CORRELATIONS BETWEEN SOME BIOCHEMICAL PARAMETERS OF CARP FISH (*CYPRINIDAE*) UP TO ONE-YEAR-OLD AGE

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KORELACIJA IZMEĐU NEKIH BIOHEMIJSKIH PARAMETARA ŠARANSKIH RIBA (CYPRINIDAE) DO STAROSTI OD JEDNE GODINE

Abstrakt

Cilj ispitivanja je da se prouče neki od biohemijskih parametara krvnog seruma i tela, kao i eventualnih korelacije između njih. Ispitivane vrste su bile šaran, tolstolobik i amur do jedne godine starosti, pre i posle zimovanja u zimovnicima. Korelacija koja je bila ustanovljena između ispitivanih parova parametara ukupnih serum protein i baktericidne aktivnosti za ispitivane tipova i starosti riba, varira od umerene do znatne, značajne i različitog pravca. Kod tolstolobika je konstatovana umerena, obrnuta i znatna korelacija između masti i glukoze krvi, kao i proteina i glukoze krvi. Korelacija koja je bila ustanovljena između protein u telu i ukupnih protein kod jednogodišnjeg šarana, tolstolobika i amura varira od umerene do znatne, obrnuta je i značajna ($r = 0.54$; $r = 0.73$; $P < 0.047$; $P < 0.0009$).

***Ključne reči**: šaranske ribe, hemijski sastav, korelacije*

INTRODUCTION

In carp farms and reservoirs in Bulgaria upon application of traditional technologies for rearing in polyculture fish production of primarily carp (*Cyprinus carpio* L.), silver carp (*Hypophtalmichthys molitrix* Val.), big-head carp (*Aristichthys nobilis Rich.*) and grass carp (*Ctenopharingodon idella* Val.) is produced.

Taking into consideration that when rearing the above species, the period of wintering is of high risk, in previous investigations (A t a n a s o v a et al. 2001; A t a n a s o v a et al. 2006; H a d j i n i k o l o v a et al. 2000) the dynamics and type specifics of a number of parameters of blood serum has been investigated, the parameters providing information on the condition of the researched organism such as bactericidal activity, blood glucose, total

serum proteins and protein fractions prior and after wintering. Some of these parameters have been also used by other authors with the purpose of determining the physiological state of fish organism (K o l m a n et. al. 1998; K o l m a n et al. 1999; S i w i c k i et al. 2003; V i c h m a n, 1996).

Researches on the physiological state of carp stocking material by studying its back-up systems (serum proteins and muscle fats) have been carried out by K o v a c h e v a and H a d j i n i k o l o v a (1990).

Bearing in mind the fact that biochemical parameters reflect the physiological state of fish organism, of particular interest is the establishment of the correlation between them with the purpose to obtain a more objective estimation as to the readiness of stock material for wintering.

Referring to the above the goal of the present investigation is to study some of the biochemical parameters of the blood serum and the body, as well as eventual correlations between them, the investigated species being carp (*Cyprinus carpio* L.), big-head carp (*Aristichthys nobilis* Rich.) and grass carp (*Ctenopharyngodon idella* Val.) up to one-year-old age prior and after wintering in carp ponds.

MATERIALS AND METHODS

Fish and rearing conditions. The investigations were carried out in the period of 1995–1999 in the Experimental Basis of the Institute of Fisheries and Aquacultures in Plovdiv with the following types of fish and their age: one-summer-old (K_0^+) and one-year-old (K_1) carp; one-summer-old (T_0^+) and one-year-old (T_1) big-head carp, one-summer-old (A_0^+) and one-year-old (A_1) grass carp. Fish was reared in polyculture in one and the same pond with an area of 0.70 ha with the density of 40 000 - 60 000 pcs. ha $^{-1}$ carp, 30 000 - 50 000 pcs. ha $^{-1}$ big-head carp and 2000 pcs. ha $^{-1}$ grass carp.

In the vegetation period to feed the fish sunflower groats (40 %) and grains forage (barley and wheat - 60 %) were used, while the daily rations has been defined on the basis of a monthly distribution of forage (June-7 %, July-26 %, August-35 %, September-30 %, October-2 %). Having been caught in November, fish was sorted and put for wintering in three carp ponds divided by type with an area of 0.13-0.15 ha and density of 200 000-300 000 pcs. ha^{-1}. During winter fish has been fed with ground sunflower groat and grains at a rations of 0.5 % of its mass and at water temperature exceeding 4^{0}C with the purpose of keeping it in good shape and preventing it from losing weight.

Upon rearing of fish in the period May-September the physical-chemical regime of water was also registered. The average seasonal values of the traced parameters lie in the technological limits for the reared species: water temperature 21.6 - 23^0 C; pH 7.61 - 8.00; oxygen dissolved in water 4.0-6.49 mg.l^{-1}.

In winter ponds the average values of water parameters are: temperature 5.7 – 7.2^0 C; pH 7.0 - 8.00; oxygen dissolved in water 15.7-16.8 mg.l^{-1}.

Analysis and processing of data. The blood for analyzing has been taken from 20-30 pcs respectively of carp, big-head carp and grass carp in each of the investigated season (autumn-spring). To eliminate the stress resulting from the manipulation fish has been anaesthetized. To have the serum separated blood was centrifuged in the course of 10 min at 3 000 turnovers min^{-1}.

