

Hematological indicators of blood and natural resistance of crossbred generations carp of Galician and Antonin-Zozulenetsk

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Abstract. Two breeds and nine intrabreed types are registered in the modern carp breeding of Ukraine, which differ in genetic origin and are adapted to different regions of Ukraine. Combining the genetic potential of these intrabreed types allows obtaining new promising industrial lines that are physiologically adapted to one or another area of fish farming. Such hybrids are the offspring obtained from the crossing of Galician and Antonina-Zozulenetsk frame carp. The conducted studies proved that crossbred carp had a higher level of natural resistance. According to indicators of the immune system, the level of phagocytic activity of blood serum in experimental carp was in the range of 28.7-39.2, while the highest level of this indicator was in year-1+. At the same time, crossbred carp both in the first and in the second year of life prevailed according to this indicator of the value of pure lines. In terms of the level of lysozyme and bactericidal activity, an increase in the level was also recorded in year-1+ compared to young-of-the-year. At the same time, the level of lysozyme activity was higher in both young-of-the-year and two-year-olds compared to bactericidal activity. At the same time, the hemoglobin content in the blood of young-of-the-year was in the range of 86.9-90.3 g L⁻¹, year-1+ 100.9-108.2 g L⁻¹, and the level of leukocytes, respectively, 1.46-1.51 million μL^{-1} and 1.95-1.98 million μL^{-1} . This indicates that in older age groups, the level of the infectious process in the body is low, so the physiological indicators of fish activity are higher than in younger age groups. Therefore, the use of this type of crossing allows to obtain promising industrial lines of carp with a high level of growth rate, commercial conditions and a level of natural resistance, which are adapted to the conditions of the Polissia of Ukraine.

Key Words: blood serum, erythrocytes, lysozyme and bactericidal activity, natural resistance, phagocytic activity.

Introduction. The history of intensive cultivation of carp fish in domestic aquaculture is almost 100 years old. However, intensive fish farming gained progressive development with the breeding of Ukrainian frame and scaly breeds of carp, which were characterized by high productivity and winter resistance, and in accordance with zoning are structured into intrabreed types (Hrytsyniak et al 2022). With the modern development of aquaculture, breeders have worked out a scheme for obtaining highly productive crossbreeds adapted to Ukrainian fish farming zones. Such crossbreeds can be the descendants of Galician and Antonina-Zozulenetsk carp. The study of hematological and immunological indicators of these hybrids will allow to obtain complete information about the stability of fish stocking material and, accordingly, to improve the economic indicators of breeding farms.

In recent years, understanding and studying the immune system of fish has become important for the fight against infectious diseases both in the natural habitat and in aquaculture (Karadaş 2023), because the physiological state of fish planting material depends on the immune system. The immune system of fish is inferior to that of vertebrates, but fish is an organism that, starting from the embryonic stage, spends its life in an aquatic environment. Therefore, they have defense mechanisms against various

microorganisms, and rely on their innate immune system throughout life, starting from the early stages of embryogenesis (Uribe et al 2011; Fraslin et al 2020).

Taking into account that the modern fishery of Ukraine is focused on the search for highly productive types of carp by crossing Ukrainian breeds, it is worth investigating the heredity of one of the main functions of the body - natural resistance, the main component of which is the presence of lysozyme blood serum. As one of the humoral factors of immunity, it has pronounced enzymatic and antibacterial properties and prevents the penetration of antigens into the internal environment of the body, stimulates phagocytosis, and enhances the activity of T-subpopulations of lymphocytes. In addition, it participates in antitoxic processes, is able to react with nucleic acids and acid mediators of inflammatory processes (Bayne & Gerwick 2001; Biller-Takahashi et al 2013; Biller-Takahashi & Urbinati 2014).

In combination with immunological values, hematological indicators are widely used to assess the health and physiological state of fish in aquaculture. Hematological parameters have been shown to be highly sensitive to various environmental factors, including diet, water quality, stress and pathogens. Although the use of hematological tests is complex and requires experience, their informativeness is one of the main advantages (Witeska et al 2022).

