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Short communcatins

SOME BIOCHEMICAL PARAMETERS OF TENCH (*TINCA TINCA* (L.)) REARED IN EARTHEN PONDS PRIOR TO AND AFTER WINTERING

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ABSTRACT. The biochemical characteristics of tench, *Tinca tinca* (L.), stocking material were examined with regard to the following parameters prior to and after wintering: bactericidal activity of the blood serum; blood glucose level (BG); total proteins (TP); blood serum protein fractions (albumins, and α-, β-, and γ-globulins). The study was conducted over the course of three years (1996-1998) on clinically healthy two-summer-old (fall) and two-year-old (spring) tench that ranged in body weight from 30 to 80 g. The study indicated that one of the parameters that characterized the resistance of tench in the wintering period was the relative stability of the bactericidal activity of the blood serum. Seasonal dynamics in the levels of blood glucose and total serum proteins were determined, and the levels of the former were higher in fall and lower in spring. The specific metabolic nature of different protein fractions in the blood serum of tench was characterized by percentage decreases of albumins and α- and γ-globulins and by increases of β-globulins.

Key words: TENCH (*TINCA TINCA*), BACTERICIDAL ACTIVITY, TOTAL PROTEINS, BLOOD GLUCOSE, ALBUMINS, GLOBULINS

In addition to reports of the economic results of rearing freshwater fish in recent years, aquaculture research has also identified some immune and biochemical parameters in the blood of freshwater fish. By determining the bactericidal activity of blood serum, blood glucose, total serum proteins, and protein fractions, it is possible to collect fast, precise information regarding the status of the examined fish thus facilitating up-to-date investigations. While the data in the literature regarding the common carp, *Cyprinus carpio* L., is more complete, that regarding tench, *Tinca tinca* (L.), is more incomplete (Amineva and Jarzombek 1984, Georgiev 1995, Silkin 1989, Fasaic et al. 1995, Erdem 1997, Ottolenghi et al. 1995, Van Muiswinkel 1996). In some of the current authors' previous investigations (Hadjinikolova et al. 2000, Atanasova 2003),

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modifications were identified in some of the immune-biochemical parameters referred to above in relation to season and fish age and type.

The wintering period is a particularly risky phase of tench rearing. Conducting more extensive experiments during this phase will result in clarifying some of the dependencies in the metabolism of the conditions of rearing and wintering in carp ponds. Thus, the purpose of the present research is to identify the biochemical characteristics of tench stocking material with regard to parameters such as the bactericidal activity of blood serum and the levels of blood glucose (BG), total proteins (TP) and blood serum protein fractions (albumins and α-, β-, and γ-globulins) prior to and after wintering.

The investigations were carried out with clinically healthy two-summer-old (November 1996, 1997) and two-year-old (April 1997, 1998) tench with body weights ranging from 30 to 80 g. The fish were reared in polyculture with carp, grass carp, *Ctenopharyngodon idella* (Val.), and bighead carp, *Aristichthys nobilis* (Rich.) in one earthen pond with an area of 0.70 ha at the experimental facility of the Institute of Fisheries and Aquaculture, Plovdiv Branch, Bulgaria. Mainly sunflower seeds, barley, and wheat were fed to the fish during the vegetation period. During the May-September rearing period, the physical and chemical water parameters were monitored, and the average seasonal values of them were within the technological limits for the types of fish reared: water temperature – 21.6-23°C; pH – 7.61-8.00; dissolved oxygen concentration – 4.0-6.49 mg O_2 l^{-1}; chemical oxygen demand (COD) – 10.4-10. 73 mg O_2 l^{-1}.

Blood for analysis was drawn from 20 tench during each season studied. To eliminate the effects of stress during manipulation, the fish were anaesthetized with hinolin (Karanikolov 1995). The serum was separated by centrifuging the blood for 10 min at 3000 G.

The natural resistance of tench was determined by the parameter of the bactericidal activity of the blood serum, with the *Aeromonas hydrophila* bacteria used as a test microbe. The investigations were conducted according to the method modified by Markov (Atanasova et al. 1995). The method was adapted by replacing the test microbe *Escherichia coli* with *Aeromonas hydrophila*, which is pathogenic for carp. The number of microbes per ml was decreased. The biochemical characteristics of the blood were identified by determining a number of parameters. Blood glucose (BG; mg 100 ml^{-1}) was determined with the spectrophotometric method (λ 366 nm) using aniline reactive and 3-chloroacetic acid

as the standard (Karakaschev and Vichev 1966). Total proteins (TP; g l^{-1}) were determined with the spectrophotometric method (λ 410 nm) and a bioretic reactive ($CuSO_4 \times 7H_2O$ + $KNaC_4H_4O_6 \times 4H_2O$ + NaOH) according to the Gornal method (Ibrischimov and Lalov 1974); they were separated with horizontal micro-electrophoresis on Cellogel strips (Chemetron, Milano, Italy) at 190 V over the course of 20 min (Pavlov et al. 1984).

