods.

Ejaculates obtained from terminal boars of the Duroc breed were taken as the studied and used semen material. The processing and preparation of ejaculates for use was carried out in the laboratory of the complex in accordance with a single standard throughout the entire study period. In the process, photocalorimetr and a microscope equipped with a high-speed digital camera were used.

To assess the economic efficiency of various methods of artificial insemination of sows and optimize the cost of semen material, an analysis of the production indicators of experimental and control groups of sows of different ages was carried out. The main evaluation criteria were the fertility level of the inseminated groups, the average number of live-born piglets per sow, as well as the volume and number of semen doses used.

The obtained digital data were analyzed using methods of variational statistics [3] and classical software tools (*Microsoft Excel*). The reliability of the differences between the study groups was determined using the Student's criterion [14].

## Results and Discussion

### Sows productivity

The starting point for the study was information on the reproductive ability of sows at the *Porco Bello SRL* complex, classified according to their age, determined by the number of parities [15].

In the course of studies conducted between 2020 and 2022, 23,991 sows were artificially inseminated (see table 1).

At the same time, the largest number of sows, namely 14114 heads, or 58%, were sows after 1<sup>st</sup>–3<sup>rd</sup> parity. The number of sows after 4–5 parities amounted to 5,884, or 24,5%, which represents a significant proportion of the total herd. It is also worth noting that these complex practices the effective use of sows up to the tenth parities.

During the study, the dynamics of the use of sows depending on their age was clearly presented, which is reflected in fig. 2. It shows that with increasing age of sows, their number gradually decreases, and after the fifth farrowing, this process becomes especially noticeable.

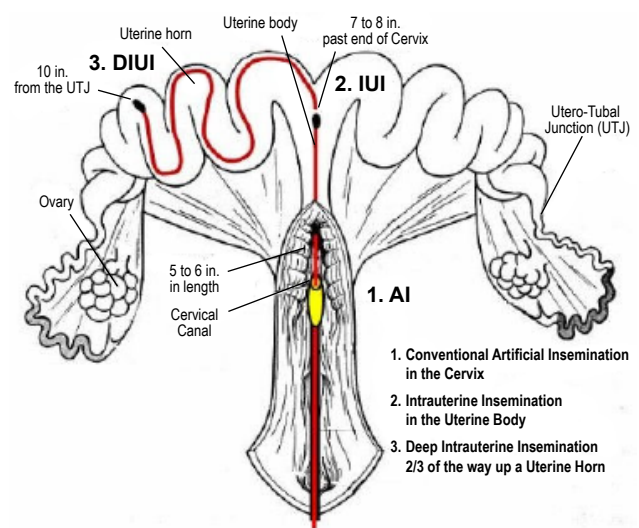


Fig. 1. Sow reproductive tract illustrating the site of semen deposition for 3 different types of artificial insemination [1]

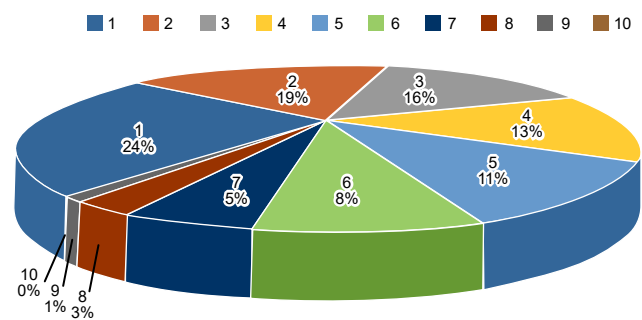


Fig. 2. Sows herd age structure by parity of investigated period 2020–2022

This is due to natural reasons — the high intensity of the use of sows in the complex (2.4 farrowing per year) leads to a faster deterioration of the animal body, which directly affects the productivity of sows. To confirm the hypothesis, an analysis of the correlation between fertility rates and the number of live-born piglets, depending on the age of the sows, was carried out. The results of this analysis are presented in the form of a diagram in fig. 2.

Table 1. Insemination structure and production results according parity number

Parity, Nr.	Sows inseminated, n	Liveborn piglets per sow, n (X±Sx)	Fertility rate, %
1	5775	13,75±0,06	85,86
2	4628	14,99±0,07	86,99
3	3711	16,09±0,07	90,73
4	3197	16,31±0,07	91,27
5	2687	16,25±0,08	92,04
6	1978	16,26±0,08	92,47
7	1221	15,65±0,11	91,4
8	564	15,50±0,16	92,38
9	184	15,16±0,26	95,65
10	46	14,50±0,53	95,65
Total	23991	X	X



Studying fig. 2, we can conclude that the farrowing carried out from the third to the sixth is the most effective. In this case, the number of live-born piglets exceeds the average value of 16 piglets per sow, and the level of actual fertility exceeds 90%. These indicators are typical for hybrid sows with Danish genetics (PIC) and can compete with the best European farms specializing in the production of high-quality pork. At the same time, the most favorable (optimal) ratios between the number of live-born piglets per sow and the level of fertility, taking into account the number of inseminated animals, are achieved in sows that have already passed the fourth and fifth farrowing.

#### *The method of artificial insemination of sows*

At this stage of the study, the effectiveness of the artificial insemination of sows was analyzed using various methods and a thorough study of their effectiveness. In each of the three cases described below, the same volume and concentration of semen dose were used.

1) *Conventional Artificial Insemination (CAI) with one repetition* — classical (cervical) artificial insemination with a single repetition 24 h after the first insemination in the presence of a immobility reflex in a sow. The volume of semen dose is 100 ml, the concentration of sperms is 30 million per milliliter.

2) *Conventional Artificial Insemination (CAI) with two repetitions* — classical (cervical) artificial insemination with a double repetition 24 and 48 h after the first insemination in the presence of a immobility reflex in a sow. The volume of semen dose is 100 ml, the concentration of sperms is 30 million per milliliter.

3) *Intrauterine Artificial Insemination* — post cervical insemination with a single repetition 24 h after the first insemination without presence of a immobility reflex in a sow. The volume of semen dose is 100 ml, the concentration of sperms is 30 million per milliliter. The results obtained during the study presented in table 2.

**Table 2.** Matting structure according artificial insemination type

Insemination techniques	Sows inseminated, n	Liveborn per sow, n	Fertility rate, %
CAI with one repetition	15924	15,13	88,57
CAI with two repetitions	5831	15,93	93,38
IAI with one repetition	866	15,16	87,3

Data presented in table 2 indicated that post cervical method of insemination of sows without considering the age of the animal, is not more effective than the traditional (CAI) method. In the course of our study, it was found that the fertility level and the number of live-born piglets in sows inseminated in the classical double-repeat method, all other things being equal,

were higher by 6.08 percentage points and 0.7 live-born piglets per sow, respectively, compared with the use of the post-cervical method.

However, the practical application of this method as the main one is impossible, due to variations in the duration of the estrous period (heat) in sows. This period is characterized by such a sign as the immobility reflex, which can last up to 48 h. In most sows, estrus lasts less than this period, so re-insemination is carried out only once. Regarding the post-cervical method, it should be noted that it is also effective and meets the criteria of productivity exceeding 15 live-born piglets per sow and 85% actual pregnancy. However, all other things being equal, it is inferior to cervical insemination.

As can be seen from the analysis of the data presented in table 2, the post-cervical method is a comparable alternative to the cervical method of insemination of sows. However, with equal volumes of semen dose, it is not optimal, which became the basis for conducting experiments to determine the optimal volume of semen dose with the post-cervical insemination method in order to achieve maximum results.

In accordance with our data presented in table 1 and information from the literature [15], sows of the 4<sup>th</sup> and 5<sup>th</sup> parity, which are the most productive and physiologically mature individuals, were selected and inseminated as experimental groups. During the experimental period, post-cervical insemination was performed using various volumes of semen dose: 100 ml, 60 ml, 50 ml, 40 ml and 30 ml, while the concentration of spermatozoa in each dose was 30 million/ml. The results obtained during the study of the level of actual pregnancy and the number of live-born piglets per sow were compared with each other, as well as between the corresponding age groups of sows that were inseminated using the classical (cervical) method with one or two repetitions (fig. 4).

Based on the information presented in the figure, a number of conclusions can be drawn.

Firstly, it was found that cervical insemination with two repetitions is more effective than with single repetition due to an increase in the fertilization coefficient. The difference in performance is about 3%.

Secondly, it is confirmed that in conditions when sows are at the most productive age, and the volume of semen dose is 100 ml, the most effective method of insemination is the cervical method, which can be repeated once or twice. At the same time, the effectiveness of this method is higher both in the number of liveborn piglets per sow (by 0.9–1 piglet) and in actual pregnancy (by 1–3%).

Thirdly, the most effective is the use of a group of sows that have 4<sup>th</sup>–5<sup>th</sup> parity age, while inseminated by the post-cervical method using a 40 ml semen dose and sperm concentration of 30 million/ml. In this case, the results obtained exceed the indicators of post-cervical insemination with a dose of 100 ml by 6% in terms of

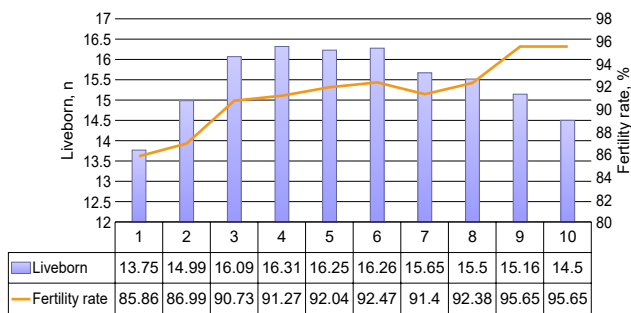


Fig. 3. Correlation of liveborn pigs vs fertility rate according parity

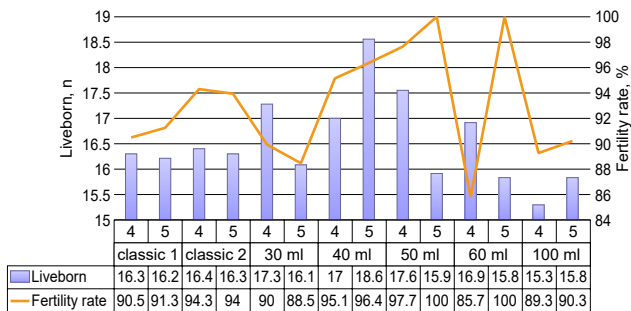


Fig. 4. Correlation between the number of live-born piglets and the fertility level of 4<sup>th</sup>–5<sup>th</sup> parity sows depending on the insemination method and the volume of semen dose

actual pregnancy and 2.7 piglets per sow in terms of the number of live births, as well as the indicators of classical (CIA) insemination with two repeats by 1–2.5% in terms of actual pregnancy and 0.6–2.2 piglets per sow.

In the course of research conducted at the *Porco Bello* SRL complex, the effectiveness of an integrated approach to the selection of the method of artificial insemination of sows was confirmed. It was found that the replacement of the traditional cervical insemination method for sows of the 4<sup>th</sup> and 5<sup>th</sup> parity with the post-cervical method leads to a significant increase in the number of piglets born while maintaining other equal conditions. This is achieved by increasing the fertility rate and prolificacy of animals.

The optimal volume of semen dose for post-cervical insemination of sows on the 4<sup>th</sup> and 5<sup>th</sup> parity is a dose of 40 ml with a concentration of 30 million sperm per milliliter. This makes it possible to significantly reduce the need for semen material and, as a result, reduce the corresponding costs for the maintenance of boars.

The results presented in this article were obtained during the implementation of a research project: “Managementul potențialului genetic și a producțiilor animalelor de rasă reproducute și exploatate în condițiile pedoclimatice ale Republicii Moldova” (code 20.800009.5107.20), Prioritatea Strategică II. Agricultură durabilă, securitate alimentară și siguranța alimentelor și în cadrul temei doctorale “Perfecționarea elementelor tehnologice în creșterea suinelor și producerea cărnii de calitate în condițiile Republicii Moldova”.

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## Оптимізація методів і технік осіменіння свиноматок

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Штучне осіменіння свиноматок у сучасних господарствах з виробництва високоякісної свинини виправдане як економічно, так і технологічно. Дослідження проводили на поголів'ї свиноматок високопродуктивного гібрида (YL) датської селекції — РІС, яке налічувало 3300 тварин. Для штучного осіменіння свиноматок використовували такі методи: цервікальне осіменіння (звичайне штучне осіменіння), внутрішньоматкове штучне осіменіння. Аналізуючи заплідненість свиноматок, багатоплідність та кількість живонароджених поросят, встановили, що за однакових умов, годівлі, термінів і способів відлучення поросят, а також отримання, оцінки, обробки та використання сперми кнурів, для всіх дослідних і контрольних груп результати дозволяють оптимізувати методи штучного осіменіння свиноматок через зменшення об'єму та кількості доз сперми та підвищення відтворних показників, максимізуючи потенціал фізіологічно статевозрілих свиноматок 4–6-го опоросу.

**Ключові слова:** свиноматка, плодючість, штучне осіменіння, доза сперми, оптимізація



# Observation of the grey heron (*Ardea cinerea*) and the great egret (*Ardea alba*) in the territory of Lviv and Cherkasy regions during the winter period

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## Authors' Contributions:

**KK:** Conceptualization; Investigation; Formal analysis; Data curation; Writing — original draft.  
**ZV:** Methodology; Investigation; Validation; Supervision; Formal analysis; Visualization: Writing — review and editing.

## Declaration of Conflict of Interests:

None to declare.

## Ethical approval:

Not applicable.

## Acknowledgements:

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The article provides brief information on observations of grey herons (*Ardea cinerea*) and great egrets (*Ardea alba*) in the Lviv and Cherkasy regions during winter. The accounted representatives of the *Ardeidae* family show a non-mass nature of wintering and were recorded only near water reservoirs not covered with ice. Such observations indicate low synanthropization and the importance of specific habitat types for these birds. In addition, the episodic nature of their detection proves their low resistance to winter conditions, therefore, in rather cold winters, representatives of the *Ardeidae* family were almost not recorded.

**Key words:** grey heron, great egret, winter avifauna, urban agglomerations, reservoirs

## Introduction

The grey heron (*Ardea cinerea*) and the great egret (*Ardea alba*) are the most common representatives of the heron family (*Ardeidae*) in Ukraine. These are wintering, migratory and nesting birds. They nest in colonies, feed on a variety of animal food: worms, insects, amphibians, fish, mammals [10].

The grey heron is the most common species in the entire territory of Ukraine, except for the mountainous part of the Carpathians and the Crimean Mountains and their adjacent territories [5, 10].

The great egret nests in almost the entire territory of Ukraine, except for the Carpathians, Mountainous Crimea and some adjacent areas, and it is the most numerous species in the Azov-Black Sea region [5, 10].

In recent years, however, information has been increasingly appearing that herons stay for the winter, which is probably caused by warm winters and climate changes.

During the literary sources analysis, we found out that the grey heron was found in the winter period in 1984. On December 1<sup>st</sup> A. A. Bokotei observed 3 in-

dividuals in Ivano-Frankove village in Yavoriv district (Lviv region). On December 31<sup>st</sup> H. V. Boyko found 1 grey heron in Dobrotvir town in Kamyanka-Buzka district (Lviv region) [6].

In 1997, M. Rahulina watched 2 grey herons on water reservoir in Staryy Dobrotvir village in Kamyanka-Buzka district (28.02.1997) [10]. According to data by D. V. Strashniuk, 2 individuals of the grey heron were observed at the water treatment facilities of the city of Ternopil (06.12.1997), as well as in the sumps of the sugar factory there was 1 individual of the great egret [8, 9].

In 1998, I. V. Shydlovskiy watched 3 great egrets in Cholhyni village in Yavoriv district (28.02.1998). D. Dubovyk observed one grey heron in Rusaniivtsi village in Letychiv district (Khmelnysky region) on Southern Bug river (01.02.1998) [11]. O. H. Hryshchuk counted one grey heron on Western Bug river between Sokal and Zvhryrka (Lviv region) (04.02.1998) [11].

In 1999, on December 12<sup>th</sup> M. V. Khymyn watched 3 grey herons in flight in Charukiv village (Lutsk region), on January 10<sup>th</sup> and December 23<sup>rd</sup> M. M. Khashchivsky observed one grey heron on Cherkhava river in Horodyshe village in Sambir district (Lviv region) [11].



In the territory of the Rivne region, the great egret is an atypical wintering species. In winter, birds were counted on lakes, ponds and rivers that do not freeze [1, 4].