Therefore, the purpose of this study is to investigate and analyze the hematological indicators of blood and natural resistance of different age groups of fish obtained from the reciprocal crossing of the Galician aboriginal massif and the Antonine-Zozulinetsky intrabreed type of carp, in order to determine the most productive type for pond aquaculture.

Material and Method. The experiments were carried out in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes".

The object of the study was evaluation of the hematological and immunological indicators of the young-of-the-year (YOY), age-1 and age-1+ carps of various genesis obtained from the reciprocal crossing of the Galician aboriginal massif and the Antonin-Zozulinets intrabreed type of carp. Cultivation of experimental groups was carried out from May 2021 to October 2023 in the conditions of fish farming of the Carpathian Vodogray LLP, Lviv Region, Pustomyty.

To conduct hematological and immunological studies, we used blood samples of carp obtained from the reciprocal crossing of the Galician aboriginal massif and the Antonin-Zozulinets intrabreed type of carp, i.e.: from the crossing of females of the Galician frame carp and males of the Antonin-Zozulinets frame carp (♀HFC x ♂AZFC); from the crossing of female Antonina-Zozulinets frame carp and male Galician frame carp (♀AZFC x ♂HFC); from the crossing of female Antonina-Zozulinets frame carp and males of Antonina-Zozulinets frame carp (♀AZFC x ♂AZFC); from the crossing of female Galician frame carp and male Galician frame carp (♀HFC x ♂HFC) grown in the conditions of Polissia of Ukraine. Blood was collected from the heart of YOYs, and from the tail vein of age-1 and age-1 + carp. The selection of biological material was carried out in compliance with the Law on Bioethics in the Conduct of Scientific Research (Yevtushenko 2013).

After blood sampling, bacteriological, lysozyme and phagocytic activity of blood serum was determined. Determination of phagocytic activity of blood was carried out according to the method described by Maslyanko et al (2001). An inactivated daily culture of a laboratory strain of *Staphylococcus aureus* (strain N 209-P (3:1)) was used as a test microbe, and in separate experiments, a microbial test culture of *Escherichia coli* (strain 1033 F41, S-MPA form). The phagocytic response of neutrophils was assessed by phagocytic activity, number and phagocytosis index (Maslyanko et al 2001).

The bactericidal activity of blood serum was determined using the photocolometric method (on FEK-56, $\lambda = 540$ nm) in relation to the microbial test culture *E. coli* (VKM strain - 125) (Dovgan 1998). Lysozyme activity of blood serum was determined by the nephelometric method, in relation to the microbial test culture *Micrococcus lysodeikticus* (VKM strain - 109) on FEK-56 in cuvettes with a working length

of 3 mm at a wavelength of 540 nm. The light transmittance of the initial suspension was 20% (Parida et al 2011).

The next task of the study was to determine the amount of hemoglobin and the number of erythrocytes in the blood of experimental carp using the Sali method. Sali's method is based on the comparison of the color of the blood solution under investigation with a standard solution. This method of measuring the amount of hemoglobin gives results with an error of up to 10%. This error is caused by the method itself and the possibility of a subjective error in determining the color. However, this method is simple and convenient. Sali's hemometer consists of a tripod with three test tubes. The outer sealed tubes contain a 1% solution of hematin hydrochloride. The average test tube has divisions from 0 to $23 \times 10 \text{ g L}^{-1}$. This is the absolute amount of hemoglobin in 100 mL of blood. Erythrocytes were counted using a Goryaev counting chamber under a microscope. The method is very time-consuming, but accurate enough (the error does not exceed 2.5%) (Dekhtyaryov et al 2001).