The data obtained was processed statistically with software for Windows 98. The significance of the differences between values was determined with the t-test at a level of probability of $P \leq 0.05$.

The data obtained regarding the bactericidal activity of the blood serum of the studied fish is presented according to year and season in Table 1. This indicates that the differences between the values for two-summer-old tench (T_1^+) (prior to wintering) in the various years had a low degree of significance ($P < 0.05$), while they were statistically significant ($P < 0.001$) with regard to the two-year-old tench (T_2) (after wintering). The data also revealed that the differences registered between the two-summer-old tench (T_1^+) (prior to wintering) and the two-year-old tench (T_2) (after wintering) were highly statistically significant ($P < 0.001$).

TABLE 1

Dynamics of bactericidal activity (BA; n=5) and blood glucose (BG; n = 5) of tench prior to and after wintering

	BA (%)			BG (mg 100 ml^{-1})		
Age of fish	mean	SD	CV (%)	mean	SD	CV (%)
Two-summer-old tench (T_1+)	51.25	0.71	2.77	72.68	1.09	3.0
	55.3	0.83	3.0	72.55	1.2	3.3
Two-year-old tench (T_2)	51.43	0.69	2.7	24.8	0.44	3.54
	62.01	0.68	2.87	24.31	1.27	7.36

CV-variation coefficient (%)

The level of blood glucose in two-summer-old tench (T_1^+) was significantly higher in comparison to that of the two-year-old tench (T_2) (Table 1). After the tench had wintered, lower absolute values of this parameter were determined in comparison with those prior to wintering (in fall). The differences were highly statistically significant ($P < 0.001$). The differences between the values registered in different years of the same season were insignificant.

Changes in levels of total protein and its fractions in the blood serum of two-summer-old tench (T_1^+) and two-year-old tench (T_2) are presented in Table 2. The quantity of total proteins in the serum of two-summer-old tench (prior to wintering) was significantly higher than those of two-year-old tench (after wintering) ($P < 0.001$).

In the same age groups, there is a low degree of significance among the values registered in the different years of the study for one-summer-old tench (T_1^+) ($P < 0.05$), but they are highly significant among two-year-old tench (T_2) ($P < 0.001$).

From determinations of the levels of albumins and α-, β-, and γ-globulins (Table 2), it was established that the contents of albumins and α- and β-globulins in the blood serum of two-summer-old tench (prior to wintering) were significantly higher ($P < 0.001$) in comparison with those of two-year-old tench (after wintering). With regard to β-globulins in two-year-old tench, higher values were detected compared to those registered in two-summer-old tench ($P < 0.001$).

TABLE 2

Total protein and protein profile of blood serum (g l^{-1}) of tench prior to and after wintering (n = 4) in 1996-1998

	Two-summer-old tench (T_1+)			Two-year-old tench (T_2)		
Specification	mean	SD	CV (%)	mean	SD	CV (%)
1996						
Total proteins	28.2	0.09	6.82	na	na	na
Albumins	na	na	na	na	na	na
α-globulins	na	na	na	na	na	na
β- globulins	na	na	na	na	na	na
γ- globulins	na	na	na	na	na	na
1997						
Total Proteins	27.6	0.06	1.13	23.40	0.07	6.48
Albumins	6.4	0.02	5.41	na	na	na
α-globulins	7.8	0.046	10.31	na	na	na
β- globulins	10.8	0.13	21.68	na	na	na
γ- globulins	2.6	0.1	67.28	na	na	na
1998						
Total Proteins	na	na	na	26.0	0.03	2.30
Albumins	na	na	na	5.6	0.014	4.44
α-globulins	na	na	na	6.3	0.009	2.39
β- globulins	na	na	na	12.7	0.023	3.11
γ- globulins	na	na	na	1.4	0.02	24.56

CV-variation coefficient (%), na – not analysed

The protein composition of tench blood serum in the fall was as follows: albumins – 23.19%; α-globulins – 28.26%; β-globulins – 39.13%, γ-globulins – 9.42% (Fig. 1). After wintering, a percentage decrease was noted, namely of albumins at 7.6%, α-globulins – 16.6%, and γ-globulins – 75.1%. However, β-globulins increased by 19.9% (Fig. 1).

