

## **CELLULAR AND HUMORAL (NON-SPECIFIC) IMMUNITY OF CARP STOCKING MATERIAL (*CYPRINUS CARPIO* L.) BEFORE AND AFTER WINTERING**

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### **Abstract**

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A biochemical characteristic of carp fish stocking material (*Cyprinus carpio* L.) has been done according to the indices bactericide activity of blood serum (BA); lysozyme activity of blood serum (LA), phagocytic activity (PhA) and phagocytic index (PhI); total proteins (TP) and  $\gamma$ -globulins of blood serum before and after wintering. The studies have been carried out by using two-summer-old (autumn) and two-years-old (spring) clinically healthy carp having a weight of 40 g to 50 g. The relative stability of bactericide activity of blood serum (BA) as an index characterizing carp resistibility during the period of hibernation has been proved. Authentically higher values of lysozyme activity of the one-summer-old carp ( $K_0^+$ ) and of phagocytic activity of the one-year-old carp ( $K_1$ ) have been established. Authentic differences concerning the phagocytic index have not been proved ( $P < 0.05$ ). Total protein level seasonal dynamics expressed in higher autumn and lower spring values has been established. The differences between  $\gamma$ -globulin values of the one-summer and one-year-old carp have been unauthentic ( $P < 0.05$ ).

*Key word:* carp, blood serum, bactericide activity, lysozyme activity, phagocytic activity, phagocytic index, total proteins,  $\gamma$ -globulins

### **Introduction**

The bactericide, lysozyme and phagocytic activity are component parts of indices, determining the natural immunity of the organism, with an outstanding bacteristatic and bactericide activity, because of which they can be used as markers for characterizing the immune status of the animals, fish and their adaptive possibilities, included (Parchomenko and Timochina, 1987; Chemshirova et al., 1999).

The serum proteins of blood play not an small role for producing the protective reactions of fish organism. The fish protein spectrum possesses a high vari-

ability and reflects the functional status of their organism (Afanasjev et al., 2001). According to Benediktov and Oljhovikova (1986) the determination of total protein level and of its fractions in blood serum has a great diagnostic and prognostic significance.

The fish humoral immune system in contrast to that of the other animals is rather variable and depends on the external conditions and fish species (Lukjanenko, 1971). The humoral factors of natural resistibility ensure the bactericide function of blood serum and tissue fluids of fish. They do not function in isolation, but are in interconnection with one another (Vikhman, 1996).

The function of protective properties of fish blood serum depends on the individual characteristics, physiological status of the organism and the season of the year (Lukjanenko, 1971; Mikrjakov et al., 1978; Silkin, 1989; Van Muiswinkel, 1996; Atanasova, 2003). According to Mikrjakov and Silkin (1978); Amineva and Jarzhombek (1984) the non-specific (humoral) protective factors reach the highest values at the end of the vegetation period.

The hibernation is a risky stage of stocking material growing. Concerning this, the purpose of this study is to investigate some indices of the cellular and humoral non-specific immunity of carp stocking material (*Cyprinus carpio* L.) before and after hibernation by means of the following indices of the blood serum: bactericide activity; lysozyme activity, phagocytic activity, phagocytic index, total proteins and  $\gamma$ -globulins.

## Materials and Methods

The studies have been carried out by using one-summer-old (October 1997) and one-year-old (April 1998) clinically healthy carps with weight of 40 g to 50 g. The fish have been grown in polyculture with tench (*Tinca tinca*), grass carp and bighead carp in one and the same pond (earthy type), having an area of 0.70 ha in the experimental basis of the Institute of Fisheries and Aquaculture - Plovdiv. For their feeding during the vegetation period mainly sunflower groats, barley and wheat have been used. During fish growing, in the period May-September, the physical and chemical mode of water has been reported, the average seasonal values of the indices traced being within the technological standards of the fish species grown: water temperature 21.6–23°C; pH 7.61–8.00; oxygen dissolved in water 4.0–6.49 mg.l<sup>-1</sup>; chemical oxygen demands (COD) 10.4–10.73 mg.l<sup>-1</sup>.

