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## **INVESTIGATIONS ON THE BIOCHEMICAL COMPOSITION OF CARP FISH (*CYPRINIDAE*) BLOOD SERUM AT CONDITIONS OF ORGANIC AQUACULTURE**

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### **Abstract**

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Having in mind that together with the productive parameters of fish production, its healthy status is of great significance, as well, then the purpose of this study is to investigate the biochemical composition of the blood serum of carp (*Cyprinus carpio* L.), bighead carp (*Aristichtuys nobilis* Rich.) and grass carp (*Ctenopharyngodon iddela* Val.), grown by applying organic production technology. At the end of the vegetation period, the blood for biochemical analysis has been taken from 15 carps and bighead carps each and from 5 grass carps during each season investigated. At conditions of organic production technology it is the bighead carp and the grass carp, which have stood out as having higher values of the parameters Bactericidal Activity (BA), Total Proteins (TP) and Blood Glucose (BG), while for the carp they have been lower concerning the two remaining species, as well as concerning the physiological standards for this species. The species differentiation between the values of the indices traced at both variants of the experiment (with and without organic fertilization of the ponds) has been more clearly expressed. The effect of this technology has been reported in the grass carp, which has been expressed in higher absolute values of the parameters BA, TP and BG in organic fertilization ponds and authenticity of the differences ( $p < 0.01$ ).

*Key words:* carp, bighead carp, grass carp, blood serum, bactericide activity, total proteins, blood glucose

### **Introduction**

The organic agriculture has been rapidly developing and nowadays it has been practiced in more than 120 countries worldwide. About 4% of the cultivated land in the EU has been organically managed (Willer and Yussefi, 2007). The development of organic production in aquacultures has amounted approximately to 9% annually, since 1970 (El-Hage Scialabba and Hattam, 2002).

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As far as organic production is concerned, one of its leading principles is that of healthfulness (of soils, of plants, of animals and of man) (El-Hage Scialabba and Hattam, 2002). The good healthy status of fish is the main element for their welfare, and in order to reach high levels, the accent should be put mostly on the application of suitable methods of fish-breeding (Boehmer et al., 2005).

Warm pond fishery is suitable for the purposes of organic production and a number of requirements

concerning the living medium – nutrition, hydrobionts welfare, interrelations with the environment, etc., which can be hardly fulfilled in some conventional aquaculture technologies, have been applicable in the traditional pond fishery of carp (Nikolova, 2006).

Having in mind that together with the productive parameters of the fish production obtained, its healthy status is of great significance, as well, then it follows that the purpose of this study is to investigate the biochemical composition of the blood serum of carp (*Cyprinus carpio* L.), bighead carp (*Aristichthys nobilis* Rich.) and grass carp (*Ctenopharyngodon iddela* Val.), grown by applying organic production technology.

## Material and Methods

The investigation has been carried out within the framework of organic production technology, developed in the Institute of Fisheries and Aquaculture – Plovdiv. For the purpose of the experiments, 8 ponds have been used, with a single area of 0.17 – 0.38 ha. The fish has been grown in polyculture, at density of stocking 500 p-es.ha<sup>-1</sup> of carp (K<sub>1</sub>) (*Cyprinus carpio* L.), (scaly and mirror form 1:1); 300 pes.ha<sup>-1</sup> of bighead carp (*Aristichthys nobilis* Rich), (T<sub>1</sub>) and 100 p-es.ha<sup>-1</sup> of grass carp (*Ctenopharyngodon iddela* Val.), (A<sub>1</sub>). Organic fertilization has been applied in the experimental ponds No. 12, 17, 19, 20, at a rate of 3000 kg.ha<sup>-1</sup>, while in the control ponds No. 8, 15, 16, 18 – fertilization has not been applied.

During the vegetation period (May-September) the water physical and chemical parameters have been reported, whose average-seasonal values have been within the technological standards for the fish species grown: temperature 21.8–23.6°C, pH 7.55–8.3; oxygen dissolved in water 4.4–12.77 mg.l<sup>-1</sup> and chemical oxygen demands (COD) 8.9–16.2 mg.l<sup>-1</sup>.

At the end of the vegetation period, blood for biochemical analysis has been taken from 15 carps and bighead carps each and from 5 grass carps during each season investigated. In order to eliminate the manipulation stress, the fish have been subjected to anaesthesia, and to separate the serum the blood has

been centrifugated at 3000 revolutions per min<sup>-1</sup> for 10 min.

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).

Biochemical characteristics of blood has been done by determining the indices: total proteins (TP) – in g.l<sup>-1</sup> colorimetrically ( $\lambda=410$  nm) by using bioretic reagent according to Gornal's method (Ibrishimov and Lalov, 1974), and blood glucose (BG) – in mg.100 ml<sup>-1</sup> by the colorimetric method ( $\lambda=366$  nm), by using anilin reagent and trichloroacetic acid as a standard (Karakashev and Vichev, 1966).

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

## Results and Discussion

Data obtained for bactericidal activity (BA), total proteins (TP) and blood glucose (BG) of fish species investigated (carp, bighead carp and grass carp) from the I<sup>st</sup> variant (without organic fertilization) and from the II<sup>nd</sup> variant (with organic fertilization) have been given in Table 1.

In the ponds of the I<sup>st</sup> variant (without organic fertilization), the values of the indices traced BA, TP and BG concerning bighead carp and grass carp have been authentically higher than those concerning carp (P<0.001), while the difference between bighead carp and grass carp as regards BA has been minimal and unauthentic (P<0.05). As far as TP contents in the blood serum are concerned, the differences between the bighead carp and the grass carp have had a lower degree of authenticity (P<0.05). The BG level of bighead carp has been authentically higher as compared to that of the grass carp (P<0.01).