The wintering of the great egret was registered in 2000–2003 in the Letychiv district of the Khmelnytskyi region. These birds were found in the valley of the South Bug River and its tributaries. The population during the winter of 2000/2001 was up to 25, in 2001/2002 it was up to 10, in 2002/2003 — up to 5 individuals [8].

On the territory of the Kremenchug reservoir, M. N. Havryliuk and his colleagues recorded wintering birds. On December 13, 2008, these authors observed 52 great egrets and 7 grey herons on the territory of the Sula Bay and on the ponds between the villages of Ly-pove and Bugaivka (Globyne district). In December 2008, 4 individuals of the great egret and 80 individuals of grey heron were observed on the territory of the Kaniv reservoir, and 8 grey herons were observed on the ponds near the Irkliiv village (Chornobaiv district, Cherkasy region) on December 12<sup>th</sup> [3].

In 2009, the authors observed one grey heron on the territory of the treatment facilities near the Chervona Sloboda village. In mid-February 2009, the authors observed a grey heron in the number of 12 individuals on the territory of Sula Bay and the nearby ponds. On the ponds near Sagunivka-Chervona Sloboda villages at the sewage treatment facilities and the adjacent areas of the reservoir in the same period, the authors observed 16 and 50 grey herons, respectively.

Therefore, the areas where herons stay for the winter differ in their geographical location, but they have one thing in common — the presence of reservoirs that do not freeze. These are mostly the man-made water bodies such as water reservoirs, sewage treatment plants, settling tanks.

There are a large number of ponds, lakes, and reservoirs in the territory of Lviv region, but representatives of *Ardeidae* are observed only in flight over the city of Lviv, they winter in waterbodies outside the city and can be seen while moving from one water reservoir to another.

On the territory of the Cherkasy region, there is the Kremenchuk Reservoir, which in turn provides the birds of the wetland complex with a territory for wintering with a large amount of feed.

Ternopil, Rivne, and Khmelnytskyi regions also have reservoirs of man-made origin, where birds often stay for the winter. In particular, on the territory of these regions there are cooling reservoirs of power plants.

Zhydachiv and Kaniv district-level agglomerations are located on rivers. Zhydachiv agglomeration is located on the Stryi River, which is a tributary of the Dniester, and the Kaniv one is located on the bank of the Dnipro River and has a large surface area due to the presence of the Kremenchuk Reservoir [13].

**The purpose** of this paper is a study of the quantitative composition of the heron family (*Ardeidae*) representatives in the territory of Lviv and Cherkasy urban agglomerations in the winter period.

## Materials and Research Methods

The research was carried out on the territory of urban agglomerations of Lviv and Cherkasy regions in the winter periods of 2020–2024. We selected 4 urban agglomerations: 2 of regional level (Lviv and Cherkasy) and 2 of district level (Zhydachiv and Kaniv).

The observations was conducted during the daylight from 8 am to 12 pm, by the method of point-counting, with a fixed detection zone, with the interval of 1–2 weeks. During the birdwatching we recorded all species of birds (including vocalization) in the radius of the circle  $R_1$  25 cm and  $R_2$  50 cm. To reduce the possible error, the points within the research areas are set accidentally with the help of qGis, which is used to create maps and store valuable information about discovering the habitats of various plant and animal species in nature conservation activities. For the birdwatching we used *Breaker 12×16* binoculars.

## Research Results

During the entire period of research, we found two species of herons — the grey heron (*Ardea cinerea*) and the great egret (*Ardea alba*) with a total number of 29 and 66 individuals, respectively.

In the territory of the city of Lviv, during the entire period of records, only six individuals of the grey heron and two of the great egret were observed. In contrast, in the territory of the Zhydachiv agglomeration representatives of the egrets were more numerous, in particular, 59 individuals of the great egret and 9 individuals of the grey heron were recorded.

On the territory of the Cherkasy agglomeration, only 17 individuals of the grey heron were observed, and the great egret was not recorded at all (although we know about its wintering in this territory, but during our research we did not manage to find it). Within the territory of the Kaniv agglomeration, we observed only 1 individual of the grey heron and 1 individual of the great egret.

This distribution of observations of wintering herons, in our opinion, indicates the importance for them of the presence of non-freezing water bodies, and at the same time with an insignificant depth. The Stryi River within the city of Zhydachiv is quite shallow and not wide, which provides birds with an opportunity to forage, unlike Lviv, where Poltava river with its confluents is almost entirely underground in collectors, but goes outside and flows within the city for more than 3 km.

There is also a significant difference between the rivers of both geographical locations (west and center of Ukraine), since the wide and deep Dnipro River flows within the borders of Kaniv and Cherkasy, where not all places within the rivulet are suitable for foraging by *Ardeidae* representatives.

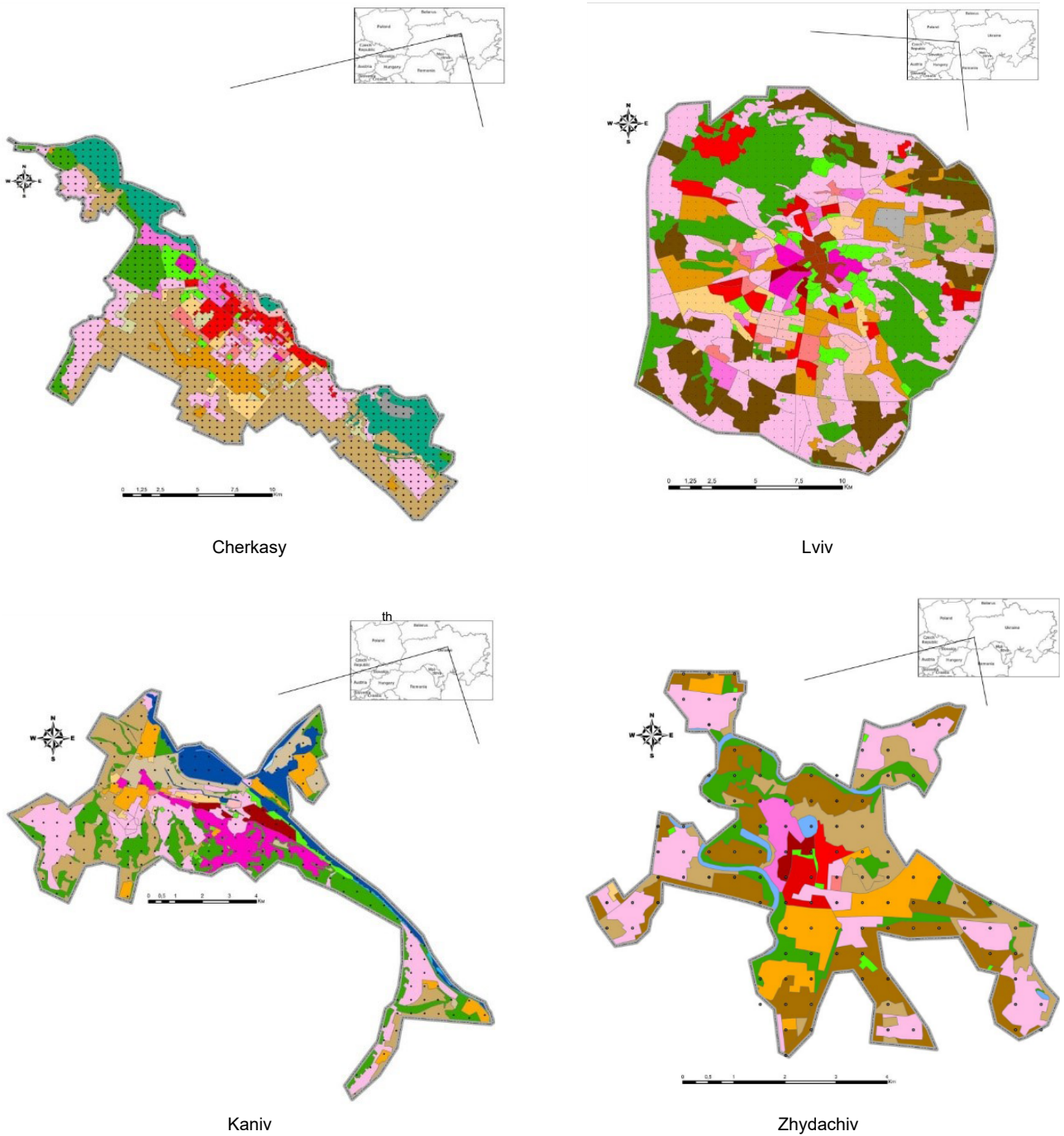


Fig. 1. Map of the research areas

According to the conducted records, wintering grey herons were more massively recorded in the territory of the Cherkasy urban agglomeration. In general, according to our data, its distribution is more uniform than that of the great egret, which is probably related to the significant, historically composed area of distribution of this heron, in contrast to the great egret, which expanded its range from the Azov-Black Sea coast to the north during the last 20–25 years.

On the territory of Ukraine, grey heron (*Ardea cinerea*) and the great egret (*Ardea alba*) can remain wintering due to the mild climate of certain regions. For example,

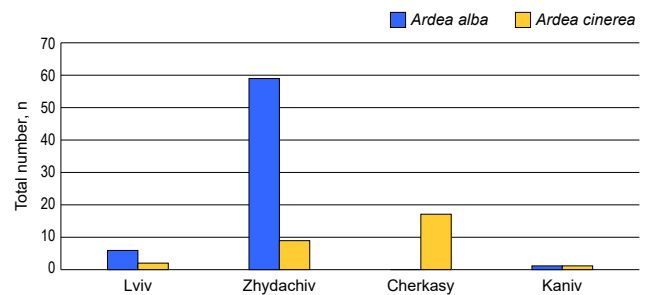


Fig. 2. The total number of *Ardea cinerea* and *Ardea alba* on the territory of the studied agglomerations in the winter periods of 2020–2024

in the southern and western regions in the valleys of large rivers, such as the Dnipro and Dniester, winters can be relatively mild. This allows the herons to stay in places where there is open water and enough forage.

The counted representatives of the *Ardeidae* family show the nature of wintering of non-mass species of birds and were recorded only near water bodies that are not covered with ice.

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## Спостереження чаплі сірої (*Ardea cinerea*) та чепури великої (*Ardea alba*) на території Львівської і Черкаської областей у зимовий період

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У статті наведена коротка інформація про спостереження у зимовий період чаплі сірої (*Ardea cinerea*) і чепури великої (*Ardea alba*) на території Львівської та Черкаської областей. Обліковані нами представники родини *Ardeidae* проявляють не масовий характер зимівлі і були зафіксовані лише біля водойм, які не вкриті кригою. Такі спостереження свідчать про низьку синантропізацію та важливість для цих птахів специфічних типів оселищ. Крім того, епізодичність їх виявлення доводить низьку стійкість до зимових умов, тому у досить холодні зими представників родини *Ardeidae* майже не фіксували.

**Ключові слова:** чапля сіра, чепура велика, зимова орнітофауна, міські агломерації, водойми



## Effectiveness of betaine, taurine, and myo-inositol in normalizing the antioxidant status of laying hens under heat stress

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### Authors' Contributions:

**PDB:** Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Validation; Visualization; Writing — original draft, review & editing.

### Declaration of Conflict of Interests:

None to declare.

### Ethical approval:

All procedures with chickens were performed in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2005), Council Directive 2010/63/EU, and the Law of Ukraine no. 3447-IV "On the Protection of Animals from Cruelty" with amendments 440-IX from 14.01.2020, following protocol no. 115a from 28.09.2022 of the Bioethics Commission for Scientific Research of the Institute of Animal Biology NAAS.

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Heat stress is a critical factor in the adaptation of animals to changing environmental temperature conditions and can significantly impact their health. Elevated ambient temperatures induce considerable stress, potentially leading to various adverse effects in poultry, including impairments in the antioxidant system. The imbalance between antioxidant and pro-oxidant processes can result in the excessive formation of free radicals, which harm cells and may contribute to the development of diseases. This study investigates the effects of artificially induced heat stress on the antioxidant system and lipid peroxidation products in the blood of laying hens. Laying hens, as commercial poultry lines selected for high egg productivity, are particularly vulnerable to high ambient temperatures due to their intensive metabolism, increased energy demand for egg production, and limited thermoregulatory capacity compared to other bird species. In intensive poultry farming, where bird density is high, these factors can exacerbate heat stress. The study aimed to identify changes in specific indicators of the antioxidant system and the content of lipid peroxidation products in the blood of chickens under the influence of betaine, taurine and myo-inositol. Analyzing parameters such as lipid hydroperoxides (LOOH), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) provides insights into the antioxidant defense system and oxidative stress levels under heat stress conditions. The study involved 15 laying hens housed in the vivarium of the Institute of Animal Biology NAAS, and was conducted in two phases. During the first phase, hens were kept at an ambient temperature of 20°C for three weeks. During the second phase, heat stress conditions were simulated by raising the temperature to 30°C for 6 hours daily over 7 days. Birds were divided into two groups: the control group (fed a standard diet) and the experimental group (supplemented with 0.5 g/kg betaine, 5 g/kg taurine, and 2 g/kg myo-inositol). Results showed that with increased ambient temperature in the control group, the content of LOOH decreased by 63% ( $P < 0.05$ ), while the activities of CAT, SOD, GSH-Px, and GR decreased by 28% ( $P < 0.001$ ), 49% ( $P < 0.01$ ), 15% ( $P < 0.01$ ), and 30% ( $P < 0.01$ ), respectively, compared to thermoneutral conditions. Conversely, GSH content increased by 37% ( $P < 0.01$ ). In the experimental group supplemented with betaine, taurine, and myo-inositol, CAT, GSH-Px, and GR activities decreased by 14% ( $P < 0.01$ ), 30% ( $P < 0.001$ ), and 23% ( $P < 0.05$ ), respectively, under thermoneutral conditions. Under heat stress conditions, LOOH content decreased by 59% ( $P < 0.05$ ), and GSH-Px activity decreased by 15% ( $P < 0.01$ ), while SOD and CAT activities increased by 55% ( $P < 0.001$ ) and 11% ( $P < 0.05$ ), respectively, compared to the control. The findings indicate the positive effects of betaine, taurine, and myo-inositol on the antioxidant system of laying hens under heat stress. The results highlight the potential of these supplements as effective strategies to maintain poultry health and productivity during heat stress.

**Key words:** laying hens, heat stress, oxidative stress, antioxidant defense system



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## Introduction

In recent years, global climate change has led to an increase in the frequency and duration of high-temperature periods, significantly impacting agriculture, particularly poultry farming [8]. Poultry are especially sensitive to heat stress (HS) due to their biological characteristics, such as high metabolic rates, elevated heat production, rapid growth, and high productivity. Commercial poultry species, particularly laying hens selected for high egg productivity, are even more vulnerable to HS due to their specific biological and physiological traits [11]. The optimal ambient temperature for their growth typically ranges between 18–24°C [15]. Temperatures exceeding this range, particularly above 30°C, can cause HS [10].

Elevated ambient temperatures can overload the thermoregulatory systems of animals, leading to increased heat production and, consequently, the activation of mechanisms that generate free radicals (FRs). These radicals are highly reactive molecules capable of damaging cells, proteins, lipids, and DNA. Under HS, the body attempts to counteract the adverse effects of high temperatures by activating antioxidant systems to neutralize FRs. These systems include both non-enzymatic low-molecular-weight antioxidants, such as reduced glutathione (GSH), and enzymatic high-molecular-weight antioxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione reductase (GR). These antioxidants limit the rate and progression of oxidation by detoxifying superoxide radicals, thereby protecting cells from oxidative damage [3]. However, intense HS can overwhelm the antioxidant system's capacity to manage these radicals, leading to increased oxidative stress (OS).

OS is characterized by an imbalance between the generation of reactive oxygen species (ROS) and their neutralization by antioxidant systems, resulting in damage to cells and tissues. ROS can react with unsaturated fats in cell membranes, forming lipid peroxides that compromise membrane integrity, increase permeability, and lead to cellular content leakage. OS also causes protein oxidation, which can result in protein denaturation and loss of biological activity. DNA oxidation may lead to mutations and strand breaks, affecting cellular division and function, increasing the risk of genetic alterations, and promoting the development of various diseases [12].