Results. The conducted studies showed that the state of the cellular and humoral links of the body's natural resistance of the studied groups depended on age and genetic characteristics. Thus, indicators of lysozyme activity in age-1+ carp were higher than in YOYs, however, lower than in age-1. The average value of serum lysozyme activity in YOYs carp over two years of research was 32.2 ± 0.89 , age-1 30.1 ± 0.70 and age-1+ carp $36.8 \pm 0.53\%$. At the same time, young-of-the-year crossbred cultivars had higher rates of natural resistance compared to pure lines, both in the first year of research and in the second. The highest lysozyme activity in blood serum (35.2% in the first year of research and 36.2% in the second year of research) was observed in crossbred YOYs obtained from crossing ♀HFC and ♂AZFC, the superiority over those of the maternal line of Galician carp was 4.7 and 6.1%. YOYs carp obtained from crossing ♀AZFC and ♂HFC had values of 32.0% and 34.5%, respectively, and exceeded the indicator of the pure maternal line of the Antoniny-Zozuleets carp by 2.5% and 4.7% respectively.

Compared to YOYs, age-1 of all experimental groups had lower values. However, it is worth noting that the decrease in this indicator in the first year of research was within the range of 0.3-2.2%, in the second year - 1.4-5.4%. At the same time, the decrease of this indicator was more significant in YOYs. Thus, in YOYs obtained from the crossing of ♀HFC and ♂AZFC, in comparison with peers of the pure line of Galician carp, the first year of research was 1.0%, the second - 3.8%. In yearlings obtained from the crossing of ♀AZFC and ♂HFC, the decrease in comparison with the indicator of the pure line of Antonina-Zozulenets carp was 2.2% and 5.4%, respectively.

The average value of the indicator of lysozyme activity in age-1+ carp was in the range of 34.1-39.0%. As in YOYs, in age-1+ this indicator was higher in crossbred individuals. The advantage of age-1+ obtained from the crossing of ♀HFC and ♂AZFC compared to YOYs of the pure line of Galician carp was 2.2 and 2.6% respectively, in YOYs obtained from the crossing of ♀AZFC and ♂HFC over peers of the pure Antoniny-Zozulenets carp line was 2.0% and 2.7% respectively (Figure 1).

A similar dependence was established for the level of bactericidal activity. Thus, indicators of bactericidal activity in age-1+ carp were higher than in YOYs, however, lower than in age-1. The average value of the bactericidal activity of blood serum in this YOYs carp over the two years of research was in the range of 28.5-32.9%, age-1 - 26.2-29.9%, age-1+ - 32.4-35.2%. At the same time, the functional state of the humoral factors of protection of crossbred carp was at a higher level than in the pure lines of Galician and Antoniny-Zozulenets *carp*. Thus, the level of bactericidal activity in crossbreeds of YOYs obtained from crossing ♀HFC and ♂AZFC was higher than in individuals of the pure line of Galician carp by 2.1% in the first year of research, and 4.3% in the second year of research. In YOYs, this advantage was 2.1% and 2.9%, in age-1 - 1+ 0.6% and 0.3%, respectively. This YOYs crossbred females obtained from crossing ♀AZFC and ♂HFC prevailed in the level of bactericidal activity of individuals of the pure line in the first year of life by 1.1-1.9%, in the second - by 0.7-0.8% (Figure 2).

Among the studied groups of carp, according to blood phagocytic activity, it was established that the highest level of this indicator was in age-1+. At the same time,

crossbred age-1+ prevailed according to this indicator of the value of pure lines. The advantage of crossbred age-1+ obtained from crossing ♀HFC and ♂AZFC was 1.8 and 2.4%, respectively, according to the years of research. In crossbred age-1+ obtained from crossing ♀AZFC and ♂HFC, a similar advantage was 1.8% and 1.3%. Crossbred strains YOYs were also characterized by a higher level of phagocytic activity in comparison with the indicators of pure lines. The advantage YOYs crossbreeds obtained from the crossing of ♀HFC and ♂AZFC compared to the pure line of Galician carp was 0.9 and 4.5%, respectively, according to the years of research. At the same time, in the YOYs of research, crossbred females obtained from the cross between ♀AZFC and ♂HFC were 0.4% inferior to YOYs of the pure Antoniny-Zozulenets carp line by 0.4%, and in the second year, on the contrary, they prevailed according to this indicator by 3.3% (Figure 3).