The blood for analysis has been taken from 35 carps during every season investigated. In order to eliminate the stress from this manipulation, the fish have been subjected to anaesthesia. In order to separate the serum, the blood has been centrifugated at 3 000 revolutions per min for 10 min.

The natural resistibility of carp has been determined by means of the index bactericide activity of blood serum (BA, %), the bacterium *Aeromonas hydrophila* being used in its quality of a test microbe. The investigations have been done by using Markov's adaptive method (Atanasova et al., 1995).

The lysozyme activity of blood serum (LA, %) has been determined colorimetrically by using Markov's method (Pljashtenko and Sidorov, 1979). *Micrococcus lysodeikticus* has been used as the test microbe.

The phagocytic activity (PhA, %) and the phagocytic index (PhI), have been determined according to Gostev's method by using the bacterium *Aeromonas hydrophila* as the test microbe (Pljashtenko and Sidorov, 1979).

The biochemical characteristics of blood has been done by determining the indices: total proteins (TP) – in g.l<sup>-1</sup> colorimetrically (410 nm), by using bioretic reagent according to Gornal's method (Ibrishimov and Lalov, 1974), and their separation – by means of horizontal micro-electrophoresis upon cellogel strips (Chemetron, Milano, Italy), at 190 V power for 20 min (Pavlov et al., 1984).

Data have been statistically processed by applying MS Office 2003 Statistical Program. The authenticity of the difference between the values of two excerpts has been determined by applying the t-test at probability degree of (P<0.05).

## Results and Discussion

The data obtained for bactericide activity of blood serum (BA) of the fish investigated, according to years and seasons have been given in Table 1. It has been proved that the differences between the values for the one-summer-old carp (K<sub>0</sub><sup>+</sup>) (before wintering) and the one-year-old carp (K<sub>1</sub>) (after wintering) have been authentic.

The lysozyme level of the one-summer-old carp (K<sub>0</sub><sup>+</sup>) (before wintering) has been authentically higher in comparison with that of the one-year-old carp (K<sub>1</sub>) (after wintering) (Table 1). After carp wintering lower absolute values of this humoral index have been established in comparison with the values reported be-

**Table 1**  
**Immunological and biochemical indices of blood serum of carp stocking material**  
**(*Cyprinus carpio* L.) before and after wintering**

Indices	Age					
	K <sub>0</sub> <sup>+</sup>			K <sub>1</sub>		
	Average weight					
	30 - 40			30 - 40		
n=10; n=5*	x	Sx	Cv	x	Sx	Cv
BA, %	72.61	2.72	11.25	62.76	0.94	4.49
LA, %	9.12	0.40	39.69	7.21	0.13	16.50
PhA, %	35.05	0.95	8.12	42.3	2.78	19.75
PhI	1.62	0.38	33.15	1.41	0.09	18.51
TP, g.l <sup>-1</sup>	36.41	0.90	7.43	27.46	0.85	9.26
γ-globuline, g.l <sup>-1</sup>	6.59*	0.35	10.75	5.39*	0.15	5.67

K<sub>0</sub><sup>+</sup> - one-summer old carp; K<sub>1</sub> -one-year old carp; BA- bactericide activity (%);

LA -lysozyme activity (%); PhA - phagocytic activity (%); PhI -phagocytic index;

TP- total proteins (g.l<sup>-1</sup>)

fore hibernation. The differences have been with high degree of authenticity (P<0.01).

In the autumn (October), we have established authentically lower phagocytic activity (PhA) of the one-summer-old carp (K<sub>0</sub><sup>+</sup>) in comparison with that of the one-year-old carp (K<sub>1</sub>) (after wintering) (P<0.001). As far as the phagocytic index (PhI) is concerned, authentic differences have not been reported (P<0.05).

