Some aspects of fish breeding for immune resistance as shown through a study of common carp

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Abstract. In this article we will review the ways and possibilities of increasing the immune resistance of fish by means of selection. We have revealed the physiological characteristics of rubella-resistant carp breeds. These breeds are distinguished by a higher level of metabolism (in terms of uric acid content, enzyme activity, such as alkaline phosphatase, creatine kinase), a lower proportion of neutrophils in the total number of leukocytes, but a higher activity of their oxygen-independent phagocytosis mechanisms in terms of the average cytochemical coefficient of the lysosomal cationic protein content. The selection of immunostable fish according to a number of physiological and immunological parameters has been proposed. A cross “Surskii low-bone” was obtained - a hybrid of a female productive Anishskaya mirror breed and a male immunostable Angelinskaya mirror breed. The cross showed a high heterotic effect on survival at different stages of ontogenesis. The results of the physiological and immunological assessment of the cross revealed the distinctive features of the cross in terms of hematological, cytochemical and biochemical parameters, namely: a significant proportion of lymphocytes (immunocompetent cells responsible for specific immunity) due to immature stab forms of neutrophils; the optimal value of CCR cationic protein; as well as a high level of protein and carbohydrate metabolism in terms of amylase activity and albumin content.

Key words: selection, immune resistance, hybrids, hematological parameters, leukogram, biochemical parameters, lysosomal cationic protein.

Introduction. Fish diseases cause significant damage to fish farms. Fertile animals are especially vulnerable, since unilateral selection for productivity leads to: an imbalance of gene potential (homozygotes); inbred depression; the development of secondary immunodeficiency states. To keep the livestock healthy, it is necessary to carry out a set of measures to strengthen the immune resistance of fish. One of these activities is selection based on a combination of traits, the most important of which is immune resistance (Hoeck & Keller 2012).

Immune resistance is the state of the body’s defense and adaptive mechanisms, capable of withstanding various adverse environmental factors (Kondrakhin 2004). Immune resistance is one of the most important integral characteristics of the body.

The body’s immune response is usually divided into two types: nonspecific innate and acquired immune responses (Syame et al 2018):

1. all multicellular organisms have nonspecific immunity, which is directed at pathogens, regardless of their type. It manifests itself in the form of humoral (due to the production of bactericidal substances) and cellular pathogens, as a result of which phagocytosis and cytotoxic effect are carried out. Nonspecific immunity is innate in origin and is carried out with the participation of neutrophils, monocytes, macrophages, eosinophils, basophils.

2. specific immunity is found only in vertebrates and works in close connection with nonspecific. As a result of specific immunity, the body increases the level of its immunity to a specific pathogen when it meets again. Specific immunity is innate and acquired. It, like non-specific, is realized in two forms: humoral (production of antibodies
by B-lymphocytes and plasma cells) and cellular, which is realized mainly with the participation of T-lymphocytes.

For selection, indicators characterizing non-specific immunity are important, since it is hereditary. In this regard, the indicator of the activity of phagocytosis, as the most phylogenetically ancient factor of nonspecific cellular defense, is of interest. The first prerequisite for the possibility of selection for the trait "resistance to disease" is the presence of variability in this trait among both domesticated and wild populations (Zhang et al 2017).

The resistance of animals to diseases usually has a polygenic type of inheritance, it is due to the action of many genes. Identification of the genetic determination of some diseases creates the basis for selection for resistance. Immune resistance is inherited as a dominant trait, the heritability coefficient often exceeds 0.3 (Meuer 1985; Kirpichnikov 1987; Reiner 2008).

Certain difficulties in breeding for the consolidation of resistance to infectious diseases of viral, bacterial and fungal etiology arise in connection with the ability of foreign agents to exhibit great variability, in which, in a short period of time, the same type of microorganism changes heredity. As a result, animals resistant to one strain are susceptible to the newly emerged strain of the microorganism (Stear et al 2012). Breeding for resistance in animals is complicated by related mating. Inbreeding leads to an increase in the homozygosity of herds and breeds, often causes inbred depression, reduces the resistance of inbred offspring, increases the spread of unwanted recessive genes and homozygous (often lethal) genotypes in the population (Freeman & Bumstead 1987; Charlesworth & Willis 2009). Despite the difficulties in breeding for resistance, encouraging results have been obtained for the creation of resistant groups of pigs and cattle (Shishkov et al 1983; Hu et al 1997; Zhao et al 2012).