The antioxidant properties of compounds such as betaine, taurine, and myo-inositol are essential for maintaining poultry health, especially under stress conditions. Betaine, a natural methyl donor, can lower homocysteine levels in poultry blood, enhancing overall antioxidant activity and reducing OS [5]. Taurine, an amino acid, plays a critical role in antioxidant defense by neutralizing FRs and regulating intracellular calcium levels, thus mitigating cellular stress and maintaining

cellular function [1]. Myo-inositol reduces OS through multiple mechanisms, including lowering pro-oxidant molecule levels, activating antioxidant enzymes (SOD and CAT), and supporting cellular metabolism, such as phospholipid synthesis and signal transduction, which helps maintain membrane integrity and reduces oxidant accumulation [2]. Collectively, these compounds may significantly reduce OS and promote overall poultry health, making them valuable dietary supplements, particularly under HS conditions.

This study aimed to evaluate the effects of betaine, taurine, and myo-inositol on selected antioxidant system parameters (GSH levels, GPxs, GR, SOD, CAT activities) and the content of lipid hydroperoxides (LOOH) in the blood of chickens under HS conditions simulated in a controlled environment.

## Materials and Methods

The study involved 15 laying hens. The first, control group (C, n=7), consisted of birds fed a standard diet without additional components. The second, experimental group (E, n=8), received a compound feed supplemented with betaine (0.5 g/kg), taurine (5 g/kg), and myo-inositol (2 g/kg) based on dry matter. Experimental work was conducted in the vivarium of the Institute of Animal Biology NAAS. The hens were housed in metal cages equipped with automatic feeders and waterers. During the study, birds were fed a complete compound feed balanced for all necessary nutrients, vitamins, and microelements, with access to clean drinking water. The vivarium conditions were controlled to maintain specified temperature, humidity, and lighting according to the experimental plan.

The study consisted of two phases. In the first phase, birds were kept under thermoneutral conditions (TN) at a temperature of 20°C and relative humidity of 60% (temperature-humidity index = 66 [6]). Blood samples were collected on day 7 for biochemical analysis. Starting on day 8, the vivarium temperature was raised to 30°C for 6 hours daily, maintaining a relative humidity of 70% to simulate heat stress (temperature-humidity index = 81 [6]). On day 14, after one week of high-temperature exposure, the birds were decapitated, and biological material was collected for further studies.

All procedures with chickens were performed in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2005), Council Directive 2010/63/EU, and the Law of Ukraine no. 3447-IV "On the Protection of Animals from Cruelty" with amendments 440-IX dated 14.01.2020, following protocol no. 115a dated 28.09.2022 of the Bioethics Commission for Scientific Research of the Institute of Animal Biology NAAS.

The lipid hydroperoxide (LOOH) content was determined using the method described in [19]. A mixture of

0.2 ml blood plasma, 0.05 ml 50% TCA, and 2.8 ml ethanol was shaken for 5–6 minutes, then centrifuged at 3500 rpm for 10 minutes. A 1.5 ml supernatant aliquot was mixed with 1.2 ml ethanol, 0.02 ml concentrated HCl, and 0.03 ml 1% ammonium thiocyanate solution, then left for 30 seconds. A 20% thiocyanate solution induced a crimson color, and optical density was measured at 480 nm for 10 minutes. LOOH concentration was calculated as the difference between control and test samples and expressed in anson unit/ml (AU/ml).

Reduced glutathione (GSH) content was measured colorimetrically using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). In the test sample, 2 ml blood hemolysate and 3 ml precipitating reagent were combined. A control used 2 ml distilled water. After 5 minutes of incubation and centrifugation, the supernatant was mixed with 0.3 M  $\text{Na}_2\text{HPO}_4$  and Ellman's reagent. Absorbance was measured at 412 nm. GSH content was calculated using a calibration graph and expressed in mmol/l [19].

Glutathione peroxidase (GPxs) activity was assessed by measuring GSH oxidation with tertiary butyl hydroperoxide. A mixture of 0.1 ml hemolysate, TRIS buffer with EDTA and sodium azide, and GSH solution was incubated at 37°C. Tertiary butyl hydroperoxide was added, followed by 10% TCA. After centrifugation, the supernatant was mixed with TRIS buffer and Ellman's reagent, and absorbance was measured at 412 nm. GPxs activity was expressed as nmol GSH/min  $\times$  mg protein [19].

Glutathione reductase (GR) activity was determined by measuring NADPH oxidation. The mixture contained  $\text{K}_2\text{HPO}_4$ , oxidized glutathione, and EDTA. The reaction was initiated with NADPH and hemolysate, and absorbance decrease at 340 nm was monitored for 1 min. Enzyme activity was expressed as  $\mu\text{mol NADPH/min} \times \text{mg protein}$  [19].

Superoxide dismutase (SOD) activity was measured based on nitroblue tetrazolium (NBT) reduction by superoxide anions. A reaction mixture included hemolysate, NBT, EDTA, and PMS in phosphate buffer, with NADH added. Samples were incubated at 20°C for 10 min, and absorbance was recorded at 540 nm. SOD activity was expressed in anson unit/mg protein (AU/ml), using a calibration curve [19].

Catalase (CAT) activity was determined using molybdenum salts reacting with  $\text{H}_2\text{O}_2$  to form a colored complex. The test sample mixed hemolysate with  $\text{H}_2\text{O}_2$ . Control samples used ammonium molybdate. After incubation and acid addition, absorbance was measured at 410 nm [19].

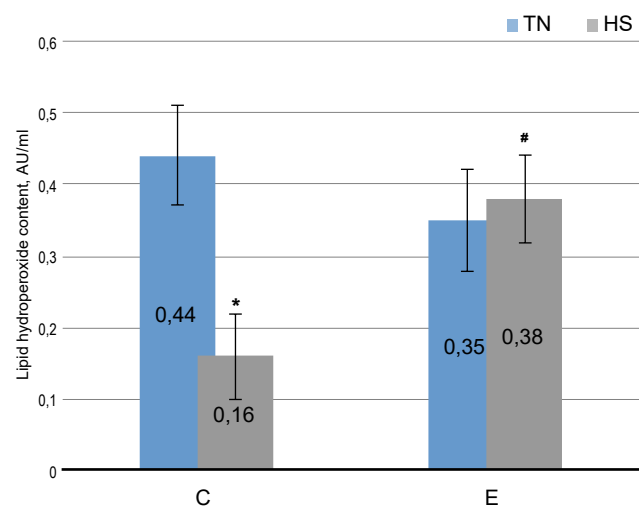
Statistical analysis was performed as described in [13]. Data are presented as mean  $\pm$  standard deviation. All data were analyzed using *Statistica 10* software. Statistical significance was determined using one-way analysis of variance (ANOVA). Student's *t*-test was used to evaluate differences between the two groups. Differences were considered statistically significant at  $P < 0.05$ .

## Results and Discussion

Lipid peroxidation is the process of oxidizing unsaturated fatty acids in cell membranes, leading to the formation of peroxides and other reactive products. This process can be triggered by free radicals and other oxidative agents [14]. Excessive activation of lipid peroxidation in biological systems increases the levels of lipid hydroperoxides and reactive aldehydes, which can be toxic to cells and tissues [16].

LOOH are the initial intermediates in cellular oxidation. They are formed when lipids react with free radicals and are unstable, quickly breaking down into other compounds such as aldehydes and ketones. Under stress, animals generally exhibit an increase in blood LOOH levels as an indicator of OS [7]. However, our study found that HS decreased LOOH levels in the blood serum of control group chickens by 2.7-fold ( $P < 0.05$ ) compared to chickens reared under thermoneutral conditions (fig.). This can be explained by HS reducing the availability of substrates for lipid peroxidation (e.g., polyunsaturated fatty acids), thereby decreasing LOOH formation. Significant GSH accumulation may also contribute to the reduction in LOOH levels [14].

The inclusion of dietary supplements under stressful conditions increased LOOH levels in chicken plasma by 2.4-fold ( $P < 0.05$ ) compared to the control group, reaching levels characteristic of TN conditions. Betaine and myo-inositol may stabilize cell membranes and maintain osmotic balance, but they also influence the metabolism of membrane lipids, particularly phosphatidylinositols containing polyunsaturated fatty acids [2, 5]. These acids are primary targets for lipid peroxidation, which could explain the increased LOOH levels.



**Fig.** Lipid hydroperoxide content in the plasma of laying hens under HS with the addition of betaine, taurine, and myo-inositol (M $\pm$ m, n=15)

*Note.* Here and further there is a statistically significant difference in data between HS and TN: \* —  $P < 0.05$ ; \*\* —  $P < 0.01$ ; \*\*\* —  $P < 0.001$ ; statistically significant difference in experimental data (E) compared to the control (C). TN — thermoneutral conditions; HS — heat stress.

SOD represents the first line of cellular defense against OS. It catalyzes the conversion of superoxide radicals ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ) and oxygen ( $O_2$ ). This process is vital for the organism as superoxide radicals are highly reactive and can damage cellular components, including DNA, proteins, and lipids. By reducing superoxide levels, SOD helps prevent cellular damage and maintains homeostasis [4].

In our study, HS in the control group caused a 49% reduction in SOD activity in the blood ( $P < 0.01$ ) compared to TN conditions (table 1). In the experimental group, dietary supplementation under HS increased SOD activity in chicken erythrocytes by 55% compared to the control group, restoring levels to those characteristic of TN conditions. This suggests enhanced antioxidant protection through taurine, betaine, and myo-inositol supplementation, which activates SOD under HS, thereby providing better protection for erythrocytes against oxidative damage.

CAT is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen, preventing the accumulation of this toxic compound in cells. Hydrogen peroxide is potentially harmful as it can initiate the formation of hydroxyl radicals, which damage cellular structures such as proteins, nucleic acids, and lipids [18]. In our study, CAT activity in the erythrocytes of laying hens under HS in the control group decreased significantly by 28% ( $P < 0.001$ ) compared to TN conditions. The addition of supplements under normal temperatures reduced CAT activity in animal erythrocytes by 14% ( $P < 0.01$ ). However, under elevated temperatures, CAT activity increased by 11% ( $P < 0.05$ ) compared to control values. This suggests a modest positive effect of supplements on the ability of erythrocytes to maintain catalase activity under stress, potentially linked

to increased cellular resistance to oxidative stress and an improved overall antioxidant status in laying hens [14].

The glutathione pathway of antioxidant defense is a key system for maintaining cellular redox homeostasis. It neutralizes ROS and prevents oxidative damage to biomolecules. The primary enzymes of this system operate in close coordination, regenerating antioxidants and maintaining a stable redox balance, which is critical for cellular function under oxidative stress [17]. This pathway includes GSH, GPxs, and GR, which we analyzed in the blood of laying hens in our study (table 2).

GSH is one of the most important non-enzymatic antioxidants, playing a crucial role in reducing LOOH through GPxs and neutralizing ROS and free radicals [16]. Under HS, we observed a 37% ( $P < 0.01$ ) increase in GSH levels in the control group and a 35% ( $P < 0.01$ ) increase in the experimental group compared to TN conditions. These findings indicate an increased need for antioxidant defense in response to heat stress.

GPxs is a key antioxidant enzyme that catalyzes the reduction of peroxides using GSH as a reducing agent [4]. As shown in table 2, HS in the control group significantly decreased GPxs activity in the blood of laying hens by 15% ( $P < 0.01$ ) compared to TN conditions, likely due to enzyme depletion under chronic oxidative stress. In the experimental group, dietary supplementation reduced GPxs activity in animal blood by 30% ( $P < 0.001$ ) under TN and by 15% ( $P < 0.01$ ) under HS compared to control values. This reduction may reflect decreased overall pro-oxidative stress due to membrane stabilization and increased GSH activity.

GR is an enzyme that catalyzes the conversion of oxidized glutathione (GSSG) into GSH using NADPH as a reducing agent [16]. GR activity analysis showed a 30% ( $P < 0.01$ ) decrease in erythrocytes of the control group under HS compared to TN. This indicates a decline in glutathione reductase functionality under HS due to prolonged oxidative stress, reducing the efficiency of the glutathione cycle. Dietary supplementation also reduced GR activity in birds' blood by 23% ( $P < 0.05$ ) compared to controls under normal temperature conditions, likely due to decreased need for GSSG reduction as peroxide and ROS levels declined. No significant changes in GR activity were observed under elevated temperatures in the experimental group, although there was a tendency for reduced activity.

Overall, the avian response to stress may involve increased production of certain antioxidants, such as GSH, to mitigate oxidative stress. However, this can deplete resources, negatively affecting other antioxidant enzymes like CAT, SOD, GPxs, and GR [3].

The reduced activity of some antioxidants in the experimental group may result from supplements such as betaine, taurine, and myo-inositol acting as direct

**Table 1.** Superoxide dismutase and catalase activities in the erythrocytes of laying hens under HS with the addition of betaine, taurine, and myo-inositol ( $M \pm m$ ,  $n=15$ )

Indicators	Condi-tions	Control	Experiment
SOD, AU/mg protein	TN	17,43±1,38	17,79±1,41
	HS	8,90±1,10**	19,57±0,87###
CAT, mmol $H_2O_2$ /min×mg of protein	TN	247,64 ± 3,35	212,16 ± 13,03##
	HS	177,10 ± 5,10***	198,04 ± 7,99#

**Table 2.** Indicators of the glutathione pathway of antioxidant defense in the erythrocytes of laying hens under HS with the addition of betaine, taurine, and myo-inositol ( $M \pm m$ ,  $n=15$ )

Indicators	Condi-tions	Control	Experiment
GSH, mmol/L	TN	0,50±0,06	0,58±0,09
	HS	0,79±0,04**	0,89±0,09**
GPxs, nmol GSH/min×mg protein	TN	145,65±7,73	101,36±6,70###
	HS	123,78±6,50**	104,58±5,41##
GR, $\mu$ mol NADPH/min×mg protein	TN	8,03±1,07	6,17±0,38#
	HS	5,61±0,69**	5,12±0,56



antioxidants. These compounds may interact with free radicals and oxidative molecules like hydrogen peroxide and superoxide anions, reducing their activity and converting them into less harmful compounds. Additionally, these compounds enhance cellular protective mechanisms, reduce inflammation, and support overall redox balance [1, 9]. Hence, antioxidant supplementation may trigger adaptive processes, reducing the need for endogenous antioxidant enzyme production due to the external antioxidant protection. This mechanism may contribute to stress mitigation and homeostasis restoration.

The study demonstrated that heat stress negatively affects the antioxidant status of laying hens, evidenced by reduced activity of key antioxidant enzymes (CAT, SOD, GPxs, GR) and LOOH levels. This indicates increased oxidative stress, disrupting the balance between pro- and antioxidant processes. Concurrently, the compensatory increase in GSH levels suggests the activation of non-enzymatic antioxidant defense pathways as a partial response to oxidative burden.

Dietary supplementation with taurine, betaine, and myo-inositol under HS normalized the antioxidant system of laying hens. Specifically, SOD activity was restored to TN levels, CAT activity moderately increased, and LOOH levels stabilized. The reduced GPxs and GR activity in the experimental group indicates a lower demand for their function due to enhanced alternative antioxidant pathways and reduced pro-oxidative stress.

Thus, the findings confirm that betaine, taurine, and myo-inositol supplements can mitigate oxidative stress, although their efficacy under HS remains limited. Further research is needed to optimize supplement composition and dosing to enhance antioxidant activity under stress conditions.

