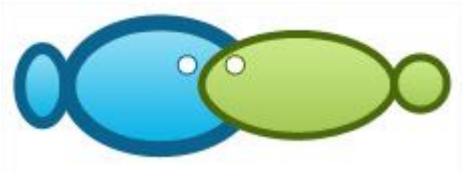

Physiological and immunological features of males and females of the immunologically resistant carp breed (*Cyprinus carpio* L.)

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Abstract. Selection on increased immune stability is one of the most radical opportunities to strengthen the resistance of animals. Especially in the actual conditions of intensive farm cultivation, with unilateral selection on efficiency when there is a decrease in resistance of the individuals against diseases. It is important in bigger measure for fishes as they are hydrobionts and water habitat leaves the mark on their activity. Besides, the poikilothermic fish increases their vulnerability to any invasion of pathogens and causative agents of diseases. From the breeds of a carp which are available now, only Angelinsky breed passed long selection on resistance to rubella (a poly etiology disease which activators can be a virus of a spring viremia carp, aero monads, pseudo-monads). Selection was carried out on a provocative background. Quantitative characteristics of the increased resistance of an Angelinsky mirror carp were shown at statement of biological tests with causative agents of diseases. The purpose of this research was the comparative analysis of hematological, biochemical and cytochemical indices of Angelinsky producers and productive carp breeds. Analysis of the results showed that the fall in adult carp, the recovery after spawning and preparing for the hibernation is expressed by the activation of hematopoiesis, the proportion of mature segmented neutrophils. The pattern for all age groups of Angelinsky carp regardless of the season of the year is the high level of metabolism, AST and albumin (protein metabolism) and triglycerides (energy metabolism).
Key Words: immune resistance, rubella of carp, hematologic parameters, lysosomal cationic protein.

Introduction. The risk of fish disease, associated with the development of secondary immunodeficiency disorders and weakening of overall physiological status under the influence of environmental factors, occurs at various stages of fish farming. A rapid growth of anthropogenic pressure and water pollution adversely affects the physiological state of aquatic organisms, the degree of their resistance and has an immunosuppressive effect, which results in the emergence of fish with deformities and immunodeficiency in the population (Reshetnikov et al 1999; Moiseenko & Lukin 1999; Kashulin et al 1999 Valedskaya 2005; Pronina & Revyakin 2015).

In addition, a one-sided selective breeding for productivity causes an imbalance between the gene complexes responsible for adaptive and productive potentials. As a consequence, highly productive fish appears to be more demanding to the environmental conditions, which results in their elimination by natural selection and hence reduction of the selection effect. The adaptation process was shown to be accompanied by a decrease in erythrocytes, leukocytes, and hemoglobin levels (Pogrebnyak 2000; Bikchentaeva et al 2012).

One way to solve the issue of a decrease in immunity in productive animals and prevent epizootic diseases is selective breeding to increase the immune resistance. This allows obtaining highly resistant breeds.

Among various breeds of carp, only Angelinskaya (mirror and scaly forms) breed was subjected to a long-term selective breeding for rubella resistance. Rubella stands for a syndrome caused by various pathogens: aeromonas, pseudomonas or spring viremia of carp virus (Golovina et al 2003).

The breed has been developed by a long-term challenge selective breeding (Ilyasov et al 1998). The selective breeding was carried out without using any medicinal products with the complete culling of diseased and recovered fish. As a result, they managed to significantly increase the resistance of this breed of carp (Kirpichnikov 1987; Ilyasov 2002). The efficiency of selective breeding was proved in the laboratory settings by infecting carps with rubella pathogens and confirmed in the field settings of the Krasnodar Territory.

The objective of this work is a comparative analysis of some biochemical and immunological parameters of broodstock of various carp breeds differing by their levels of resistance to infectious diseases.

Material and Method. The experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, ETS №123, 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 studies were carried out in the fish farm Kirya, Chuvash Republic, located in the second aquaculture zone. Currently there are Angelinskaya scaly and mirror carp breeds with resistance to rubella virus at the fish farm. These fish larvae were brought from the fish farm Angelinskaya, Krasnodar Territory.

The fish farms Angelinsky and Kirya are free from fish infectious diseases. This is confirmed by the annual reports of the animal health checks for aeromonosis and pseudomonosis. Preventive diagnostic tests for spring viraemia of carp conducted by the Federal Service for Veterinary and Phytosanitary Supervision also showed the absence of the virus.