The presence of intraspecific (for wild fish) and intra-breed (for domesticated fish) variability in resistance has been shown for a number of fish diseases. A large number of resistant lines are known among salmonids. Thus, among 79 lines of rainbow trout (*Oncorhynchus mykiss*), 10 lines are resistant to diseases: two to ceratomycoisis, three to bacterial kidney disease, one to furunculosis, one to infectious pancreatic necrosis, three to diseases in general (Ilyasov 2004; Stear et al 2012).

Common carp (*Cyprinus carpio* Linnaeus, 1758) is a fairly common type of fish, widely grown in fish farms in our country. Among the large number of infectious diseases of common carp, rubella is the most contagious, leading to epizootics and causing significant damage to farms. When this infection is detected, the fish farm is quarantined. The term “rubella” of carp is understood as a symptom complex caused by aeromonads, pseudomonads, or the virus of spring viremia of carp (Golovina et al 2003). Angelinskiy breed is the only carp breed obtained as a result of selection for immune resistance to rubella (Kirpichnikov 1987; Ilyasov 2002).

The physiological and immunological characteristics of producers of rubella-resistant fish in comparison with immune-susceptible breeds were determined by the staff of the laboratory of immuno-physiological studies of aquatic organisms of the Federal State Budgetary Scientific Institution VNIIR. It was revealed that the immune-resistant carps of the Angelinskiy scaly and Angelina mirror breeds have a higher level of protein and carbohydrate metabolism, judging by the activity of aspartate aminotransferase, creatine kinase, the content of albumin, lactate and urea. Hematopoiesis occurs more intensively in Angelinskiy carp. In the leukogram of these fish, the proportion of mature segmented forms of neutrophils is higher (Pronina 2017).

To assess the physiological and immunological status of fish, a system of methods was proposed, in which the most informative and available for determination hematological, cytochemical and biochemical parameters reflecting the state of immunity were selected (Pronina & Koryagina 2017).

The results of cytochemical studies reflect the phagocytic activity of cells, which is one of the main components of innate immunity. The body’s protective function is based on the phagocytic process, which consists in their ability to recognize, absorb, kill and digest foreign cells. As a highly sensitive indicator of norm and pathology, the
characteristics of phagocytes serve as a useful tool not only for immunological, but also for general clinical diagnostics (Mayansky & Mayansky 1989; Andersson et al 2016).

As an indicator of the potential phagocytic activity of the main microphages in blood, we use the average cytochemical coefficient (ACC) of a nonenzymatic cationic protein in blood neutrophils. Cationic lysosomal proteins - defensins have the ability to destroy microorganisms, stimulate phagocytosis, mobility and accumulation of neutrophils, regulate the activation of the complement system, and enhance the activity of innate immunity factors. Cationic peptides are involved in antimicrobial defense in two ways (Diamond et al 2009): 1. in providing a direct antimicrobial effect; 2. indirectly, inducing the mechanisms of innate and adaptive immune response.

Cationic proteins belong to the oxygen-independent cell defense system. It has been shown that even under anaerobic conditions, i.e. when cells cannot form reactive oxygen species, neutrophils kill Staphylococcus epidermidis, Pseudomonas aeruginosa, green streptococcus and other microbes. It has been shown that these proteins have universal antimicrobial activity, properties of inflammatory mediators, a permeability factor, a phagocytosis stimulator, a modifier of respiratory and enzymatic processes in a cell (Nagoev 1982; Pigarevsky 1982; Andersson et al 2016).

The purpose of this research is to create a cross of carp with a scattered (mirror) scale cover, good fertility, high natural resistance and survival. As well as a comparison of the physiological and immunological parameters of the original breeds and cross of carp, and to identify the features of immunostable fish.