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## Ефективність бетаїну, таурину та міо-інозитулу у нормалізації антиоксидантного статусу курей при тепловому стресі

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Тепловий стрес є одним із ключових чинників, що впливають на адаптацію тварин до змін температури навколишнього середовища, і може значно позначитися на їхньому здоров'ї. Підвищена температура навколишнього середовища спричиняє значний стрес, що потенційно призводить до різних негативних наслідків у птиці, зокрема до порушень у роботі антиоксидантної системи. Дисбаланс між антиоксидантними та прооксидантними процесами може спричинити надмірне утворення вільних радикалів, які шкодять клітинам і можуть сприяти розвитку захворювань. У цьому дослідженні вивчено вплив штучно індукованого теплового стресу на антиоксидантну систему та продукти пероксидного окислення ліпідів у крові курей-несучок. Курей-несучки як комерційні лінійні птиці, селекціоновані для високої продуктивності яєць, є особливо вразливими до підвищених температур через інтенсивний обмін речовин, збільшений енергетичний попит на виробництво яєць та обмежену здатність до терморегуляції порівняно з іншими видами птахів. В умовах інтенсивного птахівництва, де щільність утримання висока, ці фактори можуть посилювати тепловий стрес. Метою дослідження було визначити зміни в окремих показниках антиоксидантної системи та вмісту продуктів пероксидного окислення ліпідів у крові курей під впливом бетаїну, таурину та міо-інозитулу. Аналіз таких параметрів, як ліпідні гідроперекиси (LOOH), відновлений глутатіон (GSH), глутатіонпероксидаза (GSH-Px), глутатіонредуктаза (GR), каталаза (CAT) та супероксиддисмутаза (SOD), дає змогу оцінити стан антиоксидантного захисту та рівень оксидативного стресу в умовах теплового стресу. Дослідження проводили на 15 курках-несучках, яких утримували у віварії Інституту біології тварин НААН. Воно складалося з двох етапів: протягом першого етапу курей утримували за температури 20°C протягом трьох тижнів. На другому етапі моделювали тепловий стрес, підвищуючи температуру до 30°C на 6 годин щодня протягом 7 днів. Птицю розділили на дві групи: контрольну (годували стандартним раціоном) та експериментальну (додатково згодовували 0,5 г/кг бетаїну, 5 г/кг таурину та 2 г/кг міо-інозитулу). Результати показали, що за підвищення температури в контрольній групі вміст LOOH знизився на 63% ( $P < 0,05$ ), а активність CAT, SOD, GSH-Px та GR зменшилася на 28% ( $P < 0,001$ ), 49% ( $P < 0,01$ ), 15% ( $P < 0,01$ ) та 30% ( $P < 0,01$ ) відповідно порівняно з термонеутральними умовами. Водночас вміст GSH зріс на 37% ( $P < 0,01$ ). В експериментальній групі, де застосовували добавки, активність CAT, GSH-Px та GR знизилася на 14% ( $P < 0,01$ ), 30% ( $P < 0,001$ ) та 23% ( $P < 0,05$ ) відповідно в термонеутральних умовах. За умов теплового стресу вміст LOOH знизився на 59% ( $P < 0,05$ ), активність GSH-Px — на 15% ( $P < 0,01$ ), тоді як активність SOD та CAT зросла на 55% ( $P < 0,001$ ) та 11% ( $P < 0,05$ ) відповідно порівняно з контрольною групою. Отримані результати свідчать про позитивний вплив бетаїну, таурину та міо-інозитулу на антиоксидантну систему курей-несучок за умов теплового стресу. Це підкреслює перспективність використання цих добавок для збереження здоров'я та продуктивності птиці в умовах високих температур.

**Ключові слова:** кури-несучки, тепловий стрес, оксидативний стрес, антиоксидантна система захисту D. B. Perederiy



## Вплив екзогенних ензимів та різних форм Сульфору в раціонах курчат-бройлерів на продуктивність і якість їх продукції

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До біологічно активних чинників живлення, які позитивно впливають на перетравність і засвоєння поживних речовин кормів, належать ензимні препарати. Вони є мультиензимними і характеризуються відповідною спрямованістю на поліпшення перетравлення протеїнів, клітковини, бета-глюканів, арабіно-ксиланів тощо. Однак ефективне їх використання в раціонах для птиці деяких видів, певного віку і напрямку продуктивності потребує додаткових досліджень. Для забезпечення біологічної потреби птиці в поживних речовинах, дефіцит сірковмісних амінокислот частково можна компенсувати додатковим додаванням сульфатів, що сприяє підвищенню рівня компонентів сульфонових амінополісахаридів, а також може поліпшувати функціонування мукоїдного бар'єру травного тракту і стимулювати всмоктування поживних речовин корму. Перспективними є системні дослідження інтенсивності метаболічних процесів в організмі птиці за введення в раціон екзогенних ензимів у комплексі із Сульфуром у різних формах для кращого розщеплення, перетравлення і засвоєння поживних речовин корму та підвищення біологічної та харчової якості продукції птахівництва. Дослід проведено в умовах віварію Інституту біології тварин НААН на молодняку курей м'ясного напрямку продуктивності кросу РОСС-8, починаючи з 10-добового віку, поділених на чотири групи (по 20 голів у кожній). Утримання курчат-бройлерів у клітках і їх годівля відповідали технологічним вимогам. Уся птиця отримувала повнораціонний комбікорм. Курчата контрольної групи споживали комбікорм з додатковим уведенням 0,3% сульфату натрію. Птиці I дослідної групи до раціону додавали 0,3% сульфату натрію і Натузим; курчатам II дослідної групи — цитрат Сульфору (25% від тієї кількості, яку отримували курчата в контрольній групі, в перерахунку на Сульфур) і Натузим; курчатам III дослідної групи — цитрат Сульфору (10% від тієї кількості, яку отримували курчата в контрольній групі, в перерахунку на Сульфур) і Натузим. З'ясовано, що ефективність додаткового введення до раціону курчат-бройлерів Сульфору залежить від форми й кількості елемента. Доведено доцільність заміни в раціонах птиці м'ясного напрямку продуктивності мінеральної добавки Сульфур у формі неорганічної солі ( $\text{Na}_2\text{SO}_4$ ) цитратом елемента нанотехнологічного походження в кількості, що становить 10% від вмісту елемента в неорганічній формі, та добавки комплексного ензимного препарату Натузим. Це підвищує продуктивність птиці (маса тіла зростає на 1,7%; маса півпатраної тушки — на 4,16%; забійний вихід — на 2,12%) та якість отриманої продукції (у грудних м'язах вірогідно знижується вміст етерифікованого і вільного холестеролу ( $P < 0,01$ ) та вільних жирних кислот ( $P < 0,05$ ) за одночасного зростання кількості триацилгліцеролів ( $P < 0,01$ )).

**Ключові слова:** Сульфур, ензимна добавка, курчата-бройлери, продуктивність

## Вступ

Сучасні темпи розвитку птахівничої галузі вимагають вирішення таких завдань, як розроблення ефективної концепції годівлі сільськогосподарської птиці з внесенням відповідних корективів [16]. Адже раціональна і повноцінна годівля найістотніше сприяє росту і розвитку птиці, її збереженості й високій відтворній здатності, реалізації генетично обумовленої продуктивності та виробництву продукції відповідної якості [3, 20]. Водночас важливим важелем зниження собівартості продукції птахівництва залишається розроблення й оптимізація наявних методів годівлі, що базуються на використанні повноцінних за складом і поживністю раціонів [8].

До біологічно активних чинників живлення, які позитивно впливають на перетравність і засвоєння поживних речовин кормів, належать ензимні препарати. Саме необхідність поліпшення травлення субстратів є головним обґрунтуванням використання екзогенних ензимів у птахівництві [21]. У сучасному кормовиробництві є великий вибір ензимних кормових добавок та препаратів, які здатні покращувати перетравність і засвоєння поживних речовин в інгредієнтах і кормі загалом. Ці препарати є мультиензимними і характеризуються відповідною спрямованістю на поліпшення перетравлення білків, клітковини, бета-глюканів, арабіноксиланів тощо [3]. Однак ефективне їх використання в раціонах для птиці окремими видами, певного віку і напрямку продуктивності потребує додаткових досліджень [9].

Сульфур є важливим біогенним елементом, входить до складу амінокислот метіоніну й цистеїну і, відповідно, до складу пептидів і протеїнів. Основна його кількість надходить в організм птиці з кормом (причому близько 10% у неорганічній формі) та є важливим біогенним елементом. Джерелом сульфату стають й Сульфуромісні амінокислоти у процесі їх перетворення.

З метою забезпечення біологічної потреби птиці в поживних речовинах, дефіцит сірковмісних амінокислот частково можна компенсувати шляхом додаткового введення сульфатів, зокрема сульфату Na у кількості 0,3% [11]. Сульфат натрію сприяє підвищенню вмісту компонентів сульфонових амінополісахаридів, що може поліпшувати функціонування мукоїдного бар'єра травного тракту і стимулювати всмоктування поживних речовин корму [1, 3]. Отже, перспективними є системні дослідження інтенсивності метаболічних процесів в організмі птиці за введення у раціон екзогенних ензимів у комплексі із Сульфуром у різних формах з метою підвищення розщеплення, перетравлення і засвоєння поживних речовин корму та біологічної і харчової якості продукції птахівництва.

Метою роботи є з'ясування можливості введення до раціонів птиці Сульфуром саме у формі аквацитрату на заміну елемента у формі неорганічної солі [7, 14, 15].

## Матеріали і методи

Дослід проведено в умовах віварію Інституту біології тварин НААН на молодняку курей м'ясного напрямку продуктивності кросу РОСС-8, починаючи з 10-добового віку, з яких сформували чотири групи (по 20 голів у кожній). Курчата, яких відібрано для дослідів, були активними, зі швидкою реакцією. Пір'яний покрив — чистий, гладкий і рівномірний. Слизові оболонки цілісні, достатньо зволожені, природного кольору, без набряків. Фізіологічні показники маси й температури тіла, частоти дихання і серцебиття — в межах референтних величин. Тобто, відібрані для дослідів курчата-бройлери були клінічно здоровими, що дало підставу надалі об'єктивно оцінювати результати, отримані в експериментах.

Утримання курчат-бройлерів у клітках і їх годівля відповідали технологічним нормам. Уся птиця отримувала повнораціонний комбикорм (ПК), збалансований за поживними і біологічно активними речовинами (відповідно до схеми, поданої в табл. 1). Курчатам усіх дослідних груп (першої, другої і третьої) до раціону додавали ензимний препарат Натузим. Птиці

**Таблиця 1.** Схема дослідів на курчатах-бройлерах  
**Table 1.** Scheme of the experiment on broiler chickens

Група / Group	Характер живлення / Type of food
Контрольна Control	ПК+0,3 % Na <sub>2</sub> SO <sub>4</sub> CF+0,3 % Na <sub>2</sub> SO <sub>4</sub>
Дослідна 1 Experimental 1	ПК+0,3 % Na <sub>2</sub> SO <sub>4</sub> +Натузим CF+0,3 % Na <sub>2</sub> SO <sub>4</sub> +Natuzyum
Дослідна 2 Experimental 2	ПК+цитрат Сульфур (25 % від контролю, в перерахунку на Сульфур)+Натузим CF+Sulfur citrate (25 % of the control, in terms of sulfur)+Natuzyum
Дослідна 3 Experimental 3	ПК+цитрат Сульфур (10 % від контролю, у перерахунку на Сульфур)+Натузим CF+Sulfur citrate (10 % of the control, in terms of sulfur)+Natuzyum

**Таблиця 2.** Склад ензимного препарату Натузим  
**Table 2.** Composition of the enzyme preparation Natuzyum

Ензим Enzyme	Дія Action	Активність Activity
Целюлоза Cellulose	Перетворює клітковину на глюкозу Converts fibre to glucose	6 000 000 од./кг або 200 000 МО/кг
Протеаза Protease	Розщеплює протеїни до амінокислот Breaks down proteins into amino acids	600 000 од./кг або 700 000 МО/кг
Ксиналаза Xinalase	Гідролізує ксилан до ксилолу Hydrolyses xylan to xylene	10 000 000 од./кг або 500 000 МО/кг
α-амілаза α-amylase	Розщеплює крохмаль до простих цукрів Breaks down starch into simple sugars	400 00 од./кг або 700 00 МО/кг
β-глюконаза β-gluconase	Розщеплює глюкани Breaks down glucans	700 000 од./кг або 200 00 МО/кг
Фітаза Phytase	Вивільняє фосфор з фітатів Releases Phosphorus from phytates	600 000 од./кг або 900 000 МО/кг

другої і третьої дослідних груп вводили в раціон аквацитрат Сульфору нанотехнологічного походження (на заміну Сульфору у формі неорганічної солі  $\text{Na}_2\text{SO}_4$ ), який нам надали для досліджень співробітники ТОВ «Наноматеріали і нанотехнології» (м. Київ), відповідно до поданої схеми.

Комплексний ферментний препарат Натузим (табл. 2) утворений трьома штамми бактерій і грибів (*Trichoderma Longibrachiatum*, *Bacillus subtilis*, *Aspergillus Niger*), які продукують шість ензимних активностей, що підсилюють один одного.

Упродовж досліджу спостерігали за фізіологічним станом птиці, збереженістю поголів'я і продуктивністю. У кінці досліджу проведено забій птиці й відібрано для досліджень біологічний матеріал (взрізці грудного м'язу і печінки).

Статистичну обробку одержаних цифрових даних проводили за допомогою програми *Statistica* для *Windows XP* з використанням *t*-критерію Стьюдента [10]. У кожному з дослідів визначали ступінь вірогідності різниці (*P*) між відповідними досліджуваними показниками птиці контрольних груп та відповідними досліджуваними показниками птиці дослідних груп. Результати середніх значень вважали статистично вірогідними за  $P < 0,05$  (\*),  $P < 0,01$  (\*\*) та  $P < 0,001$  (\*\*\*).

## Результати й обговорення

Показники підвищення продуктивності курчат-бройлерів упродовж останніх чотирьох десятиліть щороку покращувалися. Така тенденція, ймовірно, продовжиться й у майбутньому, оскільки птахівництво буде впроваджувати нові технології в генетиці, селекції, біотехнології та біології розвитку птиці. У реалізації генетичного потенціалу птиці сучасних кросів і ліній щодо її здоров'я й продуктивності важливу роль відіграє годівля, збалансована за всіма поживними та біологічно активними речовинами [11, 13].

Ми провели дослідження, щоб з'ясувати ефективність комплексного введення в раціон курчат-бройлерів мінеральної добавки Сульфур у формі аквацитрату та ензимного препарату. Збереженість поголів'я птиці в усіх групах становила 100%. Водночас, за період досліджу найвищі (майже на 2%) прирости маси тіла отримано у птиці третьої дослідної групи, тобто тієї, якій випоювали Сульфур в органічній формі в кількості, що становила (в перерахунку на елемент) 10% від його вмісту у формі неорганічної солі в раціоні контрольної групи (табл. 3).

Такі ж тенденції відзначено й щодо маси напівпатої тушки (вилучені кишківник з клоакою, яєчник, яйцепровід). А саме — у курчат дослідних груп ці показники були вищими на 2,33%; 2,61% та 4,16% (табл. 4). Забійний вихід у контрольній групі становив 84,31%, а в дослідних — перевищував цей показник на 1,3%; 1,28% та 2,12% відповідно.

**Таблиця 3.** Маса курчат, до раціону яких уведено ензимний препарат і різні форми Сульфору, г ( $M \pm m$ ,  $n=10$ )  
**Table 3.** Weight of chickens after introduction of enzyme preparation and different forms of Sulfur into the diet, g ( $M \pm m$ ,  $n=10$ )

Група / Group	Маса, г / Weight, g		
	постановка на дослід (10-дбові курчата) / putting to the test (10-day-old chickens)	завершення досліджу (42-дбові курчата) / completion of the experiment (42-day-old chickens)	прирости за період досліджу / growth over the period of the experiment
Контрольна Control	184,4±6,31	2460,3±95,34	2275,9±24,11
Дослідна 1 Experimental 1	181,2±5,03	2479,5±98,98	2298,3±41,18
Дослідна 2 Experimental 2	190,0±4,92	2487,0±97,22	2297,0±37,56
Дослідна 3 Experimental 3	185,3±7,17	2499,9±104,13	2314,6±28,07

Вважається, що стан печінки є дзеркалом стану організму, адже печінка виконує різноманітні функції. Зокрема відіграє значну роль як «залоза травлення» — продукує жовч, бере участь у вуглеводному обміні й регуляції рівня цукру в крові; виступає «депо» для відкладання жиру — запасного джерела енергії; бере участь в імунігенезі, процесах зсідання крові [6]. Дуже важливою є дезінтоксикаційна функція органа, за яку відповідають клітини печінки — гепатоцити й зірчасті клітини (макрофаги) [4]. Збільшення розмірів печінки може свідчити про наявність чинників, дія яких негативно впливає на весь організм.

У курчат-бройлерів контрольної та всіх дослідних груп печінка була розташована у ділянці від другого по шостий міжреберний проміжок, права і ліва частки печінки однакові за розміром. Результати обчислення індексу маси печінки бройлерів (співвідношення маси печінки до маси тіла, виражене у відсотках) подано в табл. 4.

Варто зазначити, що в курчат контрольної групи показник індексу маси печінки наближений до верхньої межі референтних величин. Натомість показники курчат дослідних груп були нижчими. Оскільки індекс печінки пов'язаний з підвищеними енергетичними витратами, тобто його збільшення може свідчити про критичні навантаження на цей орган, а отже й на організм загалом, то отримані результати можуть свідчити про ефективність введення ензимного комплексу в раціон птиці. Водночас у курчат-бройлерів другої та третьої дослідних груп індекс маси печінки був нижчим, порівняно з контролем, на 0,25% та 0,22%. Можливо, це є наслідком дії саме цитратованої форми Сульфору на організм птиці. На думку багатьох учених, мінеральні елементи у формі нанорозмірних часточок проявляють стимулювальний вплив на метаболічні процеси в організмі птиці вираженіше, ніж їхні відомі молекулярні форми [2, 5, 18].