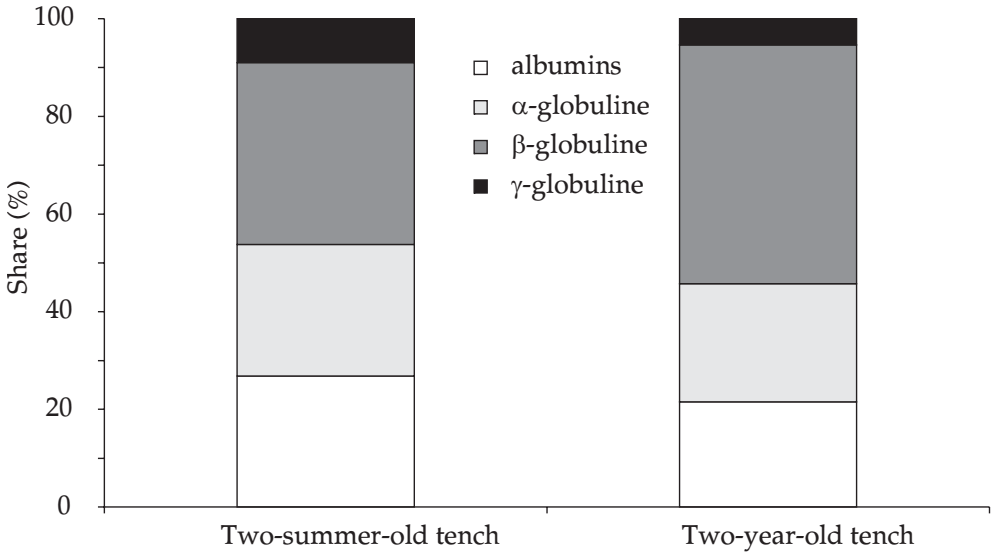

Fig. 1. Percentages of protein fractions in two-summer-old and two-year-old tench.

Data regarding the bactericidal activity of the blood serum indicate that prior to and following wintering in both seasons studied the absolute value in 67% of the tench samples studied was over 50%. This permits the conclusion that tench can be classified as fish that have a high resistance to disease according to the criteria proposed by some authors (Goncharov et al. 1974, Mikriakov and Silkin 1978, Atanasova 2003).

Tench is characterized by a seasonally dynamic blood glucose parameter which is higher in fall and lower in spring. The analysis of values of it prior to (fall) and after (spring) wintering indicates that tench utilizes blood glucose extensively in the winter period, as the quantity of it decreased by 66% in comparison to the levels recorded prior to wintering. The total protein level in the tench blood serum in fall is significantly higher in comparison to the spring level. Lower levels of total protein in spring following wintering indicate both seasonal modification and that some protein is utilized to meet energy needs during wintering. The highly significant differences between fall and spring values allow tench to be described as a species that utilizes proteins intensively to meet energy needs in the wintering period. It turn, this species can be categorized as belonging to the group of fish with an unstable physical condition that depends largely on environmental conditions.

The absolute values of total proteins obtained in two-summer-old tench (28 g l^{-1}) are close to the norms reported by a number of authors (Georgiev 1995), while the values of the blood glucose of two-summer-old tench are typical of fish reared on an intensive feeding regime as indicated by a value above 75 mg 100 ml $^{-1}$ as reported by Amineva and Jarzombek (1984). The concentrations of albumins and α-, β-, and γ-globulins reflect the specific exhaustion of proteins during wintering. The percentage ratio of albumins, and α-, β-, and γ-globulins in the blood serum of tench prior to wintering are close to those specified as norms that indicate a good physiological status (albumins 6.4 g l^{-1}; α- globulins 7.8 g l^{-1}; β-globulins 10.8 g l^{-1}; γ-globulins 9.42 g l^{-1}), and after wintering they are within tolerable limits for this species (Georgiev 1995).

The higher values of total proteins and blood glucose registered in tench in the fall are accepted as a result of the intensive feeding of this species in summer and its consequent accumulation of energetic substances. The results of this research could be summarized as follows: the protein spectrum of the blood serum provides information about both tench seasonal age peculiarity as well as its physiological status. The analysis indicates that the parameters used in the present study, including bactericidal activity, blood glucose, total proteins, and the protein fraction of blood serum, supply information regarding the status of tench, as well as of its seasonal and age differences in relation to its energy stores. When higher values of these biochemical parameters were noted in fall, this indicated that the tench was better able to winter successfully. Under the climatic conditions prevailing in Bulgaria, this is of practical significance to pond fisheries where the problem of wintering and safeguarding stocking material is of primary importance.

CONCLUSIONS

1. This study confirmed that the relative stability of the bactericidal activity of blood serum (BA) parameter indicates that tench is resistant during the wintering period.
2. It was established that there are seasonal dynamics in the levels of blood glucose and total protein, and that values of the former are higher in fall and lower in spring.
3. The specific nature of the metabolism of different protein fractions in the blood serum of tench is characterized by the percentage decrease of albumins and α- and γ-globulins and by an increase in β-globulins.

REFERENCES

Amineva V.A., Jarzombek A.A. 1984 – Fish Physiology – Legkaya i pishtevaya promishlenost, Moscow, 200 p.