Analogical tendency can be observed concerning the indices traced (BA, TP and BG) in the ponds of

**Table 1**

**Average values of bactericidic activity (BA, %), total proteins (TP, g.l<sup>-1</sup>) and blood glucose (BG, mg.100 ml<sup>-1</sup>) of the blood serum of carp (*C. carpio* L.), bighead carp (*A. nobilis* Rich.) and grass carp (*C. iddela* Val.) at different variants of growing**

Fish species	Month	BA, %			TP, g.l <sup>-1</sup>			BG, mg.100 ml <sup>-1</sup>		
		x	Sx	Cv	x	Sx		x	Sx	Cv
Carp (n=12)										
1	October	38.26	1.83	15.86	21.67	0.33	5.08	59.3	2.89	16.20
2	October	39.6	2.45	20.55	22.56	0.42	6.11	60.72	3.76	20.11
Bighead carp (n=12)										
1	October	48.32	0.68	4.67	25.04	0.47	6.21	75.47	0.96	4.21
2	October	48.13	0.79	5.50	25.20	0.62	8.11	74.56	0.77	3.42
Grass carp (n=12)										
1	October	48.44	1.20	8.22	27.15	0.64	7.42	65.06	1.81	9.21
2	October	50.11	1.87	12.39	29.58	0.54	6.08	70.13	0.44	2.06

1 – without organic fertilization

2 – with organic fertilization

the II<sup>nd</sup> variant (with organic fertilization). The established differences in the values of bactericidic activity, total proteins and blood sugar between the carp and the bighead carp and between the carp and the grass carp in the ponds of the II<sup>nd</sup> variant (with organic fertilization) have been statistically proved ( $P < 0.01$ ) and ( $P < 0.001$ ). The results obtained concerning bactericidic activity of bighead carp and grass carp have shown that the differences have a lower degree of authenticity ( $P < 0.05$ ). As regards TP contents in the blood serum, a higher level of this metabolite has been reported for the grass carp ( $P < 0.001$ ). The values of BG obtained have been authentically higher for the bighead carp as compared to those of the grass carp ( $P < 0.001$ ).

By comparing the values obtained for BA, TP and BG between carp of the I<sup>st</sup> variant (without organic fertilization) and of the II<sup>nd</sup> variant (with organic fertilization), between bighead carp of the I<sup>st</sup> variant (without organic fertilization) and of the II<sup>nd</sup> variant (with organic fertilization) and between grass carp of the I<sup>st</sup> variant (without organic fertilization) and of the II<sup>nd</sup> variant (with organic fertilization), the differences have been unauthentic ( $P < 0.05$ ).

Significant differences between the indices traced

(BA, TP and BG) have been established between the separate species in the ponds with fertilization and without fertilization. At conditions of fish organic production technology, it is the bighead carp (BA- 48.13 to 48.32%; TP- 25.04 to 25.20 g.l<sup>-1</sup> and BG -74.56 to 75 mg. 100 ml<sup>-1</sup>) and the grass carp (BA- 48.44 to 50.11%; TP- 27.15 to 29.58 g.l<sup>-1</sup> and BG- 65.06 to 70.13 mg. 100 ml<sup>-1</sup>), which have stood out as having better indices of the parameters traced (BA, TP, BG), while TP and BG values of the carp have been authentically lower than those reported for the bighead carp and the grass carp ( $P < 0.01$ ) and ( $P < 0.001$ ).

The effect of this technology has been reported concerning the grass carp, with an authentic difference between the values of the variants with fertilization and without fertilization ( $P < 0.05$ ).

The absolute values of total proteins obtained for the two-years old carp (27.67-22.56 g.l<sup>-1</sup>) have been below the values indicated by many authors as standards (Georgiev, 1995), as well as those for the blood sugar (Amineva and Jarzhombek, 1984) as a standard above 75 mg. 100 ml<sup>-1</sup>. These values have been lower than those obtained by us in some of our previous studies (Atanasova et al., 2001) at normal feeding rates in the ponds.

The lower total proteins and blood sugar values of the two-years old carp of the I<sup>st</sup> and the II<sup>nd</sup> variant can be accepted as a result of not feeding food in these ponds during the total experimental period.

By reporting that by means of bactericidic activity a complex evaluation of the non-specific humoral immunity of fish and of their resistibility towards diseases has been given, then in accordance with the accepted by some authors standards (Goncharov et al., 1972; Mikryakov et al., 1978), as well as with the developed ones by us (Atanasova, 2003), the values above 50 % have been an index for a very good resistibility of fish organism, and the values between 40 % and 50 % - for a good resistibility towards diseases.

The complex juxtaposition of the results from the blood components investigated has shown that big-head carp and grass carp have been more suitable for growing by using the organic aquaculture technology.

## Conclusion

At conditions of organic production technology it has been the bighead carp and the grass carp, which have stood out as having higher values of the parameters Bactericidic Activity, Total Proteins and Blood Glucose, while for the carp they have been lower concerning the two remaining species, as well as concerning the physiological standards for this species.

The species differentiation between the values of the indices traced in both variants of the experiment (with and without fertilization of the ponds) has been more clearly expressed.

The effect of the above technology has been reported for the grass carp, which has been expressed in higher absolute values of the parameters BA, TP and BG in organic fertilization ponds and authenticity of the differences ( $P < 0.01$ ).

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