Material and Method. The experiments were carried out between 2015 and 2019, in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, ETS Nº123, Strasbourg, 1986. The experimental protocol was approved by the Ethics Committee of the Federal State-Funded Scientific Institution All-Russian Research Institute of Irrigation Fish-Breeding. The research was carried out in the fish farm "Kirya" of the Chuvash Republic.

The objects of the study were carp of different breeds: immune-resistant (Figure 1) and immune-susceptible (Figure 2), as well as the cross "Surskiy malokostny", obtained as a result of crossing of females of the Anish mirror breed and males of the Angelinskiy mirror breed, the cross "Surskiy Low-bone", selection achievement 77898/8057049 (Figure 3).
Angelinski carps have undergone long-term selection for rubella resistance against an aggressive background at the Angelinskiy fish farm in Krasnodar. The fish were brought to the Kirya fish farm at the larval stage.

Carps of the Chuvash scaly and Anishskaya mirror breeds were obtained by an accelerated method of selection for productive growth with selection for the level of serum enzyme alanine aminotransferase with control for hematological parameters (Maslova & Petrushin 2005).

The survival rate of eggs was determined by the percentage of living embryos in Petri dishes and Weiss apparatus.

The effect of heterosis was determined by the formula (Gorin & Epishko 1992; Gixhari & Sulovari 2010; Vlasov & Maslova 2015):

$$H = \frac{(Sg - Sb) \times 100}{Sb}$$

where:
- $H$ stands for absolute (true) heterosis;
- $Sg$ is a sign of a hybrid;
- $Sb$ is a sign of the best breed.

Physiological and immunological assessment of fish was carried out according to hematological, biochemical and immunological parameters.

The leukocyte formula (WBC differential) was determined by differential counting in Pappenheim-stained peripheral blood smears. The level of fish’ hematopoiesis was defined based on the share of immature forms of erythrocytes and leukocytes.

Phagocytic activity of fish neutrophils was determined according to the principle of Astaldi & Verga (1957) by the cytochemical method with bromophenol blue according to Shubich (1974), adapted for hydrobionts by Pronina (2008). The content of non-enzymatic cationic protein in lysosomes of peripheral blood neutrophils was determined. According to the degree of phagocytic activity, the cells under study were divided into 4 groups (Figure 4):

- 0 degree - no cationic protein granules;
- 1st degree - single granules;
- 2nd degree - granules occupy about 1/3 of the cytoplasm;
- 3rd degree - granules occupy 1/2 of the cytoplasm and more.

The Mean Cytochemical Coefficient (MCC) was calculated with the formula:

$$MCC = \frac{(0 \times N_0 + 1 \times N_1 + 2 \times N_2 + 3 \times N_3)}{100}$$

where:
- $N_0, N_1, N_2, N_3$ are numbers of neutrophils with 0, 1, 2 and 3 point activity;
- $N_0 + N_1 + N_2 + N_3 = 100$.

Biochemical analysis of blood serum was carried out on the device Chem Well Awareness Technology, using VITAL reagents.

The mathematical treatment of the digital materials was performed by statistical methods according to Student’s test with the application of such software as Excel from the Microsoft Office package. The differences were considered significant at $p < 0.05$. 

Figure 3. Cross “Surskiy malokostny”. 

Angelinskiy carps have undergone long-term selection for rubella resistance against an aggressive background at the Angelinskiy fish farm in Krasnodar. The fish were brought to the Kirya fish farm at the larval stage.

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Figure 3. Cross “Surskiy malokostny”。
Results and Discussion. When studying three-year old immunostable Angelinskiy and susceptible Chuvash scaly and Anish mirror carp breeds, it was found that fish did not differ significantly in body weight (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Index</th>
<th>Chuvash scaly</th>
<th>Angelinskiy scaly</th>
<th>Anish scaly</th>
<th>Angelinskiy mirror</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>1.98±0.05</td>
<td>2.16±0.18</td>
<td>1.84±0.06</td>
<td>2.44±0.28</td>
</tr>
<tr>
<td>Body standard length, cm</td>
<td>43.9±0.5</td>
<td>46.9±0.5</td>
<td>42.8±0.4</td>
<td>46.8±1.3</td>
</tr>
</tbody>
</table>

Comparative analysis showed that Angel immune-resistant carp differ from fertile breeds in a number of indicators.