Atanasova R. 2003 – Investigations on the natural resistance of carp (*Cyprinus carpio* L.) reared in ponds – PhD Thesis, National Centre for Agrarian Sciences, Sofia, 123 p. (in Bulgarian).

Atanasova R., Hadjinikolova L., Christev Ch. 1995 – Calorimetric method for determining bacterial activity in carp blood serum (BABS) – Proc. Fresh. Fish. Res. Inst., Plovdiv 19: 105-108 (in Bulgarian).

Erdem M. 1997 – The possibilities of using cotton seed oil meal in place of fish meal in carp diets – Ankara Univ. Ziraat Fak. Yilligi 29 (2-4): 675-689.

Fasaic K., Debeljak L., Adamek Z. 1995 – Neki hematoloski pokazatelji u uzgoju dvogodisnjeg sarana (*Cyprinus carpio* L.) – Ribarstvo 3: 95-103.

Georgiev G. 1995 – Fishery and sea farming – Zemizdat, Sofia, 277 p. (in Bulgarian).

Goncharov G., Vladimirov V., Mikriakov B. 1972 – Fish immunology – In: Parasites and diseases of fish and other aquatic invertebrates. Moscow, pp. 79-96 (in Russian).

Hadjinikolova L., Atanasova R., Hubenova-Siderova T. 2000 – Comparative immuno-biochemical characteristic of stocking material of carp (*Cyprinus carpio* L.), bighead carp (*Aristichthys nobilis* Rich.), grass carp (*Ctenopharyngodon idella* Val.) and tench (*Tinca tinca* L.) raised in polyculture – Bulg. J. Agric. Sci. 6(6): 675-679.

Ibrischimov N., Lalov Ch. 1974 – Clinical investigations in veterinary medicine – Zemizdat, Sofia, 390 p. (in Bulgarian).

Karanikolov J. 1995 – A new preparation for inducing anesthesia in freshwater fishes – Proc. Fresh. Fish. Res. Inst., Plovdiv, 19: 113-119 (in Bulgarian).

Karakaschev A., Vichev E. 1966 – Micromethods in clinical laboratories – Medicine and Sport, pp. 113-114 (in Bulgarian).

Mikriakov V. R., Silkin N. F. 1978 – Seasonal dynamics in the antimicrobial features of the blood serum in fish from different ecological habitats – Biol. Inland Wat. 39: 63-68 (in Russian).

Ottolenghi C., Puviani A.C., Ricci D., Brighenti L., Morsiani E. 1995 – The effect of high temperature on blood glucose level in two teleost fish (*Ictalurus melas* and *Ictalurus punctatus*) – Comp. Biochem. Physiol. A 11(2): 229-235.

Pavlov D., Romanov M., Vasilev M., Popov I. 1984 – Clinical laboratory investigations – Medicine and Physical Exercises, 256 p. (in Bulgarian).

Silkin N.F. 1989 – Seasonal dynamics in the unspecific immunological parameters and its relationship with some physiological features in matured fishes – Proc. Biol. Ins. Inland Wat., ANSSSR, Borok, 114 p.

Van Muiswinkel W.B. 1996 – Immunogenetics of disease resistance in fish comparative approach – Am. Prev. Develop. Comp. Immunol. 20(6): 365-381.

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STRESZCZENIE

WYBRANE BIOCHEMICZNE PARAMETRY LINA (*TINCA TINCA* (L.)) PODCHOWYWANEGO W STAWACH ZIEMNYCH PRZED I PO OKRESIE ZIMOWANIA

Biochemiczna charakterystyka materiału zarybieniowego lina, *Tinca tinca* (L.), była wykonywana przed i po okresie zimowym z uwzględnieniem następujących parametrów: aktywność bakteriobójcza surowicy krwi, poziom glukozy we krwi (BG), białka całkowitego (TP), frakcji białek surowicy krwi (albumin, α-, β-, i γ-globulin). Badania były prowadzone w okresie trzech lat (1996-1998) na klinicznie zdrowych dwuletnich – jesiennych i wiosennych linach o masie ciała od 30 do 80 g. Badania wykazały, że jeden z parametrów plazmy krwi odpowiedzialny za odporność lina (bakteriobójcza aktywność plazmy krwi) był relatywnie stabilny w okresie zimowym. Poziom glukozy we krwi i białka całkowitego w surowicy był zależny od sezonu (tab. 1). Poziom pierwszego z parametrów był wyższy w okresie jesiennym, zaś niższy w okresie wiosennym. Szczególne zmiany dotyczyły różnic poziomu frakcji białek surowicy krwi lina i charakteryzowały się procentowym spadkiem albumin, α- i γ-globulin i wzrostem β-globulin (tab. 2, rys. 1).