**Таблиця 4.** Показники маси тіла та печінки курчат-бройлерів, г  
**Table 4.** Indicators of body weight and liver of broiler chickens, g

Показники /Indication	Групи курчат-бройлерів / Groups of broiler chicks			
	Контрольна Control	Дослідна 1 Experimental 1	Дослідна 2 Experimental 2	Дослідна 3 Experimental 3
жива маса, г / live weight, g	2460,3±95,3	2479,5±98,98	2487,0±97,22	2499,9±104,13
маса напівпатраної тушки, г / weight of semi-gutted carcass, g	2074,27±73,3	2122,69±82,4	2128,37±91,6	2160,66±88,6
забійний вихід, % / slaughter yield, %.	84,31	85,61	85,58	86,43
маса печінки, г / liver weight, g	58,81±1,14	56,27±0,23	53,32±0,44	54,37±0,64
індекс маси печінки, % / liver weight index, %	2,39	2,26	2,14	2,17

**Таблиця 5.** Хімічний склад грудного м'яза курчат-бройлерів, до раціонів яких вводили ензимний препарат і різні форми Сульфуру, % (M±m, n=5)  
**Table 5.** Chemical composition of the pectoral muscle of broiler chickens after the introduction of an enzyme preparation and different forms of Sulfur into diets, % (M±m, n=5)

Показник / Indication	Група птиці / Groups of poultry			
	Контрольна Control	Дослідна 1 Experimental 1	Дослідна 2 Experimental 2	Дослідна 3 Experimental 3
Суша речовина / Dry matter	26,5±0,37	26,1±0,22	26,9±0,44	27,5±0,48
Протеїн / Protein	20,1±0,09	19,6±0,32	20,3±0,18	20,9±0,14
Жир / Fat	4,32±0,01	4,98±0,13**	5,12±0,03***	4,87±0,08**
Глікоген / Glycogen	1,2±0,07	1,1±0,09	1,3±0,04	1,3±0,03
Зола / Ash	1,7±0,07	1,7±0,06	1,6±0,05	1,7±0,07

**Таблиця 6.** Вміст загальних ліпідів та співвідношення їх окремих класів у тканинах печінки курчат-бройлерів, %, (M±m, n=5)  
**Table 6.** The content of total lipids and the ratio of their individual classes in liver tissues of broiler chickens, %, (M±m, n=5)

Показник / Indication	Група птиці / Groups of poultry			
	Контрольна Control	Дослідна 1 Experimental 1	Дослідна 2 Experimental 2	Дослідна 3 Experimental 3
Загальні ліпіди / Total lipids	8,40±0,21	9,13±0,18	9,06±0,13	8,68±0,19
Класи ліпідів / Lipid classes:				
Фосфоліпіди / Phospholipids	30,48±1,05	31,29±1,78	30,32±1,92	29,42±1,65
Моно- і диацилгліцероли / Mono and diacylglycerols	9,16±0,29	8,74±0,25	9,57±0,37	8,58±0,20
Вільний холестерол / Free cholesterol	7,65±0,24	7,68±0,41	9,27±0,18*	8,02±0,25
Вільні жирні кислоти / Free fatty acids	15,74±0,10	16,54±0,15	16,42±0,21	14,10±0,09*
Триацилгліцероли / Triacylglycerols	19,05±0,17	19,51±0,21	20,47±0,30*	19,85±0,11*
Етерифікований холестерол / Esterified cholesterol	15,84±0,22	14,97±0,17	16,16±0,19	16,54±0,26*

**Таблиця 7.** Вміст загальних ліпідів та співвідношення їх окремих класів у грудних м'язах курчат-бройлерів, %, (M±m, n=5)  
**Table 7.** The content of total lipids and the ratio of their individual classes in the pectoral muscles of broiler chickens, %, (M±m, n=5)

Показник / Indication	Група / Group			
	Контрольна Control	Дослідна 1 Experimental 1	Дослідна 2 Experimental 2	Дослідна 3 Experimental 3
Загальні ліпіди / Total lipids	4,32±0,01	4,98±0,03**	5,12±0,0***	4,87±0,02**
Класи ліпідів / Lipid classes:				
Фосфоліпіди / Phospholipids	33,84±0,89	34,14±1,33	34,42±1,75	35,23±1,50
Моно- і диацилгліцероли / Mono and diacylglycerols	14,37±0,40	12,29±0,11*	12,09±0,18*	11,61±0,26*
Вільний холестерол / Free cholesterol	6,56±0,09	5,13±0,05*	5,93±0,04	5,43±0,04
Вільні жирні кислоти / Free fatty acids	7,85±0,08	8,25±0,04	6,22±0,08*	5,31±0,05**
Триацилгліцероли / Triacylglycerols	28,72±0,11	35,18±0,14	35,02±0,19	36,05±0,12**
Етерифікований холестерол / Esterified cholesterol	8,25±0,02	5,01±0,01**	5,84±0,01**	6,37±0,02*

Щодо хімічного складу м'яса курчат-бройлерів дослідних груп, то встановлено (табл. 5), що вміст сухої речовини, протеїну і глікогену в тканинах грудного м'яза був на рівні показників птиці контрольної групи. Тоді як вміст загальних ліпідів, порівняно з контролем, був вищим у м'язах курчат другої і третьої дослідних груп ( $P < 0,5-0,001$ ).

Не виявлено (табл. 6) вірогідних змін кількості загальних ліпідів, фосфоліпідів, моно- і диацилгліцеролів у печінці курчат-бройлерів дослідних груп, порівняно з аналогічними показниками контролю. Щодо рівня триацилгліцеролів, то він зростав у печінці курчат другої і третьої дослідних груп відповідно на 1,42% та 0,8% ( $P < 0,05$ ). Уміст вільного холестеролу дещо підвищувався лише в печінці птиці другої дослідної групи ( $P < 0,05$ ), а етерифікованого холестеролу — третьої дослідної групи ( $P < 0,05$ ).

Холестерол — стабілізувальна складова біологічних мембран і вихідний матеріал для синтезу стероїдних гормонів. Він є найважливішим прекурсором жовчних кислот. Етерифікований холестерол також неполярний, гідрофобний і виконує роль резервної чи транспортної форми холестерину. Очевидно, отримані в досліді зміни зумовлені тим, що печінка вважається своєрідним «фондом» холестерину і служить одночасно як основним джерелом, так і головним центром розподілу холестерину в організмі, може використовуватися для синтезу жовчних кислот, включатися в мембрани гепатоцитів, секретуватися в жовч, а далі в кишківник, потрапляти в кров у складі ліпопротеїнів і переноситися до позапечінкових органів [19].

Встановлено, що за комплексного впливу цитрату Сульфору в різних концентраціях та ензимного препарату в м'язах більшості тварин збільшується вміст ліпідів. Зокрема, у підсумку проведених досліджень виявлено незначне, але вірогідне збільшення рівня загальних ліпідів у тканинах грудних м'язів курчат усіх дослідних груп в 1,15; 1,18 та 1,13 рази ( $P < 0,01-0,01$ ) відповідно (табл. 7), порівняно з контролем. Причиною цих різниць може бути інтенсифікація обміну речовин в організмі курчат-бройлерів.

Результати визначення окремих класів ліпідів показали, що додавання цитрату Сульфору як в кількості, еквівалентній 25% від контролю, в перерахунок на Сульфур (друга дослідна група), так і 10% (третья дослідна група), мали помітний вплив на співвідношення окремих класів ліпідів (табл. 7). Так, у грудних м'язах курчат-бройлерів усіх дослідних груп виявлено менший вміст моно- і диацилгліцеролів та вільних жирних кислот ( $P < 0,01$ ) і більший вміст триацилгліцеролів ( $P < 0,01$ ). Ці дані свідчать про посилення синтезу триацилгліцеролів і підвищення їх депонування у грудних м'язах курчат-бройлерів за умов застосування цитратів у їхньому раціоні. Підтвердженням цього є обернена залежність між вмістом триацилгліцеролів, їх попередників моно- і диацилгліцеролів та вільних жирних кислот у грудних м'язах курчат другої і третьої дослідних груп, яким згодовували комбікорм з дода-

ванням цитрату й ензимного препарату. Поряд із цим відомо, що жирнокислотний склад триацилгліцеролів значною мірою залежить від жирнокислотного складу корму. Загалом, саме триацилгліцеролі є ідеальним субстратом для депонування енергії [19].

Цікавими є отримані результати про те, що на протигагу динаміки аналогічних показників у печінці (табл. 6), додавання до корму курчат-бройлерів дослідних груп вказаних вище добавок сприяло деякому зниженню відносного вмісту вільного й етерифікованого холестеролу в грудних м'язах дослідних груп ( $P < 0,01-0,05$ ), порівняно з контролем.

Отже можемо зробити висновок, що дія цитратів Сульфору в комплексі з ензимним препаратом Натузим на вміст ліпідів у грудних м'язах курчат-бройлерів, якщо їх додавати до комбікорму, позитивно впливає на харчову цінність м'яса внаслідок зменшення в ньому кількості холестеролу. Одержані результати також свідчать про регуляторний вплив цитратів Сульфору на синтез триацилгліцеролів і холестеролу в грудних м'язах курчат-бройлерів.

Доведено доцільність заміни в раціонах птиці м'ясного напрямку продуктивності мінеральної добавки Сульфур у формі неорганічної солі ( $\text{Na}_2\text{SO}_4$ ) цитратом елемента нанотехнологічного походження в кількості, що становить 10% від вмісту елемента в неорганічній формі та добавки комплексного ензимного препарату Натузим. Так забезпечується підвищення продуктивності птиці (маса тіла зростає на 1,7%; маса півпатраної тушки — на 4,16%; забійний вихід — на 2,12%), якості отриманої продукції (у грудних м'язах вірогідно знижується вміст етерифікованого та вільного холестеролу ( $P < 0,01$ ) і вільних жирних кислот ( $P < 0,05$ ), водночас спостерігається зростання кількості триацилгліцеролів ( $P < 0,01$ )).

Ефективність додаткового введення до раціону курчат-бройлерів Сульфору залежить від форми та кількості елемента.

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## Influence of exogenous enzymes and different forms of Sulfur in the diets of broiler chickens on productivity and quality of poultry products

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Biologically active feeding factors that positively influence the digestibility and absorption of nutrients from feed include enzyme preparations. These preparations are multi-enzyme and are characterized by their specific focus on improving the digestion of proteins, fiber, beta-glucans, arabinoxylans, and others. However, their effective use in the diets of certain species of poultry, of specific ages and productivity types, requires further research. At the same time, to meet the biological needs of poultry for nutrients, the deficiency of sulfur-containing amino acids can be partially compensated by the additional inclusion of sulfates, which contributes to an increase in components of sulfonated amino-polysaccharides, potentially improving the functioning of the mucosal barrier of the digestive tract and stimulating the absorption of feed nutrients. Therefore, systemic studies on the intensity of metabolic processes in the bodies of poultry when introducing exogenous enzymes in combination with sulfur in various forms are promising, aimed at enhancing the breakdown, digestion, and absorption of feed nutrients, as well as improving the biological and nutritional quality of poultry products. The study was conducted in the vivarium of the Institute of Animal Biology NAAS on young meat-type chickens of the ROSS-8 cross, starting from 10 days of age, divided into four groups (20 birds each). The broiler chicks were kept in cages and fed according to technological requirements. All poultry received a complete compound feed. The chicks in the control group consumed compound feed with an additional 0.3% sodium sulfate. The first experimental group had 0.3% sodium sulfate + Natuzym added to their diet; the second experimental group received sulfur citrate (25% of the control, calculated as sulfur) + Natuzym; and the third experimental group received sulfur citrate (10% of the control, calculated as sulfur) + Natuzym. It was shown that the effectiveness of the additional inclusion of sulfur in the diets of broiler chicks depends on the form and amount of the element. The appropriateness of replacing the mineral sulfur additive in the diets of meat-type poultry with sulfur in the form of an inorganic salt ( $\text{Na}_2\text{SO}_4$ ) with sulfur citrate of nanotechnological origin in an amount constituting 10% of the content of the element in its inorganic form, along with the complex enzyme preparation Natuzym, was proven. This leads to increased poultry productivity (body weight increased by 1.7%; weight of the slaughtered carcass increased by 4.16%; slaughter yield increased by 2.12%) and improved quality of the obtained products (the content of both esterified and free cholesterol in breast muscles significantly decreased ( $P < 0.01$ ) as well as free fatty acids ( $P < 0.05$ ), while the quantity of triglycerides increased ( $P < 0.01$ ).

**Key words:** Sulfur, enzyme additive, broiler chicks, productivity



## The effect of drugs “Enteronormin” and “Zeleris” on the antioxidant potential of young calves

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**POO:** Conceptualization.  
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**VOI:** Conceptualization.  
**MDI:** Investigation.  
**BNA:** Investigation.  
**MMB:** Investigation.  
**SOO:** Writing — original draft.  
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None to declare.

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The article deals with study of the effect of the synbiotic drug “Enteronormin” in a complex with the trace elements iodine and selenium on the indicators of lipid peroxidation and the activity of antioxidant protection system in the body of calves and to the comparison of its action with the antibiotic “Zeleris”. In the last decade, the study of the role of various essential trace elements in various aspects of the regulation of metabolic homeostasis and the state of the immune potential of young cattle remains relevant. In this concern, it is important to emphasize the role of iodine and selenium, as well, as biologically active compounds of new products, among which synbiotic drugs are becoming widespread. In view of this, the development of new effective immunotropic drugs, their comparative study with existing drugs and traditional antimicrobial drugs, such as antibiotics, is actual. Therefore, the purpose of the research, the results of which are presented in this article, was to conduct a comparative study of the effect of the antibiotic “Zeleris” and the complex use of the synbiotic drug “Enteronormin” together with iodine and selenium on the indicators characterizing the activity of lipid peroxidation processes and the level of antioxidant protection system in the body of young calves. As a result of the research, it was found that feeding calves the drug “Enteronormin” in combination with selenium and iodine leads to an increase in the activity of the enzyme glutathione peroxidase and the level of reduced glutathione in the erythrocytes of calves, which is noted at 50- and 60-day age. Such changes, in turn, logically lead to a decrease in the number of lipid peroxidation products in their body. In contrast, we did not detect similar effects from the use of the antibiotic “Zeleris” during the study. Thus, it is concluded that feeding calves with the drug “Enteronormin” together with iodine and selenium leads to an increase in the activity of the key enzyme of the antioxidant defense system — glutathione peroxidase and the level of reduced glutathione in the erythrocytes of the blood of calves. These changes, in turn, lead to a decrease in the number of lipid peroxidation products in their body. However, similar effects from the use of the antibiotic “Zeleris” were not detected. The data obtained may indicate a positive effect of the drug “Enteronormin” in combination with iodine and selenium on the activity of the antioxidant defense system in the body of calves.

**Key words:** calves, glutathione peroxidase, reduced glutathione, TBA-active products, hydroperoxides, iodine, selenium



## Introduction

An important problem in the Western region of Ukraine remains the insufficient intake of dietary elements such as iodine and selenium into the human and animal body. Deficiency of trace elements in the animal body leads to the development of microelement diseases, which are endemic diseases, which, in turn, is associated with the insufficient content of active forms of trace elements in soils, water sources and plants [1, 2, 8, 9]. In order to ensure the proper level of iodine intake into the human and animal body, a large number of methods have been developed for enriching human food and animal feed with this trace element. However, the vast majority of these methods are based on the use of inorganic iodine compounds, which is not effective in terms of their absorption by the body, and the compounds themselves are unstable and have time to decompose to a large extent before they enter the body. Another problem is the balance of such drugs and supplements with the another important element, such as selenium, which significantly affects the absorption of iodine in the body [13].