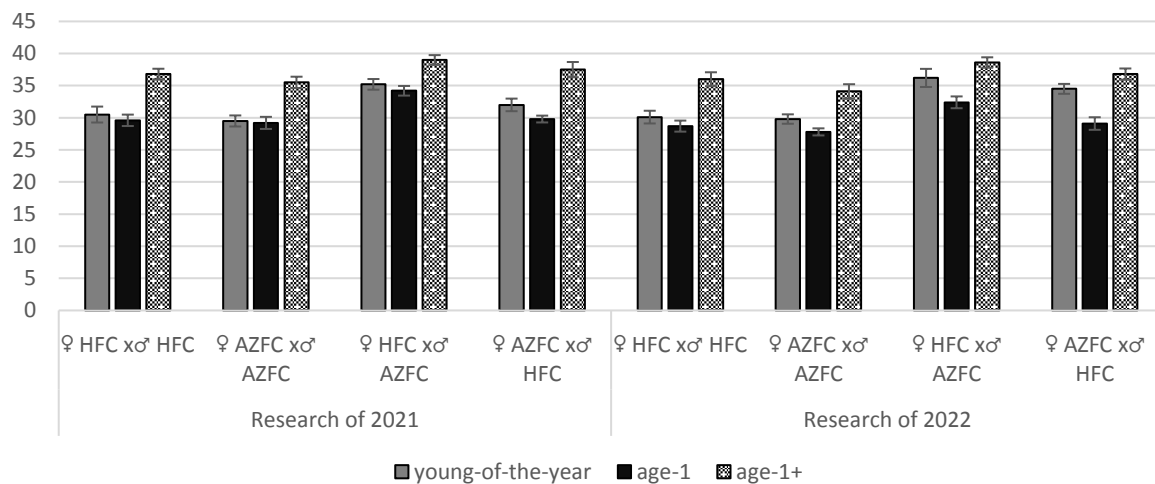


Figure 1. Indicators of lysozyme activity of different age groups of carp of different genesis ($x \pm SD$, $n = 6$).

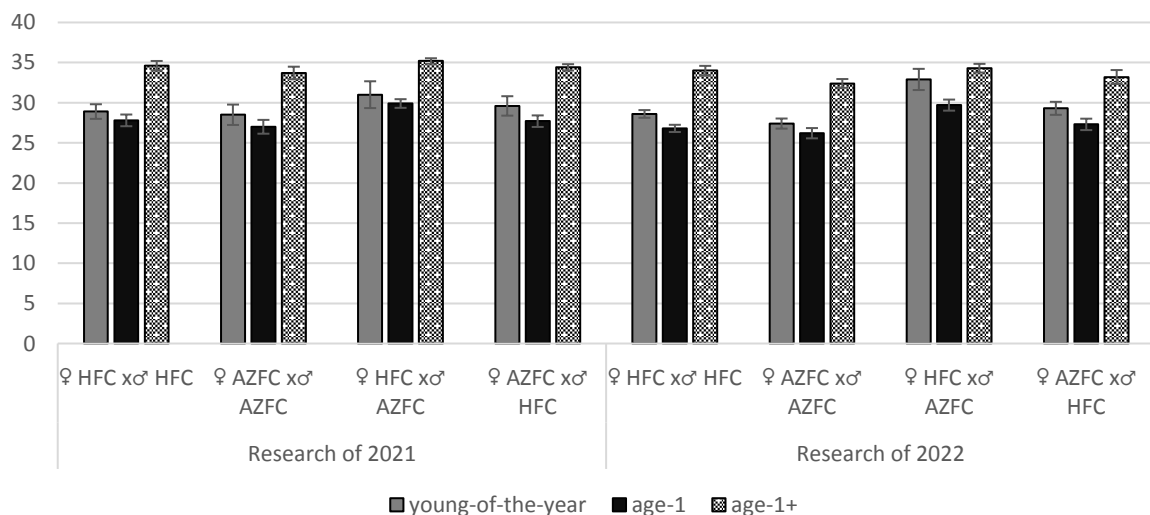


Figure 2. Indicators of bactericidal activity of different age groups of carp of different genesis ($x \pm SD$, $n = 6$).