The proportion of neutrophils in fish of the Angelinskiy breed is significantly lower than in other studied breeds due to immature stab forms (Table 2). This indicates a better formation of the microphage system of the blood of angelina fish.

**Table 2**

<table>
<thead>
<tr>
<th>Index</th>
<th>Chuvash scaly</th>
<th>Angelinskiy scaly</th>
<th>Anish scaly</th>
<th>Angelinskiy mirror</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte formula, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>-</td>
<td>0.4±0.3</td>
<td>-</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>-</td>
<td>0.6±0.4</td>
<td>0.2±0.2</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>5.0±0.8</td>
<td>3.6±1.0</td>
<td>7.4±1.6</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td>Banded neutrophils</td>
<td>4.4±1.3*</td>
<td>0.8±0.4*</td>
<td>5.2±1.7**</td>
<td>0.4±0.3**</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>3.6±1.4</td>
<td>3.0±0.6</td>
<td>1.8±0.9</td>
<td>2.6±1.0</td>
</tr>
<tr>
<td>Total neutrophils</td>
<td>8.0±1.1*</td>
<td>3.8±0.7*</td>
<td>7.0±1.4**</td>
<td>3.0±1.0**</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.4±0.3</td>
<td>-</td>
<td>0.2±0.2</td>
<td>-</td>
</tr>
<tr>
<td>Basophils</td>
<td>-</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.6±1.4</td>
<td>5.0±0.6</td>
<td>3.4±0.8</td>
<td>4.2±0.7</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>85.0±1.9</td>
<td>86.4±1.6</td>
<td>81.6±3.1</td>
<td>87.8±1.6</td>
</tr>
</tbody>
</table>

**Lysosomal cation test**

| MCC, units                         | 1.65±0.08*    | 1.93±0.07        | 1.69±0.05** | 1.88±0.06**       |

Note: hereinafter * the differences between the scaly rocks; ** the differences between the mirror rocks are significant (p < 0.05).
It is the mature segmented forms of neutrophils that effectively perform the functions of nonspecific cellular defense.

The proportion of lymphocytes in carp of various breeds as follows: Amur wild carp, Ropsha scaly carp, Ukraine scaly carp, Northern mirror carp, Hungarian mirror carp, Israeli mirror carp, South Bohemian mirror carp and Tata scaly carp, as well as in individuals of the wild carp population (Svobodova et al 2008) is at the same level as in Chuvash scaly, Angelinskiy scaly, Anish mirror, Angelinskiy mirror, and cross "Surskiy malokostny." The percentage of neutrophils in scaly and mirror Angelinskiy carps is lower than in other fish (studied in the present study and according to Svobodova et al (2009)). Comparison of leukopoiesis is difficult, since most authors do not isolate immature forms of granulocytes in the blood.

The potential phagocytic activity of these cells (according to the SCC of the content of non-enzymatic cationic protein in lysosomes) in Angelinskiy fish is significantly higher than in fertile breeds.

Angelinskiy carp have a high level of alanine aminotransferase, which reflects a significant potential for protein growth (Table 3). The difference is significant only for scaly groups. The activity of creatine kinase of the Chuvash scaly and Anish mirror breeds is approximately twice as high as compared to the same age Angelinskiy carp. The enzyme reflects energy metabolism in the muscles, and such an increase in its level may be associated with the activation of growth and immunity, in connection with this possible damage to cells after wintering. The values of the indicator were high in all carp and most likely due to the peculiarities of fish metabolism (in comparison with homeothermic organisms such as mammals).