In this aspect, it is relevant to conduct research to determine the role of iodine and selenium and other trace elements and biologically active compounds in the form of new drugs and feed additives in the regulation of metabolism and the state of the immune potential of the body of farm animals, in particular young cattle. The experiments that we conducted in previous years confirmed the powerful modulating effect of selenium compounds on the functional potential of immunocompetent cells, as well as the associated activity of the antioxidant defense system of the body as a whole. In addition, it should be noted that the effect of selenium on immune function is primarily associated with its antioxidant properties [6, 9, 10]. Iodine is no less important in this regard, which prevents metabolic disorders in tissues and supports the body's protective reactions, accelerates the formation of new immunocompetent cells [3]. The class of so-called synbiotic drugs deserves special attention [7]. In our case, the basis of such a synbiotic preparation is a complex, which includes probiotics, in particular, lactic acid bacteria *Lactobacillus* spp. and *Enterococcus* spp., spore-forming bacteria *Bacillus subtilis* and prebiotics — water-soluble chitosan and microbiological chitons. It must be noted that such preparations are used in a wide range of domestic animals, including pond fish and bees. At the same time, it is extremely important to clarify the role of the components of the preparation in the regulation of immune function in animals. In view of this, the development of new effective immunotropic agents, their comparative study with existing methods for restoring the body's immune potential and traditional antimicrobial drugs, such as antibiotics, and a comprehensive study of their effect on the animal body are relevant. The above undoubtedly justifies the importance and relevance of studying another aspect

of such an impact, namely, the study of the effects of a synbiotic preparation containing trace elements on the activity of the antioxidant defense system in the body of young cattle.

Thus we conducted a comparative study of the dynamics of the effect of the antibiotic “Zeleris” and the complex use of the synbiotic drug “Enteronormin” together with the trace elements iodine and selenium on indicators characterizing the activity of lipid peroxidation processes and the level of antioxidant protection in the body of young calves.

## Materials and Methods

The study was conducted in a private farm (TF Dmytriv LLC “Barkom”) on calves of the black-and-white dairy breed of 10-day age. It was formed three groups of calves-analogues weighing 40–50 kg: a control and two experimental groups of 15 animals each. Feeding and keeping of animals of the control and experimental groups met the existing requirements. Animals of the control group at the age of 10 days were injected intramuscularly with a 0.9% sodium chloride solution at a dose of 5 ml/animal. Calves of the first experimental group during the specified period were injected once with the antibacterial drug “Zeleris” at a dose of 1 ml/10 kg of body weight. The calves of the second experimental group were given the drug “Enteronormin” in the amount of 1 g/10 kg of body weight per day according to the following scheme: the first time the studied remedy was given with water at 10-day age, for six consecutive days, the next time the drug was given at 24-day age for two days. Before use, to activate the synbiotic drug, the required amount of “Enteronormin” was dissolved in the solution “Iodis concentrate + Se” (water containing biologically active iodine ions and selenium citrate) in a ratio of 1 to 5 and was exposed for 16 hours at room temperature. At the same time, calves of this group, starting from 10- to 65-day age, were given an aqueous solution of Iodine and Selenium, using the drug “Iodis-concentrate + Se” as a source of trace elements, at a dose of 25 mg /l t of water. At 10, 25, 50, and 60 days of age, blood was taken from the jugular vein from each group of calves before morning feeding for biochemical studies.

In blood plasma, the content of lipid hydroperoxides was determined in the reaction with ammonium thiocyanate after precipitation of proteins with a solution of trichloroacetic acid and extraction of lipids with ethanol. The content of TBA-active products in blood plasma was determined in the reaction of malondialdehyde with thiobarbituric acid. In blood erythrocytes, the activity of glutathione peroxidase was determined by the rate of glutathione oxidation in the presence of tert-butyl hydroperoxide. The level of reduced glutathione (GSH) was determined by the level of formation of thionitrophenyl anion as a result of the interaction

of SH-groups of glutathione with 5,5'-Dithiobis-2-nitrobenzoic acid. All of the above mentioned methods are described in the proper sections of the "Handbook of Laboratory Research Methods" [12]. The obtained digital results were processed by the method of variational statistics using the Student's criterion with the *Microsoft Excel* program.

## Results and Discussion

As can be seen from the data presented in tables 1 and 2, we found significant changes in glutathione peroxidase activity, reduced glutathione content, and lipid peroxidation products under the action of the studied drugs.

In particular, as can be seen from the data presented in table 1, the decrease in the content of TBA-active products and lipid hydroperoxides in the blood of 50-day age calves of the second experimental group, which received the synbiotic preparation "Enteronormin" and the trace element complex of iodine and selenium, was observed. Thus, the content of TBA-active products in the blood of calves of the experimental group was 1.35 times lower, the content of lipid hydroperoxides, respectively, was 1.5 times lower, compared with calves of the control group ( $P \leq 0.001$ ). At the same time, it was found that the use of the antibiotic "Zeleris" in 50-day-old calves led to a certain increase in the level of peroxidation products

in their blood. In particular, the content of TBA-active products in the blood of 50-day-old calves of the first experimental group was 1.26 times higher, the content of lipid hydroperoxides was 1.42 times higher than in calves of the control group ( $P \leq 0.001$ ). It is known that antibiotics have a certain toxic effect on the body of animals in which they are used, which is also accompanied by a pronounced prooxidant effect [4]. During the next 10 days of the experiment, this negative effect of the antibiotic was leveled. The explanation of such changes requires further research, but it can be assumed that this may be associated with the intensive postnatal development of the organism, which is accompanied by the active formation of the protective adaptive systems of the organism during this period.

A similar picture of a decrease in the content of lipid peroxidation products under the influence of a synbiotic preparation in a complex with trace elements was observed in calves of 60-day age, however, these changes were expressed to a somewhat lesser extent ( $P \leq 0.05$ ). These data indicate that the use of a synbiotic preparation in a complex with microelements leads to a decrease in lipid peroxidation products in the blood of calves during the studied period. The following results allow us to reveal the reason for such a decrease in the intensity of the formation of peroxidation products. Unlike animals that were treated with the drug "Enteronormin", the use of the antibiotic "Zeleris" led to a certain increase in lipid peroxidation processes, in particular in 50-day-old calves.

**Table 1.** Content of TBA-active products and lipid hydroperoxides in the blood plasma of calves ( $M \pm m$ ,  $n=5$ )

Animal groups	Indicators	
	TBA-active products, nmol/ml	Lipid hydroperoxides, U/ml
10-day-old calves		
Control	3.79±0.25	0.32±0.013
Experiment 1	3.80±0.21	0.30±0.017
Experiment 2	3.60±0.14	0.31±0.014
25-day-old calves		
Control	3.87±0.20	0.32±0.023
Experiment 1	3.83±0.15	0.31±0.012
Experiment 2	3.72±0.13	0.33±0.015
50-day-old calves		
Control	4.2±0.05	0.33±0.013
Experiment 1	5.3±0.10***	0.47±0.012***
Experiment 2	3.01±0.07***	0.21±0.007***
60-day-old calves		
Control	4.0±0.05	0.30±0.010
Experiment 1	3.9±0.06	0.33±0.015
Experiment 2	3.1±0.08***	0.27±0.005*

Note. \* –  $P \leq 0.05$  the statistically significant differences in animals of this experimental group compared to animals of the control group; \*\* –  $P \leq 0.01$  the same; \*\*\* –  $P \leq 0.001$  the same

**Table 2.** Glutathione peroxidase activity and the level of reduced glutathione in erythrocytes of calves ( $M \pm m$ ,  $n=5$ )

Animal groups	Indicators	
	Glutathione peroxidase, mmol GSH/min. mg protein	Reduced glutathione, $\mu\text{mol/g}$
10-day-old calves		
Control	30.45±0.22	0.46±0.02
Experiment 1	29.00±0.54	0.50±0.03
Experiment 2	32.00±0.20	0.53±0.02
25-day-old calves		
Control	30.61±0.20	0.49±0.005
Experiment 1	28.53±0.92	0.54±0.3
Experiment 2	30.23±0.29	0.58±0.03*
50-day-old calves		
Control	30.72±0.34	0.47±0.01
Experiment 1	29.41±0.61	0.42±0.02
Experiment 2	44.24±2.22***	0.79±0.02***
60-day-old calves		
Control	31.65±0.38	0.60±0.08
Experiment 1	30.66±0.38	0.52±0.01
Experiment 2	37.26±0.88***	0.73±0.02

From the data presented in table 2, it can be seen that the activity of the enzyme glutathione peroxidase in the blood erythrocytes of calves of the second experimental group, which received “Enteronormin” with trace elements at 50-day age, was 1.44 times higher, and the level of reduced glutathione in blood plasma was 1.68 times higher ( $P \leq 0.001$ ) than these indicators in the blood of calves of the control group.

At the next stage of the experiment, namely in calves of 60-day age, only a slight increase ( $P \leq 0.001$ ) in the activity of glutathione peroxidase was observed in the second experimental group, which received the synbiotic preparation together with trace elements. It is noteworthy that the use of the antibiotic did not affect the activity of glutathione peroxidase at all stages of the study. We also noted a slight increase in the content of reduced glutathione in calves of 25-day age, which received the synbiotic preparation together with trace elements ( $P \leq 0.05$ ). These data may to some extent indicate the positive effect of the drug “Enteronormin” together with iodine and selenium on the content of glutathione, which plays a certain role in the antioxidant defense system. In our opinion, the main antioxidant effect of the studied drug is primarily associated with the content of the trace element selenium in its composition, which is part of the active center of glutathione peroxidase, the key enzyme of the glutathione link of the antioxidant system [5]. Our study demonstrates a more pronounced positive effect on the balance of pro- and antioxidant processes when using the synbiotic drug in a complex with the trace elements iodine and selenium, compared to the effect of the antibiotic “Zeleris”.

Summing up the obtained results, we can conclude that feeding calves the drug “Enteronormin” in combination with selenium and iodine leads to an increase in the activity of the key enzyme of the antioxidant defense system — glutathione peroxidase and the content of reduced glutathione in the erythrocytes of calves. Such changes, in turn, logically lead to a decrease in the number of LPO products in their body. In contrast, similar effects from the use of the antibiotic “Zeleris” were not detected during our study.

In connection with the results obtained, it would be advisable to establish the influence of individual components of the drug on the activity of antioxidant defense processes in the body of calves in further studies, namely to determine the role of individual microelements and the synbiotic component of the feed additive. A comprehensive study of the state of the antioxidant and immune systems of the body under these conditions is also considered promising.

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## Вплив препаратів «Ентеронормін» та «Зелеріс» на антиоксидантний потенціал організму телят раннього віку

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Стаття присвячена вивченню впливу синбіотичного препарату «Ентеронормін» у комплексі з мікроелементами Йодом і Селеном на показники перекисного окиснення ліпідів та активність антиоксидантного захисту в організмі телят та порівнянню його дії з антибіотиком «Зелеріс». В останнє десятиріччя залишається актуальним дослідження ролі різних есенціальних мікроелементів, зокрема Йоду і Селену, а також біологічно-активних сполук у складі нових засобів, широкого розповсюдження серед яких набувають синбіотичні препарати, у різних аспектах регуляції метаболічного гомеостазу та стану імунного потенціалу молодняка великої рогатої худоби. З огляду на це, актуальними є розробка нових ефективних імунотропних препаратів, порівняльне їх дослідження із вже наявними засобами та традиційними антимікробними препаратами — такими, як антибіотики. Тому метою досліджень, результати яких представлені у цій статті, було проведення порівняльного дослідження впливу антибіотика «Зелеріс» та комплексного застосування синбіотичного препарату «Ентеронормін» разом із Йодом та Селеном на показники, які характеризують активність процесів пероксидації ліпідів, та рівень антиоксидантного захисту в організмі телят раннього віку. В результаті проведених досліджень встановлено, що випоювання телятам препарату «Ентеронормін» у комплексі з Селеном та Йодом призводить до зростання активності ензиму глутатіонпероксидази та рівня відновленого глутатіону у еритроцитах телят, що спостерігали у 50- та 60-добовому віці. Такі зміни логічно призводять до зменшення кількості продуктів ПОЛ в їхньому організмі. На противагу цьому, подібних ефектів від застосування антибіотику «Зелеріс» у дослідженні нами не виявлено. Тож робимо висновок, що випоювання телятам препарату «Ентеронормін» разом з Йодом та Селеном призводить до зростання активності ключового ензиму системи антиоксидантного захисту — глутатіонпероксидази та рівня відновленого глутатіону в еритроцитах крові телят. Ці зміни, у свою чергу, ведуть до зменшення кількості продуктів пероксидації в їх організмі. Водночас аналогічних ефектів від застосування антибіотику «Зелеріс» не виявлено. Отримані дані можуть свідчити про позитивний ефект препарату «Ентеронормін» у комплексі з Йодом та Селеном на активність антиоксидантної системи захисту в організмі телят.

**Ключові слова:** телята, глутатіонпероксидаза, відновлений глутатіон, ТБК-активні продукти, гідропероксиди, Йод, Селен





## Genetic resources of local chicken in Georgia

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The preservation of local poultry gene fund has both scientific and practical importance for Georgia. High genetic potential is important for breeders from national, state, scientific and economic points of view. It has been determined by scientists that modern poultry farming in the world is represented by a small genetic line of birds, which is why the breeds of agricultural birds are almost uniform in origin. According to the breeders, the mentioned fact will definitely lead to the disappearance of valuable and necessary alleles, which local birds are the protectors of. Further progress in poultry breeding is impossible without the use of genetic diversity. The breeding of new crosses necessarily requires extensive involvement in the selection of the “less economical” local bird gene pool. The intensification of the poultry industry, with the wide spread of imported highly productive hybrid birds, created a threat to the preservation of the gene pool of local birds, which almost created the danger of its degeneration and extermination for such breeds of chicken that have been widespread in Georgia since time immemorial, including the following local breeds: Chalisferi, Shavi, Megrula, Natsara, Keltitvela. These local breed chicken are characterized by valuable genetic traits. They are a source of rare marker genes, characterized by auto-sexuality, good adaptation to hot climates, the best quality of eggs and meat, less demand for nutrition and care, and high resistance to a number of diseases.

**Key words:** preservation, poultry, alleles, genotype, breeds, selection, productivity

## Introduction

According to the data of the United Nations Food and Agriculture Organization (FAO), over the last 100 years, 39 breeds of birds have completely disappeared across Europe, and 481 are at risk of extinction. These are breeds that are characterized by good adaptability, high resistance to various diseases, less demand for storage conditions, high taste qualities of eggs and meat.

A decrease in genetic diversity is manifested by the loss of valuable genes and their alleles. All this significantly limits the possibilities and efficiency of further selection work. In addition, local breeds and populations are valuable living cultural heritage. No less

important are the issues of keeping reserve lines of industrial poultry. That is why it is necessary to develop methods and ways of preserving the genetic diversity of agricultural poultry.

World practice shows that it is possible to use different methods: *in situ*, *ex situ*, *in vivo*, and *in vitro*. It is also worth noting the existing organizational forms in this regard: fancier-birders' associations, state and private gene pool farms, monetary incentives at the national level, and others.

Legislation regulating the use of genetic resources in our country requires improvement, which means that there should be a law on breeding livestock. It is also necessary to create a law on the preservation of genetic resources of agricultural animals and poultry

and a mechanism for its implementation. Regrettably, it is a fact that today the country does not have an organizational system for storing genetic resources.

In Georgia there are suitable conditions for the development of poultry farming, such as: moderate climate, abundance of sunny days, diversity of plants and their long growing season. The most common domestic bird is the chicken. Information about chicken farming and some taxes related to it, is also reflected in historical documents. Chicken was one of the important sources of providing the population with meat products, which contributed to the breeding of meat, egg-laying and combined breeds of chicken.

Today, the following populations of Georgian chicken are still preserved in Georgia and have been identified by scientists and named according to their color: Chalisferi, Shavi, Megrula, Keltitvela, Natsara.

### Population — Georgian Chalisferi chicken

**Common name:** chicken. **Latin name:** *Gallus domesticus*.

**Origin:** mostly spread in Kartli and Kakheti. The breeding of this population in Georgia has a centuries-old history, and at the same time, its origin is still unknown.