In addition, the objects of study were males and females of the Chuvash scaly and Anishskaya mirror carp breeds developed by accelerated selective breeding for productivity (Maslova & Petrushin 2005).

Fish were divided into 8 groups with 7 animals in each. Two groups were formed from each of the four breeds (Chuvash scaly, Anishskaya mirror, Angelynskaya scaly and Angelynskaya mirror breeds): males and females.

A physiological and immunological evaluation of mature carp breeding stock included haematological, biochemical and immunological parameters was performed.

Blood was aseptically collected from the caudal vein of fish in vivo in autumn (September). Blood smears (2 pcs. for each fish: one for leukocytic formula (WBC) differential, the second one for a cytochemical test for cationic protein determination) were prepared immediately after the blood withdrawal. The WBC differential was performed by differential counting on Pappenheim stained peripheral blood smears.

Non-enzymatic lysosomal cationic protein in neutrophils from fish blood was determined by the Shubich (1974) cytochemical method with bromophenol blue, adapted by Pronina (2008) for aquatic organisms. The test cells were divided into four groups (0-3 points) according to their phagocytic activity: 0 — no cationic protein granules, 1 — individual granules, 2 — granules occupy approximately 1/3 of cytoplasm, 3 — granules occupy 1/2 of cytoplasm or more.

The mean cytochemical coefficient (MCC) was calculated using the following formula (Kaplow 1955):

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

Where N_0, N_1, N_2, N_3 are numbers of neutrophils with 0, 1, 2 and 3 point activity.

In order to obtain the blood serum, fish blood was collected into dry sterile test tubes. The test tubes with blood were left in the stand for 1 hour at room temperature. During this time, the clotting process was completed, and a clot retraction occurred. After this, the serum was withdrawn by a syringe with a thin needle, transferred to an Eppendorf tube and frozen in a freezer at -18 to -20°C. The serum was transported to the

laboratory frozen in insulated shipping containers. Immediately before the analysis, the serum was thawed for 1 hour at room temperature.

Serum chemistry was carried out on the Chem Well analyzer, Awareness Technology, using Vital reagents.

The statistical data processing was performed by variational statistics using Excel. The results were evaluated using Student test. The differences were considered significant at $P \geq 0.05$.

Results and Discussion. There were no significant inter-fish differences by size and weight criteria, such as body weight and length (Tables 1, 2).

Table 1

Size, weight, hematological and cytochemical parameters in producers of different breeds of scaly carp

Parameters	*Chuvash scaly*		*Angelinskaya scaly*	
	Males	*Females*	*Males*	*Females*
	a	*b*	*c*	*d*
Body weight (kg)	3.5±0.4	4.5±0.5	3.8±0.6	4.1±0.5
Body length (cm)	56.0±1.5	59.4±1.8	55.9±1.8	57.0±1.2
Erythropoiesis (%)				
Hemocytoblasts, erythroblasts	-	0.8±0.2	-	-
Normoblasts	4.0±0.6	2.2±0.2[a]	4.1±0.5[b]	4.3±0.4[b]
Basophilic erythrocytes	9.2±1.4	9.0±1.1	11.5±0.9	9.3±2.5
Total mature and polychromatic erythrocytes	86.8±1.9	88.0±1.4	84.4±1.7	86.4±2.6
WBC differential (%)				
Myeloblasts	0.2±0.2	-	-	-
Promyelocytes	-	-	0.9±0.3	-
Myelocytes	0.3±0.4	-	2.1±0.6[a]	-
Metamyelocytes	1.5±0.6	1.0±0.1	2.0±0.8	2.3±0.7
Band neutrophils	0.8±0.5	2.2±0.2	1.1±0.3	2.3±0.5
Segmented neutrophils	2.7±0.6	2.4±0.3	4.9±0.7[a]	4.1±0.5[b]
Total neutrophils	3.5±0.6	4.6±0.4	6.0±0.5[a]	6.4±0.6[ab]
Eosinophils	-	0.4±0.3	-	-
Basophils	-	0.4±0.3	0.5±0.3	1.2±0.5
Monocytes	2.5±0.7	3.2±0.4	4.2±0.5	4.4±0.9
Lymphocytes	92.0±0.7	90.4±0.6	84.3±1.2[ab]	85.7±0.9[ab]
Phagocytic activity				
MCC (units)	1.74±0.06	1.77±0.09	1.85±0.03	2.01±0.04[abc]

Values in the same row with different superscripts (a, b, c) indicate significant differences.