The carp breeds studied by us, in contrast to Amur wild carp and breeds derived from it (Ropsha scaly carp, Ukrainian scaly carp and Northern mirror carp (according to Svobodova et al (2009)), had consistently low values of glucose and triglyceride levels in their blood, that is, a fairly intensive carbohydrate and lipid metabolism. This is in favor of the species of the Kirya fish farm.

### Table 3

<table>
<thead>
<tr>
<th>Index</th>
<th>Chuvash scaly</th>
<th>Angelinskiy scaly</th>
<th>Anish scaly</th>
<th>Angelinskiy mirror</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, units L⁻¹</td>
<td>43.0±2.7*</td>
<td>63.6±4.3*</td>
<td>45.2±3.7</td>
<td>60.5±10.1</td>
</tr>
<tr>
<td>AST, units L⁻¹</td>
<td>287±55</td>
<td>230±15</td>
<td>248±20</td>
<td>243±36</td>
</tr>
<tr>
<td>Glucose, mmol L⁻¹</td>
<td>4.1±1.1</td>
<td>5.4±0.4</td>
<td>4.7±1.5</td>
<td>4.8±1.3</td>
</tr>
<tr>
<td>CC, unit L⁻¹</td>
<td>454±237*</td>
<td>2874±339*</td>
<td>4868±495**</td>
<td>2807±493**</td>
</tr>
<tr>
<td>Creatinine, μmol L⁻¹</td>
<td>14.3±2.6</td>
<td>8.9±2.9</td>
<td>17.4±7.5</td>
<td>5.6±4.6</td>
</tr>
<tr>
<td>Lactatate, mg dL⁻¹</td>
<td>47.9±4.3</td>
<td>56.8±8.2</td>
<td>40.0±10.2</td>
<td>77.5±12.4</td>
</tr>
<tr>
<td>ALP, units L⁻¹</td>
<td>30±3*</td>
<td>185±41*</td>
<td>32±12</td>
<td>145±67</td>
</tr>
<tr>
<td>Albumin, g dL⁻¹</td>
<td>11.7±1.5</td>
<td>11.2±0.3</td>
<td>14.1±2.1</td>
<td>16.1±3.1</td>
</tr>
<tr>
<td>Amylase, u L⁻¹</td>
<td>24.7±10.8</td>
<td>26.7±7.4</td>
<td>13.4±8.1</td>
<td>42.5±17.2</td>
</tr>
<tr>
<td>Urea, mg dL⁻¹</td>
<td>5.6±1.8</td>
<td>13.6±0.5</td>
<td>10.3±1.5</td>
<td>17.8±3.1</td>
</tr>
<tr>
<td>Total protein, g L⁻¹</td>
<td>23.5±2.0</td>
<td>30.8±3.6</td>
<td>27.4±3.1</td>
<td>34.8±6.4</td>
</tr>
<tr>
<td>Triglycerides, mg dL⁻¹</td>
<td>134±23</td>
<td>134±19</td>
<td>162±34</td>
<td>145±42</td>
</tr>
<tr>
<td>Cholesterol, mg dL⁻¹</td>
<td>148±6</td>
<td>70±3</td>
<td>163±17</td>
<td>85±12</td>
</tr>
</tbody>
</table>

Note: hereinafter * - the differences are significant (p < 0.05).

The intensity of protein metabolism in Angelinskiy carp is relatively high. Thus, the activity of alanine aminotransferase (ALT) in Angelinskiy three-year-olds is significantly higher than in other studied carps.

All the studied parameters did not go beyond the reference values determined for the corresponding age categories of fish (Pronina & Koryagina 2015).

On the basis of the research carried out, the criteria for the selection and selection of carp with high immunity were identified according to the following indicators:
1. the proportion of stab neutrophils in the leukogram (0-3%);
2. segmented neutrophils (2-8%);
3. MCC of lysosomal cationic protein in peripheral blood neutrophils (1.4-1.8);
4. ALT (25-60 units L⁻¹);
5. AST (150-250 units L⁻¹).

Studies of the obtained cross "Surskiy malokostny" showed that due to its high immune properties, it has good survival at the embryonic, larval stages. Tables 4 and 5 show the results of incubation of eggs in Petri dishes and Weiss apparatus, respectively.