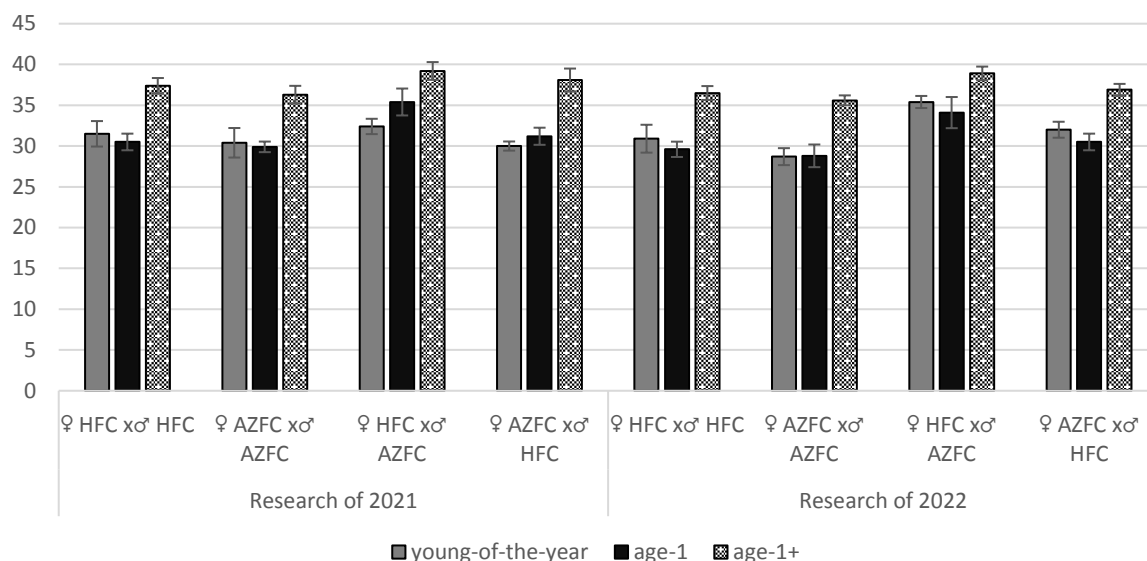


Figure 3. Indicators of phagocytic activity of different age groups of carp of different genesis ($x \pm SD$, $n = 6$).

The obtained results showed that the studied groups of fish differ among themselves in terms of hemoglobin content at all stages of cultivation. During the two periods of research, among this YOYs carp, the highest level of hemoglobin - 90.3 g L^{-1} was found in crossbred carp obtained from crossing ♀HFC and ♂AZFC, the lowest - 86.9 g L^{-1} in individuals of the pure line of Antonina-Zozulenetsk carp. In general, the average indicator of hemoglobin content in YOYs was 88.5 g L^{-1} and was higher than the average value of age-1 by 3.9%. At the same time, among YOYs, the highest value was also observed in crossbred individuals obtained from the crossing ♀HFC and ♂AZFC. Compared to the pure lines of Galician carp, the advantage of this hybrid was 2.11% in the first year of research, and 1.45% in the second. In age-1+ carp of all experimental groups, there was a significant increase in hemoglobin concentration compared to YOYs. In the first year of research, the maximum value was characterized by the crossbred age-1+ obtained from the crossing of ♀HFC and ♂AZFC - 108.3 g L^{-1} and the pure line of Galician carp - 108.2 g L^{-1} . At the same time, according to this indicator, crossbred age-1+ dominated the value of pure lines, both for crossing ♀HFC and ♂AZFC and ♀AZFC and ♂HFC. The following year, the superiority of crossbred groups over the pure line was observed only in the crossing of ♀HFC and ♂AZFC, the crossbred group of age-1+ carp obtained from the crossing of ♀AZFC and ♂HFC was inferior to the pure line of Antoniny-Zozulenets carp by 1.1% (Table 1).