Females of the Chuvash scaly breed had some specific characteristics in relation to erythropoiesis. Namely, there was a small fraction of blast forms of red blood cells with a lower (2-fold) normoblast percentage as compared to the other fish groups studied. Furthermore, their WBC differential contained eosinophils and basophils.

The proportion of neutrophils in the WBC differential of Angelinskaya carp was significantly higher than that of other breeds studied due to mature segmented forms. The content of lysosomal cationic protein in cells of Angelinskaya carp is somewhat larger, as assessed by the MCC. The accumulation of this cytotoxic product in macrophage lysosomes occurred probably due to intensive protein metabolism during the growing season. Thus, there is an increasing potential for phagocytosis in rubella resistant fish before hibernation.

Our previous studies have also shown that lysosomal cationic protein MCC in Angelinskaya carp decreases in spring and increases in autumn, as opposed to this

parameter in carp of native Chuvash breeds, whose parameter varies insignificantly in different seasons (Pronina et al 2013; Pronina & Petrushin 2013; Pronina 2014).

Alanine aminotransferase (AST) levels in Angelinskaya carp were significantly higher than those in other species (both males and females). We also noticed this pattern in other age groups of fish (Pronina 2012; Pronina et al 2013).

The activity of creatine kinase (CK) in Angelinskaya carp was also higher than in the Chuvash carp breeds.

Table 2

Size, weight, hematological and cytochemical parameters in producers of different breeds of mirror carp

Parameters	*Anishskaya mirror*		*Angelinskaya mirror*	
	males	*females*	*males*	*females*
	a	*b*	*c*	*d*
Body weight (kg)	4.9±0.4	5.1±0.4	3.8±0.8	6.2±0.4^{ac}
Body length (cm)	59.0±1.2	60.8±1.1	57.4±2.1	67.1±1.4^{abc}
Erythropoiesis (%)				
Hemocytoblasts, erythroblasts	0.4±0.2	0.2±0.2	1.0±0.1^{ab}	1.0±0.1^{ab}
Normoblasts	4.6±0.3	4.2±0.3	5.5±0.3	4.2±0.6
Basophilic erythrocytes	12.4±2.1	11.0±1.8	9.6±1.4	15.7±1.8^{b}
Total mature and polychromatic erythrocytes	82.6±1.8	84.6±1.8	83.9±1.8	79.1±2.1
WBC differential (%)				
Myeloblasts	-	-	-	-
Promyelocytes	-	-	1.5±0.5	-
Myelocytes	0.6±0.3	0.2±0.2	2.1±0.4^{ab}	2.2±0.6^{ab}
Metamyelocytes	1.4±0.8	1.0±0.5	2.2±0.9	2.3±0.5
Band neutrophils	1.4±0.6	0.5±0.4	0.5±0.3	1.2±0.4
Segmented neutrophils	2.2±0.4	2.3±0.7	6.5±0.5^{ab}	6.3±0.8^{ab}
Total neutrophils	3.6±0.8	2.8±0.8	7.0±0.8^{ab}	7.5±1.2^{ab}
Eosinophils	-	0.2±0.2	0.5±0.3	-
Basophils	-	-	-	-
Monocytes	3.8±0.4	2.8±0.5	3.5±1.1	4.5±0.9
Lymphocytes	90.6±1.6	93.0±0.6	83.2±0.8^{ab}	83.5±1.3^{ab}
Phagocytic activity				
MCC (units)	1.88±0.04	2.01±0.08	1.79±0.02^{b}	1.92±0.03

Values in the same row with different superscripts (a, b, c) indicate significant differences.

Albumin and urea levels (final product) in Angelinskaya carp were higher than in native species of fish (Tables 3, 4).

However, no significant differences were observed in the total protein content between the groups. The lactate content in rubella resistant fish is almost two-fold higher than in the other groups of fish studied. On the other hand, uric acid levels are relatively lower in Angelinskaya carp. The cholesterol levels in Angelinskaya carp were significantly lower, while triglyceride levels were higher than those in other fish.

The group of Angelinskaya mirror carp had higher levels of serum glucose than those in native species, with levels in males being higher than in females.