**Brief description:** meat and egg-laying chicken direction, but, as noted, leans more towards meat chicken. The body is medium-sized, the head is medium-sized, the face is slightly puffy, the beak is yellow, the comb is simple (leaf-like), the mouth and ears are red, the chest is wide, the back is relatively short, the legs are not feathered and covered with yellow scales, the skin is pigmented yellow. Feather color varies from straw to light red (more carrot) in different individuals, males are somewhat darker than females. As usual, the cover is white, and on the ends they do not have mixed black feathers. The average live weight of 1-year-old females is 1.8–2.0 kg, and that of males is 2.5–2.8 kg; for adults it is 2.4–2.7 and 3.0–3.4 kg, respectively. Adolescents start laying eggs from the age of 6–7 months. The average annual egg production is 135–145. The average weight of the egg in the middle of the laying period is 53–56 g, and at the end — 58–61 g. The egg is characterized by high incubation properties: the percentage of hatching out of every 100 laid eggs is 85–88. Chicken grow quickly and are characterized by good plumage. Adolescents reach the live mass for slaughter at the age of 2.5–3.5 months. Like other local populations, one of the main virtues of the Chalisferi chicken is its ability to adapt to changing environments, high liveliness and less demanding. Along with this, in separate trials, the increased resistance of local chicken to such infectious diseases as plague, pulurosis and marek has been established. It is characterized by the high taste qualities of the meat, which is due to the fact that fat

accumulates not only under the skin and in the abdominal cavity, but also between the muscles. Its egg has a high content of lysine, leucine and isoleucine, which leads to high incubation and taste properties.

**Current state:** the number of Chalisferi chicken has not been established, since they are reared together with other populations of local chicken in private farms. It is very similar in appearance and structure to the Rhode Island variety bred in the USA.

### Population — Georgian Black chicken

**Common name:** chicken. **Latin name:** *Gallus domesticus*.

**Origin:** it is one of the varieties in five local chicken populations. It is characterized by a broad head, a short and thick neck, a broad chest, a long back, dense feathers, a comb that is leaf-like and blood-red in color, and the comb behind the ear is also red. Limbs below the ankle joint are not inflated. The scales on the foot are small and densely covered. Brief description: The body length of chicken is 21–23 cm, the length of the breastbone is 12.5–15.5 cm, and the circumference of the chest is 30.5–32.5 cm. The corresponding measurements for roosters are: 24.0–26.0, 16.5–18.5 and 42.0–45.0 cm. The feathers on the whole body are pigmented black; there may be single white or yellowish patches (dots or short stripes) on the neck. In roosters, the black color of the feathers on the main part of the body, on the neck, wings and tail, turns into a greenish-purple-shiny-glossy color. Beak and legs blackish-grey (dark). It is resistant to changes in environmental conditions. It does not require any special maintenance conditions and shows high productivity when kept in simple poultry houses. The live weight of chicken is 2.2–2.4 kg, roosters — 2.8–3.1 kg. They start laying eggs from the age of 6–7 months. Average egg production is 130–142 pieces/year; the average mass of 1 egg is 55.5 g. Under natural conditions (under the hook), 85–88 chicken hatch from every 100 eggs. In the “Tushuri” population of black chicken, Georgian researchers have revealed a fairly high rate of resistance to bird plague.

**Current state:** like other local chicken populations, black chicken are raised in family and farm farms, and no official information is available on their numbers.

### Population — Megrula chicken

**Latin name:** *Gallus domesticus*.

**Origin:** they have been popular since ancient times in one of the regions of Georgia — Samegrelo (the origin of the name of the chicken). It can be found in other regions of the country as well. It is durable and easily adapts to changing climates, both heat and cold. This is what caused it to be widespread in Armenia, Russia, and Ukraine in the last century.

**Brief description:** characteristics of physical features: medium-sized head, long neck, broad chest and relatively short back. The color of birds of both sexes is the same in adulthood: feathers are blackish-gray on the whole body, with white tips or feathers (short stripes). In individual specimens, the wing feathers are slightly golden. In roosters, the comb is large, leaf-like, red in color and often folded. It is desirable that the upper comb be leaf-like and folded. The beak and legs are light yellow, but dark coloring is also allowed. The legs are padded up to the ankle joint. The feathers of newly hatched chicken of different sexes are of different colors: on the head of the male chicken, it has white feathers, in the male chicken, such feathers are almost absent, while the rest of the body is pigmented black in the juveniles of both sexes. Such differentiated coloration allows choosing the sex of a one-day-old chicken. It is worth noting that from the age of two weeks, the chicken acquires a striped coloration. Average live weight of males — 2.8–3.2 kg, females — 2.4–2.7 kg.

Among the chickens of the Georgian population, it is the most early-maturing and productive: it starts laying eggs somewhat earlier — from the age of 160–170 days. On average, it gives us 160–165 eggs weighing 55.3 g per year. Out of every 100 laid eggs, the specific share of hatching is 80–82% of the eggs laid in the initial period of egg laying, and 88–90% of the eggs laid in the middle period. Chicks grow fast but fledge slowly.

Megrula eggs are characterized by high incubation properties, chicken grow quickly and reach slaughter age at the age of 3–4 months. Both young and adult birds are characterized by high-tasting qualities of meat, which is due to the fact that fat accumulates not only under the skin and in the abdominal cavity, but also between the muscles, which is not characteristic of other cultural breeds and crosses. Unlike other cultured varieties, its egg has a high content of lysine, leucine and isoleucine, which determines its high incubation and taste properties.

**Current state:** the number is greatly reduced. They are raised on family farms, together with chicken from other Georgian populations.

### Population — Georgian “Keltitvela” chicken

**Common name:** Chicken. **Latin name:** *Gallus domesticus*.

**Origin:** it is assumed that the Keltitvela chicken generally originated in the territories of today's Austria, Germany, Hungary and Romania. The period of their distribution in Georgia has not been determined. In some parts of the country, Keltitvela chicken are also called Chinese (“Kitaika”).

**Brief description:** Georgian “Keltitvela” chicken differs from other Georgian populations in its appearance:

the head is round, the beak is short and slightly curved, the comb is simple, medium-sized, blood-red and mostly upright, the earrings are small and red, the neck is of medium length, not inflated and red, the chest is wide and rounded, the back is long and broad, the wings are tightly attached to the body, and the part of the foot is quite long and not inflated. Feather pigmentation in both roosters and hens repeats the coloring of all Georgian chicken populations. The beak and part of the foot are mostly yellow.

One of the main conditions for belonging to the Georgian “Keltitvela” group is that the chicken should not have white feathers in its coat. In terms of productivity, “Keltitvela” chicken are combinative, egg-meat oriented. Among individuals of different colors, the straw-colored keltitvela is the largest. On average, the live weight of roosters is 3.2–3.5 kg, chicken — 2.7–2.8 kg. It starts laying eggs at the age of 5–6 months, and reaches the peak in the 7<sup>th</sup>–8<sup>th</sup> month. The average production is 155–160 eggs, egg mass is 56.8–58.5 g, hatching egg yield is 90%, and the number of hatched chicken from every 100 eggs reaches 86–88. The chick is growing fast. It has tender, tasty and “white” flesh, which is also characterized by good technological/culinary properties. It is established that the “Keltitvela” chicken is characterized by high vitality, when kept in family conditions, it easily “finds” food and tolerates extremely high air temperature well.

**Current state:** it is not found as a separate groups in any farm/household. According to the expedition research of 2009–2012, the number of hens in Dusheti and Tianeti municipalities is 24–30% of the total mass. Probably, the situation is similar to this in other regions of the country.

### Population — Georgian Natsara chicken

**Common name:** chicken. **Latin name:** *Gallus domesticus*.

**Origin:** it is one of the local chicken populations. Compared to the Black chicken, it has a longer body. It is characterized by a wide chest, medium height, dark colored and un-feathered legs, a short, dark colored and slightly curved beak, a leaf-like and mostly erect comb and a red colored earlobe.

**Brief description:** in females, the body is completely covered with uniformly gray feathers, which may be slightly darker in the neck area. Roosters also have body feathers that are a solid gray that fades to dark on the tail and neck areas, and some individuals may have a slightly noticeable light golden color on the wings. The live weight of adult females is 2.4–2.7 kg, males — 3.0–3.4 kg. They start laying eggs from the age of 160–170 days. In this period, the intensity of eggs is on average 14.6%, and the peak of egg production occurs at the age of 8–9 months and reaches 63.2%. The average annual egg production rarely

exceeds 150–155 eggs, and the average egg weight is 55.8 g. The hatching egg yield reaches 88.6%, and the hatching % is 83.6.

Birds of this population are characterized by high vitality and good endurance. In the experiments, it was determined that the rate of mortality of Natsara chicken in the period from 5 to 17 months of age is quite low and amounts to 15.4%. It should be considered as an important positive feature that the laying of eggs in the summer months is quite even, which indicates the high ability of chickens to adapt to the high temperature.

**Current state:** Natsara chicken are raised together with other local populations in family/household farms. There is no official information on their number.

As we can see, the local chicken populations spread in Georgia are characterized by a number of important positive features, such as vitality and durability, high resistance to various diseases, resistance to high temperatures, good productive indicators, etc. Today, when scientists from all over the world, including specialists in the field of animal husbandry, fight with the negative consequences caused by climate change, local chicken populations are able to breed highly productive lines and

crosses well adapted to new natural and climatic conditions as a result of targeted selection work.

It should be emphasized that the local chicken populations spread in Georgia are not only well adapted to the local natural and climatic conditions, but they can also be used in foreign countries which once again points to the need to preserve these populations. Accordingly, we believe that it is necessary to create a genetic bank in the country, where the genetic material of valuable Georgian chicken populations will be preserved.

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## Генетичні ресурси місцевих курей в Грузії

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Збереження генофонду місцевої птиці має для Грузії як наукове, так і практичне значення. Високий генетичний потенціал важливий для селекціонерів з національної, державної, наукової та економічної точок зору. Вченими встановлено, що сучасне птахівництво у світі представлене невеликою генетичною лінією птахів, тому породи сільськогосподарської птиці майже однорідні за походженням. На думку селекціонерів, згаданий факт однозначно призведе до зникнення цінних і потрібних алелів, носіями яких є місцеві птахи. Подальший прогрес у птахівництві неможливий без використання генетичного різноманіття. Виведення нових схрещувань обов'язково вимагає широкого залучення до відбору «менш економічного» місцевого генофонду птахів. Інтенсифікація птахівництва з широким розповсюдженням імпортової високопродуктивної гібридної птиці створила загрозу збереженню генофонду місцевої птиці, що майже створило небезпеку його виродження та винищення для таких порід курей, які споконвіку поширені в Грузії, в тому числі місцевих порід: Чалісфері, Шаві, Мегрула, Нацара, Кельтвела. Ці місцеві породи курей характеризуються цінними генетичними ознаками. Вони є джерелом рідкісних генів-маркерів, характеризуються автосексуальністю, хорошою адаптацією до жаркого клімату, найкращою якістю яєць і м'яса, меншою вимогливістю до годівлі та догляду, високою стійкістю до низки захворювань.

**Ключові слова:** збереження, птиця, алелі, генотип, породи, селекція, продуктивність





## Екологія мікобактерій в умовах впливу абіотичних та біотичних чинників

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**RMM:** Formal analysis; Data curation.

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У статті проаналізовано сучасний стан екологічної ситуації щодо різних видів мікобактерій, їх систематику, епідемічне й епізоотологічне значення, зокрема в Україні, з урахуванням динаміки змін природно-кліматичних умов, а також за впливу антропогенних факторів. Розглянуто основні ризики для здоров'я людини, які можна очікувати від цих мікроорганізмів, а також необхідні заходи для запобігання їх виникненню. У праці узагальнено низку власних досліджень, а також результати, які одержали науковці, як в Україні, так і в інших країнах світу.

**Ключові слова:** мікобактерії, довкілля, мікобактеріози, екологія, туберкульоз, алергія, хіміорезистентність

## Вступ

Відповідно до *Bergey's Manual of Systematic Bacteriology*, мікобактерії належать до порядку *Actinomycetales*, родини *Mycobacteriaceae* і роду *Mycobacterium*, що включає патогенні, умовно-патогенні та сапрофітні види [1, 32]. Для всіх представників роду *Mycobacterium* характерною ознакою є кислото- і спиртостійкість, подібні культурально-морфологічні особливості, антигенна спорідненість, тинкторіальні властивості, а також здатність до зафарбовування методом Циль-Нільсена. Мікобактерії є нерухомими аеробами, не утворюють спор і капсул, не мають на своїй поверхні ворсинок. Особливістю мікобактерій є те, що вони дуже повільно ростуть на штучних поживних середовищах — до 90 діб. Крім того, ці мікроорганізми здатні до зворотної дисоціації у R-, L- та S-форми, що робить їх ще більш підступними.

Ці мікроорганізми є важливими агентами для медицини та ветеринарії через їхню здатність викликати інфекційні захворювання, як-от туберкульоз, паратуберкульоз, проказа та інші мікобактеріози. Основним способом боротьби макроорганізму при потрапленні патогенних культур мікобактерій є клітинний імунітет, а первинним маркером їхньої патогенної дії — незавершений фагоцитоз [2, 3]. Проте значення мікобактерій не обмежується тільки патогенністю; їх екологія в природних середовищах є предметом постійних досліджень, які покликані краще зрозуміти їхню роль у природних і штучних екосистемах [6].

## Різноманітність та адаптація мікобактерій до довкілля

На сьогодні у світі ідентифіковано понад 300 видів і підвидів мікобактерій, а в Україні виділяють

понад 50 [1]. Також вивчено понад 20 видів мікобактерій, які є патогенними або умовно-патогенними для людей. Ці мікроорганізми поширені на всіх континентах земної кулі, фактично, вони є убіквітарними. Навіть у тих країнах, які мають стійке епізоотичне благополуччя щодо туберкульозу, час від часу виникають спорадичні випадки виділення патогенних видів мікобактерій від тварин або птахів [11]. За культурально-морфологічними ознаками мікобактерії розподіляють на чотири групи (класифікація Раньона, 1959): фотохромогенні (I група), скотохромогенні (II), нефотохромогенні (III) і швидкорослі (IV) [32]. Цікавим є той факт, що на території України ізолюють лише мікобактерії II, III і IV груп за класифікацією Раньона. Види, що належать до I групи (фотохромогенні), в Україні не виділяють. Що стосується видового розмаїття, то найчастіше в Україні від тварин, людей або з об'єктів довкілля ізолюють такі види атипичних мікобактерій: *M. scrofulaceum*, *M. fortuitum*, *M. gordonae*, *M. vaccae*, *M. phlei*, *M. avium-intracellulae complex*, *M. thyfimurium*, *M. flavescens*, *M. triviale*, *M. xenopi*, *M. smegmatis* та інші [35].

Мікобактерії демонструють високу екологічну пластичність, пристосовуючись до широкого спектра середовищ — від ґрунтів і вод до середовищ із екстремальними умовами, як-от висока чи низька температура або високий вміст металів [15]. Деякі види мікобактерій, як, наприклад, *Mycobacterium smegmatis*, мешкають у ґрунті й активно беруть участь у процесах біодеградації органічних речовин. Інші, зокрема *Mycobacterium avium*, виявляють у природних і штучних водних системах, зокрема в очисних спорудах, де вони можуть стати збудниками для сприйнятливих організмів [4, 7]. Що стосується екогеографічних особливостей, то найбільше атипичні (сапрофітні) види мікобактерій на території України персистують на заболоченій місцевості, торф'яниках, у місцях з підвищеною вологістю повітря [9, 34, 35]. Після їх потрапляння до макроорганізму відбувається лише короткочасна сенсibiliзація — стан гіперчутливості (алергія) до дії споріднених в антигенному відношенні речовин, розвиток інфекційного патологічного процесу при цьому не відбувається [14]. Механічними переносниками мікобактерій, що сприяють їхньому розповсюдженню, найчастіше є синантропна або дика птиця (горобці, голуби, ворони, качки, ластівки та інші) [31, 34].

#### Взаємодія з біоценозами

Мікобактерії можуть утворювати складні симбіотичні або патогенні зв'язки з іншими організмами [18, 19]. Наприклад, у ґрунтових системах вони здатні співіснувати з грибами або найпростішими, сприяючи розщепленню органічних речовин і мінералізації. У водних екосистемах — колонізувати поверхні біоплівки, де конкуренція за ресурси визначає їхню життєздатність. Залежно від певних умов довкілля мікобактерії можуть зберігати свої властивості впродовж багатьох років [9].

Найбільш близькими до мікобактерій у антигенному відношенні є такі мікроорганізми, як коринібактерії, нокардії, родококки, ерсинії [1, 32].