According to the content of erythrocytes in the blood of the studied groups of carp, the difference between them was recorded at all stages of cultivation. The average value of crossbreeds YOYs was $1.59 \text{ million } \mu\text{L}^{-1}$ and exceeded the indicator of pure lines by 28.4%. At the age of age-1, the dynamics of the content of erythrocytes in the blood tended to increase in all experimental groups. So, in age-1, the number of erythrocytes was in the range of $1.80\text{-}1.88 \text{ million } \mu\text{L}^{-1}$. At the same time, as in YOYs, in the first year of research, crossbred age-1 obtained from crossing ♀HFC and ♂AZFC and ♀AZFC and ♂HFC had an advantage over the pure lines of Galician and Antoniny-Zozulenets carp. On the other hand, in the second year of research, crossbred annuals obtained from ♀AZFC and ♂HFC had lower values than the indicators of the pure line of Antoniny-Zozulenets carp. In the second year of life, the number of erythrocytes in the blood of experimental carp was in the range of $1.95\text{-}1.99 \text{ million } \mu\text{L}^{-1}$, and it was higher in crossbred individuals. Thus, in crossbred age-1+ obtained from crossing ♀HFC and ♂AZFC, the number of erythrocytes was higher in comparison with individuals of the pure line of Galician carp by 0.5%, during two years of research. Crossbred age-1+ obtained from crossing ♀AZFC and ♂HFC had a similar advantage over individuals of the pure line of Antoniny-Zozulenets carp (Table 2).

Table 1

Indicators of hemoglobin content in the blood of carp of different genesis in the first and second year of cultivation, g L⁻¹ (x±SD, n = 6)

Age category	Research of 2021				Research of 2022			
	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x
	♂ HFC	♂ AZFC	♂ AZFC	♂ HFC	♂ HFC	♂ AZFC	♂ AZFC	♂ HFC
YOYs	87.69±0.81	88.99±1.18	90.27±1.04	89.41±1.29	89.01±0.90	86.92±0.72	88.35±1.33	87.74±0.84
Age 1	84.82±0.85	85.77±1.61	86.61±1.54	85.52±1.54	84.11±1.11	84.64±1.17	85.33±1.63	84.82±1.54
Age 1+	108.17±0.47	100.91±1.25	108.31±0.54	106.65±0.72	104.82±1.54	103.50±1.15	107.86±1.29	102.39±0.95

Table 2

Indicators of the content of erythrocytes in the blood of carp of different genesis in the first and second year of rearing, million μL⁻¹ (x±SD, n = 6)

Age category	Research of 2021				Research of 2022			
	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x	♀ HFC x	♀ AZFC x
	♂ HFC	♂ AZFC	♂ AZFC	♂ HFC	♂ HFC	♂ AZFC	♂ AZFC	♂ HFC
YOYs	1.47±0.09	1.50±0.08	1.51±0.06	1.46±0.07	1.47±0.13	1.46±0.14	1.49±0.07	1.47±0.06
Age 1	1.83±0.04	1.85±0.04	1.86±0.04	1.88±0.03	1.81±0.04	1.83±0.04	1.84±0.06	1.80±0.05
Age 1+	1.98±0.06	1.95±0.05	1.99±0.07	1.97±0.05	1.97±0.03	1.95±0.04	1.98±0.04	1.96±0.03

Discussion. Fish contain natural, relatively non-specific proteins that differ from immunoglobulins and may confer some degree of immunity against natural infection. In most cases, the cause of the antigenic stimulus is not obvious, although the formation of these "antibodies" could be caused by exposure to various microorganisms (Ingram 2006). Previous studies have established that carp of the Ukrainian scaly breed are more adaptable and hardy to artificial growing conditions compared to representatives of the frame breed. Accordingly, the level of natural resistance in them is higher (Loboyko 2013). However, taking into account that the market requires products with a low-scaly type of scaly coating, the main direction of selection work is the development of new industrial lines that would be characterized by high indicators of the immune system (Beh & Hrytsyniak 2011). Obtaining this result is possible using genetic methods. Thus, among the herd, individuals with increased indicators of natural resistance are selected and offspring are obtained from them, among whom, at the age of puberty, re-selection is carried out for this trait (Fjalestad et al 1993; Mohanty et al 2007; Houston 2017; Buchmann 2022).