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Surskiy malokostny hybrid</th>
<th>Anish mirror breed</th>
<th>Angelinskiy mirror breed</th>
<th>True heterogosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>93.6</td>
<td>87.0</td>
<td>85.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Two days</td>
<td>86.4</td>
<td>78.4</td>
<td>84.4</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Anish mirror female x Angelinskiy mirror male</th>
<th>Angelinskiy mirror female x Anish mirror male</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 o'clock</td>
<td>1.3±0.5</td>
<td>2.9±0.8</td>
</tr>
<tr>
<td>36 o'clock</td>
<td>21.4±1.8</td>
<td>29.2±3.6</td>
</tr>
</tbody>
</table>

Table 5

Two-year-olds of the "Surskiy low-bone" cross showed advantages over the same-aged parental forms (breeds) in a number of hematological parameters (Table 6).

<table>
<thead>
<tr>
<th>Index</th>
<th>Surskiy malokostny hybrid</th>
<th>Parent breeds</th>
<th>Leukocyte formula, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anish mirror</td>
<td>Angelinskiy mirror</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>0.2±0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>0.3±0.2</td>
<td>0.3±0.3</td>
<td>-</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>0.5±0.3</td>
<td>0.6±0.5</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>1.2±0.4</td>
<td>2.0±0.9</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>Banded neutrophils</td>
<td>1.0±0.3</td>
<td>3.1±0.5*</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>2.0±0.9</td>
<td>1.7±0.7</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>Total neutrophils</td>
<td>3.0±0.9</td>
<td>4.7±0.3</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>-</td>
<td>0.3±0.3</td>
<td>-</td>
</tr>
<tr>
<td>Basophils</td>
<td>-</td>
<td>0.7±0.3</td>
<td>0.1±0.6</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.2±0.6</td>
<td>5.0±0.3*</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>92.6±1.3</td>
<td>86.3±1.2*</td>
<td>90.4±1.3</td>
</tr>
</tbody>
</table>

Lysosomal cation test

| MCC, units | 1.63±0.03 | 1.87±0.09* | 1.83±0.07* |

Judging by the presence of blast forms of myeloid cells in the blood, leukopoiesis is more active in the fish of the "Surskiy low-bone" cross. Nevertheless, there are significantly fewer immature stab forms of neutrophils in the cross than in the productive Anish mirror breed, which indicates the inheritance of this trait in the immunostable Angelinskiy breed. The predominance of mature segmented neutrophils (within the reference values), which are more capable of phagocytosis than stab neutrophils, characterizes nonspecific cellular defense. A smaller percentage of stab neutrophils and monocytes is provided due...
to a larger proportion of lymphocytes in the leukogram, which are responsible for acquired immunity.

The hybrid lacks eosinophils and basophils, in contrast to the Anish Mirror breed and Angelinskiy Mirror (a small proportion of basophils).

The MCC of the cationic protein in blood neutrophils in the cross is significantly less than in the initial forms. In Angelinskiy carp, this indicator also tended to decrease in different periods. In this sense, the cross has surpassed the parent breeds. Indicator values less than 1.7 (the lower limit recommended for selection is 1.4) (Pronina & Koryagina 2015) characterizes a good level of immune protection, at which the lysosomal cationic protein is consumed for the destruction (phagocytosis) of foreign agents.

The coefficient of variability of hematological parameters is mostly quite high due to the high liability of the blood system. A low coefficient of variability, and, therefore, a greater heritability, was noted in the proportion of lymphocytes in the WBC differential and MCC.

Comparative characteristics of the cross "Surskiy malokostny" and parental forms in terms of biochemical parameters are presented in Table 7.

There were no significant differences in the activity of transaminases and glucose in the blood serum between the cross and parental forms.