High levels of AST, albumin, lactate and urea in Angelinskaya carp are indicative of more extensive protein metabolism. More active CK and higher cholesterol levels in blood serum of Angelinskaya carp are probably associated with increased energy metabolism during the body recovery after the spawning season and preparation for winter.

Thus, rubella resistant carp, unlike rubella-susceptible fish, have higher levels of protein and carbohydrate metabolism, as assessed by the enzyme activity (aspartate aminotransferase, creatine kinase), albumin, lactate and urea levels. Angelinskaya carp

has more intensive hematopoiesis. WBC differential counts of these fish contain a larger proportion of neutrophils, particularly mature segmented forms. These features of Angelinskaya carp should be considered while growing and breeding this breed.

Table 3

Biochemical parameters in producers of different breeds of scaly carp

Parameters	*Chuvash scaly*		*Angelinskaya scaly*	
	males	*females*	*males*	*females*
	a	*b*	*c*	*d*
ALT (U/L)	30.8±0.5	36.4±2.1[a]	31.4±0.4	36.5±0.8[ac]
AST (U/L)	132.2±14.4	141.1±7.9	200.9±12.1[ab]	173.9±9.4[ab]
Glucose (mmoL/L)	5.9±0.5	5.4±0.2	5.9±0.3	6.9±0.4
CK (U/L)	2494±3	2962±172[a]	3499±38[ab]	2955±56[ac]
LDH (U/L)	656±149	844±147	777±112	896±98
Lactate (mg/dL)	43±7	31±8	95±6[ab]	82±5[abc]
Uric acid (mmoL/L)	429±4	350±56	228±59[a]	306±44[a]
ALP (U/L)	55±18	75±21	167±12[ab]	23±19[c]
Albumin (g/dL)	7.3±0.9	6.2±0.4	13.1±0.6[ab]	13.2±0.3[ab]
Urea (mg/dL)	4.7±0.9	7.4±1.3	12.9±1.7[ab]	11.2±0.7[ab]
Total protein (g/L)	17.6±0.6	19.5±2.8	17.9±0.7	17.4±1.3
Triglycerides (mg/dL)	114±19	82±9	124±12[b]	145±8[b]
Cholesterol (mg/dL)	106.0±4.4	84.6±7.1[a]	70.3±5.2[ab]	64.4±6.8[ab]

Table 4

Biochemical parameters in producers of different breeds of mirror carp

Parameters	*Mirror carp*		*Angelinskaya mirror*	
	males	*females*	*males*	*females*
	a	*b*	*c*	*d*
ALT (U/L)	42.8±7.6	28.5±3.1	46.0±0.9[b]	31.4±1.2
AST (U/L)	148.2±9.4	133.9±10.8	211.9±8.3[a]	163.5±5.1[b]
Glucose (mmoL/L)	4.1±0.6	4.5±0.5	9.1±0.4[ab]	7.6±0.4[abc]
CK (U/L)	3079±180	2735±231	1713±156[ab]	2252±98[ac]
LDH (U/L)	638±142	548±123	1159±96[ab]	872±88
Lactate (mg/dL)	46.0±8.4	46.2±6.6	86.6±5.3[ab]	73.8±4.5[ab]
Uric acid (mmoL/L)	416±54	267±31a	294±45	220±25[a]
ALP (U/L)	124±22	40±16[a]	82±15	16±2[ac]
Albumin (g/dL)	8.1±0.8	6.4±0.5	13.2±0.7[ab]	12.0±0.6[ab]
Urea (mg/dL)	6.5±1.4	6.4±1.3	12.9±0.9[ab]	11.2±1.2[ab]
Total protein (g/L)	24.2±2.1	16.3±0.6[a]	20.3±2.2	14.2±1.2[ac]
Triglycerides (mg/dL)	103±5	92±7	129±6[ab]	115±4[b]
Cholesterol (mg/dL)	125.0±6.9	86.9±7.4[a]	80.9±7.8[a]	84.7±5.5[a]

Conclusions. According to the activity of enzymes (aspartate aminotransferase, creatine phosphokinase) and level of albumin, lactate and urea, carps resistant to rubella (Angelinskiy carp - AC), in contrast to susceptible to rubella, having higher levels of protein and carbohydrate metabolism. AC has more intensive hematopoiesis and leukocyte formula, also has higher number of neutrophils, especially segmented mature forms compared with carps susceptible to rubella. This specificity of AC should be considered in breeding of this breed and especially in the selection work with such fish and its hybrids.

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