In the body of fish, circulating immune complexes characterize the degree of synthesis of antibodies and, accordingly, are aimed at the destruction of pathogens. At the same time, phagocytosis is the first phase of a specific immune reaction in the formation of the cellular link of non-specific resistance of the fish organism, as a result of which the antigen undergoes changes, accordingly, the result is the formation of a large number of antigenic determinants (Bowden et al 2007). Research within the scope of this work established that, according to indicators of the immune system, the level of phagocytic activity of blood serum in experimental cross-breed carp was in the range of 30.0-39.2%, and pure lines - 28.7-37.4%. At the same time, experimental individuals of the pure line and crossbred with the mother line of the Galician carp had higher indicators compared to the individuals of the Antonin-Zozulenetsk carp. The research results show that the selected crossbreeding makes it possible to obtain crossbred groups of carp with increased indicators of natural resistance compared to pure lines of this inbred type (Gurbyk et al 2017).

Bactericidal activity of blood serum (BASC) is an integral factor of the body's natural resistance of the humoral type, which is due to the presence of lysozyme and properdin, the main function of which is to neutralize microbial cells (Dissasa et al 2023). In experimental individuals, the level of bactericidal activity increased with age in all experimental groups, but the indicator of this activity in crossbred groups compared to pure lines decreased in age-1+ compared to YOYs. A similar dynamic of indicators was recorded for the level of lysozyme activity in blood serum. Age-1+ were characterized by the maximum indicators. At the same time, it is worth noting that the experimental individuals of the Antoniny-Zozulenets carp had the minimum indicators during two years of research, while crossbred groups due to the influx of Galician carp blood had higher values. Since the level of bactericidal and lysozyme activity reflects the level of the humoral link of immunity, this indicates that in older age groups, the level of the infectious process in the body is low, respectively, the physiological indicators of fish activity are higher than in younger age groups. This feature is characteristic of breed groups of carp grown not only in the conditions of Ukraine, but also European breeds (Stosik et al 2002).

The analysis of indicators of the hematological formula confirms that the hemoglobin content in the blood of YOYs was in the range of 86.9-90.3 g L⁻¹, age-1+ 100.9-108.2 g L⁻¹, and the level of leukocytes, respectively, 1.46-1.51 million μ L⁻¹ and 1.95-1.98 million μ L⁻¹. At the same time, the maximum values for this indicator were characterized by crossbred individuals obtained from crossing ♀HFC and ♂AZFC. Therefore, the use of this type of crossing allows obtaining promising industrial lines of carp with a high rate of growth, commercial conditions (Krasnopolska & Kurinenko 2023, 2024) and a level of natural resistance adapted to the conditions of the Polissia of Ukraine.

Conclusions. As a result of the conducted research, the main links of natural resistance of crossbred carp obtained from the reciprocal crossing of Galician and Antoniny-

Zozulenets carp were studied, and a comparative analysis of the obtained results with YOYs of pure lines was carried out. According to the research results, it was established that crossbred carp had a higher level of natural resistance. According to the analysis of indicators of the immune system, the level of phagocytic activity of blood serum in experimental carp was in the range of 28.7-39.2, while the highest level of this indicator was in year-1+. At the same time, crossbred carp both in the first and in the second year of life prevailed according to this indicator of the value of pure lines. In terms of the level of lysozyme and bactericidal activity, an increase in the level was also recorded in year-1+ compared to YOYs. At the same time, the level of lysozyme activity was higher in both YOYs and year-1+ compared to bactericidal activity. At the same time, the hemoglobin content in the blood of YOYs was in the range of 86.9-90.3 g L⁻¹, year-1+ 100.9-108.2 g L⁻¹, and the level of leukocytes, respectively, 1.46-1.51 million μL⁻¹ and 1.95-1.98 million μL⁻¹.

Conflict of interest. The authors declare that there is no conflict of interest.

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