#### Антропогенний вплив на етіологічні й екологічні властивості мікобактерій

Мікобактерії активно реагують на антропогенні зміни в довкіллі, зокрема на забруднення води, зміну температурного режиму або зростання рівня токсичних сполук. В умовах забруднення водних екосистем підвищується поширення умовно-патогенних видів мікобактерій, як-от *Mycobacterium intracellulare*, що може стати загрозою для здоров'я людини. Трапляються також повідомлення про виділення мікобактерій від риб [8, 13, 16, 17, 20, 25]. Діяльність людей має великий вплив на різні мікроорганізми, зокрема це стосується і мікобактерій. Цей вплив умовно можна розподілити на локальний і глобальний. Глобальний вплив пов'язаний зі змінами клімату (глобальне потепління), появою антибіотикорезистентних і хіміорезистентних форм, що зумовлюють інфекційні захворювання, зокрема туберкульоз, які важко піддаються лікуванню за допомогою традиційних лікарських засобів. Глобалізація світу також призводить до швидкого розповсюдження патогенних видів мікобактерій між континентами земної кулі [11].

#### Локальний вплив антропогенних факторів

Локальний вплив антропогенних факторів являє собою зміни еко-географії та імуніо-біохімічних властивостей мікобактерій внаслідок господарської діяльності людини, наприклад, осушення або, навпаки, створення штучних водних екосистем, будівництво тваринницьких або птахівничих комплексів з високою концентрацією поголів'я тощо [3, 6]. Також воєнні дії, особливо якщо вони точаться протягом тривалого часу, значно впливають на екологію мікобактерій, зумовлюючи, зокрема, ризик спалахів туберкульозної інфекції та інших небезпечних захворювань.

Однією з екологічних і соціальних проблем в Україні під час війни стало забруднення повітря, води та ґрунту. Постійні бомбардування й обстріли міст і населених пунктів призвели до викиду великої кількості токсичних хімічних речовин у довкілля. Знищення лісів, пожежі на полях, нафтопереробних підприємствах, заводах, електростанціях тощо — усе це призводить до нищівного впливу на екосистему не тільки в зоні ведення бойових дій, а й загалом на теренах усієї країни та за її межами. Хімічні речовини забруднюють ґрунт, джерела води й повітря, що негативно впливає на тварин і спричиняє низку проблем з погіршення здоров'я населення.

Руйнування ландшафтів, інфраструктури, розливи і витіки небезпечних матеріалів, як-от нафта, хімікати, токсичні й радіоактивні речовини, у навколишнє середовище чинять негативний вплив на ґрунтову

мікрофлору та всю екосистему мікроорганізмів. Не з'ясованим залишається вплив перерахованих вище абіотичних і біотичних чинників на екологію мікобактерій, позаяк щоб це з'ясувати, треба провести спеціальні дослідження, зокрема визначити їхнє поширення та патогенність. Однак брак стабільності та безпеки ускладнює доступ науковців, екологів і представників зацікавлених установ та організацій до територій, які потребують уваги й досліджень щодо етології і розповсюдження мікобактерій, виникнення у них можливих мутаційних змін тощо.

### Патогенність для людей і тварин

Деякі види мікобактерій є збудниками небезпечних захворювань, зокрема і туберкульозу в людей, що зумовлює вид *Mycobacterium tuberculosis*.

Відомо, що туберкульоз у великої рогатої худоби здатні викликати збудники видів *Mycobacterium bovis* (*M. bovis*) і *M. tuberculosis*. Водночас характер перебігу захворювання у тварин суттєво відрізняється. Також немає єдиної думки серед науковців щодо ролі деяких видів атипичних мікобактерій, а також збудника виду *M. avium* у виникненні патологічного процесу в організмі великої рогатої худоби. Трапляються повідомлення, що культури *M. avium* здатні зумовлювати в людей, особливо у дітей, туберкульозні ураження в кістковій тканині [21, 22, 23, 36].

Світова концепція сучасної системи боротьби з інфекційними захворюваннями передбачає принцип «One health», максимальне викоринення зооантропонозних захворювань з метою розриву зворотного кола зараження тварин і людей, тобто здоров'я людей — через здоров'я тварин. Підготовлений Глобальний план боротьби з туберкульозом людей на 2023–2030 рр. (*Global Health Campus*) [10] неможливо успішно реалізувати без приділення уваги до подолання туберкульозної інфекції також і серед тварин.

Окремо варто зупинитися на проблемі неспецифічних реакцій як у тварин, так і в людей, на мікобактеріальні алергени (туберкулін, ААМ), які використовуються у медицині для прижиттєвої діагностики захворювання на туберкульоз [33]. Переважна більшість атипичних видів цих мікроорганізмів, маючи спільні антигенні детермінанти зі збудниками туберкульозу, є причиною так званих параалергічних реакцій внаслідок формування у макроорганізмі стану гіперчутливості сповільненого типу. Цей феномен значно ускладнює діагностику туберкульозу, яка має бути комплексною, а у тварин, зокрема у великої рогатої худоби (ВРХ) призводить до забою, який здійснюється з діагностичною метою, здорових продуктивних особин, що своєю чергою спричинює значні економічні затрати у галузі тваринництва [24, 30, 35].

У людей явище параалергії також суттєво ускладнює контроль туберкульозної інфекції, особливо це стосується дітей, щеплених культурою вакцинного

штаму *BCG*, у яких часто виявляють позитивну реакцію Манту, але при цьому розвитку інфекційного процесу не відбувається [2, 3, 25, 26].

Що стосується галузі тваринництва, то в Україні, починаючи з жовтня 2016 р., за офіційними даними, проведено її повне оздоровлення від туберкульозної інфекції, проте, наприклад, у першому півріччі 2024 р., за даними Державного науково-дослідного інституту лабораторної діагностики та ветсанекспертизи (Київ), під час планових алергічних досліджень (туберкулінізація) було виявлено 208 голів ВРХ із 44 господарств у восьми областях, які позитивно реагували на внутрішньошкірне введення *PPD*-туберкуліну для ссавців у стандартному розчині. Причини цього явища потребують ретельних досліджень у кожному конкретному випадку — з метою диференціації специфічних від параалергічних або псевдоалергічних реакцій на туберкулін для ссавців. Це необхідно для запобігання розвиткові інфекційного й епізоотичного туберкульозного процесів, які можуть бути непоміченими на тлі гіперсенситибілізації тварин іншими антигенами, що мають спільні антигенні детермінанти зі збудниками туберкульозу. Особливої актуальності ці питання набувають під час дії воєнного стану, коли поширеними є випадки масового переміщення поголів'я ВРХ із зони бойових дій у безпечніші регіони, а водночас, із об'єктивних причин, карантинні діагностичні дослідження тварин здійснюються неякісно або взагалі не проводяться. До того ж ситуацію ускладнює загальне зниження рівня життя людей, а також умов утримання й годівлі сільськогосподарських і свійських тварин. У зв'язку з цим на сьогодні питанням контролю епідемічної та епізоотичної ситуації в нашій країні слід приділяти особливу увагу [29]. Це також стосується й обережних прогнозів щодо появи паратуберкульозу в Україні, який може бути спричинений *M. avium-paratuberculosis complex* [4, 33, 35].

Погіршується й епідемічна ситуація щодо туберкульозної інфекції. Враховуючи антропозоонозний характер туберкульозу, поліпшення епідемічної ситуації неможливе без ужиття відповідних протитуберкульозних заходів і у тваринництві. Це стосується не лише галузі скотарства, але й птахівництва, свиначства тощо [5, 27, 28].

В останні роки в Україні набуває популярності утримання в домашніх умовах різних видів екзотичних птахів-компаньйонів (зокрема папуг). Однак такі птахи часто є джерелом, що виділяє в довкілля різні види мікобактерій, серед яких і ті, що можуть становити небезпеку для здоров'я людей. Передусім це стосується мікобактерій комплексу *M. avium-intracellulae*, які можуть спричинити в людей, і особливо в дітей, захворювання на туберкульоз трубчастих кісток. Найбільш небезпечні з огляду на це птахи, яких утримують у домашніх умовах упродовж багатьох років (літні особини). На особливу увагу заслуговує факт виділення від птахів-компаньйонів в Україні впродовж останніх трьох-п'яти років нового виду мікобактерій, який за



культурально-морфологічними ознаками попередньо ідентифіковано як *M. genavense* [12]. Слід зазначити, що раніше повідомлень про ізоляцію цього виду в нашій країні не було. Отже, небезпечним є те, що патогенність для людини виду *M. genavense* залишається не до кінця з'ясованою.

Екологія та еволюційні механізми адаптації різних видів мікобактерій сприяють формуванню їх підвищеної стійкості до дії протимікробних препаратів, зокрема антибіотиків, а також до дезінфекційних засобів, що значно ускладнює або навіть унеможлиблює лікування зумовлених ними захворювань. Так, унаслідок тривалого застосування протимікробних і дезінфекційних засобів утворюються хіміорезистентні форми мікобактерій, які стають невразливими до дії традиційних лікувальних засобів або дезінфектантів. Це питання в сучасних умовах набуває глобального характеру і вимагає глибокого вивчення на молекулярно-генетичному рівні механізмів резистентності й адаптації мікобактерій до сучасних динамічних змін довкілля, а також до розроблення концепції нових протоколів лікування, заходів боротьби і профілактики мікобактеріозів [10, 11, 24].

Мікобактерії є екологічно важливими мікроорганізмами, які відіграють важливу роль у природних екосистемах, а також мають значний вплив на здоров'я людей і тварин. Враховуючи високу стійкість цих мікроорганізмів до змін довкілля, їхній повільний ріст і розвиток, а також здатність викликати низку небезпечних інфекційних захворювань, вважаємо, що стратегію боротьби з поширенням мікобактеріозної інфекції слід вибудовувати на підставі комплексних підходів і завчасно планувати заходи профілактики та діагностики.

Подальші дослідження екології та взаємодії мікобактерій з іншими організмами, а також пошуки нових способів і засобів боротьби з їхніми патогенними формами, є надзвичайно актуальними для сучасної науки.

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## Ecology of mycobacteriums under conditions of abiotic and biotic factors

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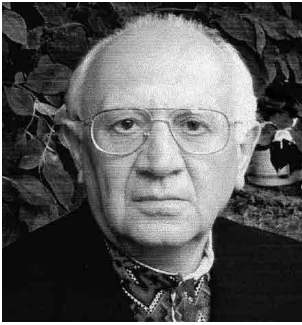
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The article provides an analysis of the current state of the ecological situation regarding various types of mycobacteriums, their systematics, epidemic and epizootological significance, in particular, in Ukraine, taking into account the dynamics of changes in natural and climatic conditions, as well as the influence of anthropogenic factors. The risks to human health that are likely to be expected from these microorganisms, as well as the necessary measures to prevent the realization of these risks, have been studied. The work summarizes a number of own researches, as well as the results obtained by scientists, both in Ukraine and in other countries of the world.

**Key words:** mycobacteriums, environment, mycobacteriosis, ecology, tuberculosis, allergy, chemoresistance



## Ратичу Іринієві Борисовичу — 85!

**15 жовтня 2024 року**

**доктору сільськогосподарських наук, професору,  
члену-кореспонденту НААН,  
директору Інституту (1998–2001 рр.),  
засновнику наукового журналу «Біологія тварин»  
Ратичу Іринієві Борисовичу  
виповнилося 85 років**

Іриней Борисович Ратич народився 15 жовтня 1939 р. в с. Орхівець Підволочиського р-ну Тернопільської обл. 1956–1959 рр. — студент Львівського зооветеринарного технікуму; 1959–1960 рр. — старший лаборант відділу біохімії сільськогосподарських тварин Інституту землеробства і тваринництва західних районів України; 1959–1966 рр. — студент ветеринарного факультету Львівського зооветеринарного інституту; 1960–1976 рр. — старший лаборант та молодший науковий співробітник лабораторії фізіологічних основ утримання сільськогосподарських тварин, а в період 1976–1994 рр. — старший та провідний науковий співробітник лабораторії білків і амінокислот Інституту фізіології і біохімії сільськогосподарських тварин НААН; 1994–1998 рр. — заступник директора з наукової роботи та в період 1998–2001 рр. — директор Інституту землеробства і біології тварин УААН та Інституту фізіології і біохімії сільськогосподарських тварин НААН. З 2001 р. — головний науковий співробітник лабораторії фізіології, біохімії та живлення птиці Інституту біології тварин НААН. У 1971 р. захистив кандидатську дисертацію на тему «Дослідження показників білкового обміну в крові і шкірі телят за ультрафіолетового опромінювання», а у 1994 р. отримав науковий ступінь доктора сільськогосподарських наук на підставі захисту матеріалів, викладених у монографії «Біологічна роль сірки і метаболізм сульфату у птиці». З 1994 до 1998 рр. — член експертної ради Вищої атестаційної комісії України. У 1999 р. обраний членом-кореспондентом Національної академії аграрних наук України.

І. Б. Ратич зосередив основну увагу на вивченні впливу штучних джерел ультрафіолетового випромінювання на процеси білкового обміну в шкірі і крові молодняку великої рогатої худоби.

Іриней Борисович вперше провів імунохімічну ідентифікацію розчинних протеїнів шкіри і сироват-

ки крові великої рогатої худоби. Під час імуноелектрофорезу розчинних протеїнів шкіри опромінюваних і неопромінюваних тварин виявлена однакова кількість дуг преципітації.

Новий етап науково-дослідної роботи І. Б. Ратича розпочався після переходу до лабораторії білків і амінокислот. Дослідження цього періоду були спрямовані на вивчення обмінних процесів і продуктивність птиці у зв'язку з протеїновим, амінокислотним та мінеральним живленням різних вікових, видових і продуктивних груп птиці. Пошуки наукових підходів до вирішення поставлених завдань дали можливість вперше методом авторадиографії амінокислотних хроматограм курячого яєчного білка встановити використання мінеральної сірки, міченої за  $^{35}\text{S}$ , для синтезу цистину вже через 24 годин після її парентерального введення.

Проведені І. Б. Ратичем дослідження з використанням радіоактивних сірковмісних сполук стали теоретичною основою для практичного застосування мінеральної сірки у живленні птиці.

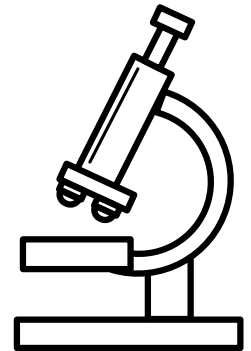
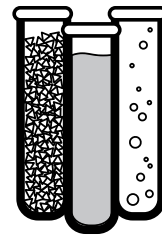
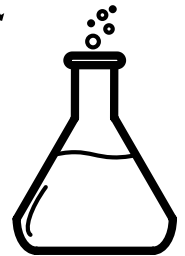
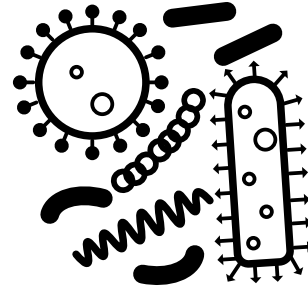
Іриней Борисович Ратич є лауреатом премії ім. С. З. Гжицького. Нагороджений відзнакою Краківського аграрного університету за спільні дослідження та публікації, Почесною відзнакою УААН, медаллю «Знак пошани» Мінагрополітики України, Почесною грамотою Кабінету Міністрів України, грамотою Верховної Ради України, Золотим гербом міста Львова від міського голови Львова Андрія Садового, Грамотою від глави Української Греко-Католицької Церкви Блаженнішого Святослава за «плідну багаторічну наукову працю, що увінчалася численними здобутками в галузі аграрної науки».

І. Б. Ратич опублікував понад 330 наукових праць, 7 книг, 2 посібники, 10 методичних рекомендацій, отримав 11 авторських свідоцтв і патентів. Під його керівництвом захищена одна докторська та 6 кандидатських дисертацій.

*Колектив працівників Інституту біології тварин НААН щиро вітає ювіляра,  
зичить міцного здоров'я і творчого довголіття!*

# ІНСТИТУТ БІОЛОГІЇ ТВАРИН НААН ПРОВОДИТЬ:

- Дослідження біохімічних показників (аналізатор *Humalyzer 2000*, Німеччина)
- Гематологічний аналіз (аналізатор *Mythic-18Vet*, Швейцарія)
- Мікробіологічні дослідження (посів на стерильність, антибіотикограма, склад мікрофлори кишечника тварин, мікробіологічний аналіз кормів, води, повітря)
- Імуноферментні дослідження (аналізатор *Stat Fax 3000*, Німеччина)
- Оцінка репродуктивної здатності тварин, штучне осіменіння, трансплантація ембріонів
- Селекційно-генетичні дослідження
- Дослідження кормів
- Дослідження молока
- Дослідження яєць
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