The changes in total protein levels of the blood serum of the one-summer-old carp (K<sub>0</sub><sup>+</sup>) and the two-summer-old carp (K<sub>1</sub>) have been indicated in Table 1. Total proteins quantity in the serum of the one-summer-old carp (before wintering) has been authentically higher than those of the one-year-old carp (after wintering) (P<0.001). As far as γ-globulins are concerned, authentic differences have not been reported (P<0.05) between the one-summer-old carp (K<sub>0</sub><sup>+</sup>) and one-year-old carp (K<sub>1</sub>).

The data of the blood serum bactericide activity (BA) indicate that before and after wintering, in both seasons investigated, the absolute values in 100% of the samples of carp from the ages studied are above 62%. Because of that it can be counted among the fish category having a very good resistibility towards diseases, in accordance with the standards accepted

by some authors (Goncharov et al., 1974; Mikriakov et al., 1978; Atanasova, 2003).

An authentically higher level of lysozyme activity has been established in the one-summer-old carp (K<sub>0</sub><sup>+</sup>) (before wintering), in comparison with that of the one-year-old carp (K<sub>1</sub>) (after wintering). Our investigations have confirmed the concept of other authors, as well, that in the autumn, the level of lysozyme in the blood serum is the highest one (Amineva and Jarzhombek, 1984). Having in mind that lysozyme plays an important role in ensuring organism's resistibility and that it increases its stability towards stress factors effect, the values obtained have characterized it as healthy and well prepared for wintering.

Having in mind that the phagocytic reaction has a protective function for the organism, the levels established have characterized the carp stocking material as healthy, and the higher spring level has been due most probably to the age variations.

The level of total proteins of carp blood serum in the autumn samples has been authentically higher as compared to the spring one. The lower spring values of total proteins after wintering have been an index of their seasonal variability, and also that a part of the protein has been used to cover its energy needs dur-

ing wintering. The high level of authenticity between the differences of the autumn and spring values have characterized the carp as species, which during the period of wintering utilizes more intensively proteins for its energy needs. This ranks it among the fish group with labile physiological condition, depending to a high degree on environmental conditions.

Total proteins absolute values obtained for the one-summer-old carp ( $36.41 \text{ g.l}^{-1}$ ) have been close to the standards, indicated by many authors (Kovatcheva, 1989; Georgiev, 1995) and have been typical for fish grown by intensive nutrition.

$\gamma$ -globulins are among the most interesting fractions in plasma composition, because they are connected with the protective processes of the organism and especially with the humoral immunity. The percent ratio of  $\gamma$ -globulins in the carp blood serum before and after wintering have been close to those indicated as standards of good physiological status ( $\gamma$ -globulins from  $4$  to  $14 \text{ g.l}^{-1}$ ) (Georgiev, 1995).

The higher values of carp total proteins reported in the autumn have been accepted as a result of the intensive summer nutrition of this species and of the accumulation of plastic and energetic substances in its organism. From the investigations done it can be summarized that the  $\gamma$ -globulin fraction of blood serum has given the idea either for the seasonal age peculiarity, or for the physiological status of carp, as well.

The analysis has pointed out that the bactericide, lysozyme and phagocytic activities, the phagocytic index, the total proteins and the  $\gamma$ -globulin fraction of blood serum indices used, have been carriers of information for the carp status, as well as for its seasonal and age variations as regards the plastic and energetic reserves in its organism. The higher values of the biochemical parameters traced in the autumn have been an index for the greater depot of these substances in the carp organism and have been a prerequisite for the good fish wintering.

Concerning this, the practical significance of climatic conditions in Bulgaria has been connected with the pond fishery where the problem of wintering and protection of the stocking material has been of a primary importance.

## Conclusion

A relative stability of the blood serum bactericide activity in the period of hibernation has been proved.

The values of lysozyme activity of the one-summer-old carp ( $K_0^+$ ) and those of the phagocytic activity of the one-year-old carp ( $K_1$ ) have been authentically higher. Authentic differences regarding the phagocytic index have not been proved ( $P < 0.05$ ).

Total protein level seasonal dynamics expressed in higher autumn and lower spring values has been established. The differences between the  $\gamma$ -globulins values of the one-summer-old and one-year-old carp have been unauthentic ( $P < 0.05$ ).

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