### Table 7

<table>
<thead>
<tr>
<th>Biochemical parameters of two-year-old carp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index</strong></td>
</tr>
<tr>
<td>ALT, units L(^{-1})</td>
</tr>
<tr>
<td>AST, units L(^{-1})</td>
</tr>
<tr>
<td>Glucose, mmol L(^{-1})</td>
</tr>
<tr>
<td>CC, unit L(^{-1})</td>
</tr>
<tr>
<td>Creatinine, μmol L(^{-1})</td>
</tr>
<tr>
<td>Amylase, u L(^{-1})</td>
</tr>
<tr>
<td>Total protein, g L(^{-1})</td>
</tr>
<tr>
<td>Albumin, g dL(^{-1})</td>
</tr>
<tr>
<td>Urea, mg dL(^{-1})</td>
</tr>
<tr>
<td>Triglycerides, mg dL(^{-1})</td>
</tr>
<tr>
<td>Cholesterol, mg dL(^{-1})</td>
</tr>
</tbody>
</table>

The peculiarity of two-year-olds of the "Surskiy malokostny" cross is a high level of creatinine and creatine kinase (CK). It can be assumed that the effect of heterosis of crosses provides a significant rate of metabolic processes that require additional energy. It is known that CC is an enzyme that catalyzes the synthesis of a high-energy compound creatine phosphate from ATP and creatinine, which is consumed by the body under increased stress levels. Creatine phosphate is a depot of high-energy bonds and is used for rapid resynthesis of ATP during cell work.

By the content of amylase in the blood, the cross "Surskiy malokostny" is ahead of the parental forms in which the enzyme activity is below normal. This indicates an intense carbohydrate metabolism of the cross.

The total protein content of the cross did not significantly differ from the parental breeds, however, the albumin fraction of the cross, which was close to the immunostable Angelinskiy breed, significantly exceeded that of the productive Anish mirror breed, which confirms the high level of protein metabolism of the cross.

The cross "Surskiy malokostny" and the Anish mirror breed in the blood have a lower level of urea - the end product of protein metabolism, than in Angelinskiy fish. This appears to be due to intense growth, in which the body needs large amounts of protein and nitrogen, essential building blocks. Moreover, all indicators were within the physiological norm for this fish species. Differences in the content of urea can be associated with the characteristics of selection groups, as well as different degrees of tissue hydration.
Indicators of lipid metabolism: the content of triglycerides and cholesterol in the cross "Surskiy malokostny" are within the reference values for fish. The triglyceride level is intermediate between the original breeds, the cholesterol level is close to the Angelinskiy immunostable breed and is about two times lower than that of the productive Anish mirror breed.

A low coefficient of variability was noted for the following biochemical parameters: CK activity, total protein, albumin, triglyceride, cholesterol content.

**Conclusions.** Thus, we have obtained a cross "Surskiy low-bone": a hybrid of female Anish mirror and male Angelinskiy mirror rubella-resistant breeds (selection achievement 77898/8057049). Cross has a mirrored scale cover, has high fertility and good survival at different stages of ontogenesis.

Physiological and immunological studies of the original breeds showed a high differentiation of neutrophilic granulocytes of Angelinskiy rubella-resistant carps in comparison with other breeds: their leukogram contains significantly less immature forms of neutrophils. Judging by the indicators of enzyme activity: ALT (1.5 times more), CK (about 2 times lower), Angelinskiy rubella-resistant carp have a high level of protein metabolism, compared to other studied breeds.

The potential phagocytic activity of neutrophils in three-year-old rubella-resistant carps is higher than in productive breeds of the same age, since according to the cytochemical reaction, the content of lysosomal cationic protein in their blood neutrophils is 15-17% higher.

The cross, as well as the immuno-resistant parent breed, in comparison with the productive parental Anish mirror breed, has a lower proportion of immature stab forms of neutrophils, a relatively low (optimal) value of CCR of cationic lysosomal protein in blood neutrophils, as well as a high level of protein and carbohydrate metabolism: a high level QC, amylase, albumin; relatively low content of urea, triglycerides, cholesterol.

The data obtained allow us to assume that the cross "Surskiy low-bone" has inherited high immune resistance from the Angelinskiy mirror carp.

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