The natural resistance of fish has been determined by means of the parameter bactericidal activity of the blood serum (BA,%) with the using of *Aeromonas hydrophila* as a testing mi-

crobe. Investigations were carried out by applying a modified method of Markov (A t a n a s o v a et al. 1995). The method was adapted with exchanging of the testing microbe *Escherichia coli* with *Aeromonas hydrophila*, which is pathogenic for carp when microbe bodies are reduced in 1 ml. Biochemical characteristics of the blood serum were identified by determining the total proteins (TP, $g.l^{-1}$) which were defined by Gornal's method, colourmetrically (λ = 410 nm), by the use of Bioretic reactive ($CuSO_4 x\ 7H_2O + KNaC_4H_4O_6 x\ 4H_2O + NaOH$), (I b r i s h i m o v and L a l o v, 1974). Blood glucose (BG, mg 100 ml^{-1}) was determined by application of the spectrum-photometrical method (λ = 366 nm), with the use of aniline reactive and 3-chloroacetic acid as a standard (K a r a k a s h e v and V i c h e v, 1966).

Samples of meat that had been previously extracted from the body of the fish (without head and insides) were prepared and having been homogenized, those were analyzed for defining the contents of protein (Kjeldahl and Parnas-Wagner distillation of nitrogen) and fat (Soxhlet). Standard methods for fish analyzing were applied.

Fish included in the present investigation is with an average single mass of 30 up to 50 g and has been selected occasionally.

Data was processed statistically by means of software on statistical processing of Microsoft Office 2003. The average arithmetical value (x) of parameters, its error (Sx) and variation coefficient (Cv, %) were registered.

Correlations have been determined and correlation coefficients between biochemical parameters of the blood serum and the body of the investigated types of fish were indicated. The correlation coefficient (r) and the significance rate (p) were determined by means of a statistical package "Statistica 6.0". Upon comparison of two samples the significant differences were analyzed upon application of t-test according to Student, the probability rate being $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

RESULTS AND DISCUSSION

The results obtained for the BA of the blood serum (Table 1) point out that in the autumn samples the absolute values for grass carp are significantly higher ($P < 0.01$) compared to those for carp and big-head carp. The difference between the latter is not significant ($P > 0.05$). In the spring samples the differences between the absolute values of the examined species are insignificant ($P > 0.05$). With a low degree of significance are characterized the differences between autumn and spring samples in carp (K_{0+} / K_1) and big-head carp (T_{0+} / T_1), ($P < 0.05$), while in grass carp (A_{0+} / A_1) the difference is insignificant. ($P > 0.05$).

Table 1. Average values of biochemical composition of the blood serum of carp fish up to one-year-old age.

Fish spesies, age	Bactericidal activity (%)			Total proteins ($g.l^{-1}$)			Blood glucose ($mg.100\ ml^{-1}$)		
	x	Sx	$Cv_{(\%)}$	x	Sx	$Cv_{(\%)}$	x	Sx	$Cv_{(\%)}$
K_{0+}	45.52	1.35	17.29	29.46	1.602	31.72	93.56	1.498	9.34
T_{0+}	47.78	0.98	7.64	26.73	0,548	7.67	78.00	2.202	10,57
A_{0+}	52.75	1.14	8.05	33.63	0,651	7.25	74.51	0.777	3,91
K_1	56.13	4.52	24.15	27.30	0.545	5.99	40,72	0.926	6.825
T_1	50.86	0.90	5.31	18.60	0.450	7.26	67.27	2.746	12.25
A_1	55.14	3.39	18.46	23.30	0.473	6.08	52.39	0.645	3.70

K_{0+} -one-summer-old carp (n=35); T_{0+} - one-summer-old big-head carp (n=15); A_{0+} - one-summer-old grass carp (n=15); K_1 – one-year-old carp (n=10); T_1 – one-year-old big-head carp (n=10); A_1 – one-year-old grass carp (n=10);

As far as the level of the total proteins is concerned, it has been registered that the autumn values for grass carp are significantly higher than those for carp and big-head carp ($P < 0.05$; $P < 0.001$). The differences between the latter are insignificant ($P > 0.05$). In the spring samples the absolute values for carp are significantly higher ($P < 0.001$) than those registered for big-head carp and grass carp. The differences between the latter are mathematically proved ($P < 0.01$). The autumn values of the total proteins for big-head carp and grass carp are significantly higher than those established for the respective type in spring ($P < 0.01$), while in carp the difference is insignificant. ($P < 0.05$)

The level of blood glucose in one-summer-old carp is significantly higher in comparison with that of one-summer-old big-head carp and grass carp ($P < 0.01$). The difference between the latter two species is insignificant ($P < 0.05$). In one-year-old big-head carp the level of blood glucose is significantly higher compared to that of one-year-old carp and grass carp ($P < 0.001$), the differences between the latter two types also being mathematically proved ($P < 0.01$). For the three examined types of fish after wintering there have been established lower absolute values for the above parameter compared to those prior to wintering (in autumn), the values being specific for each particular type. The differences are characterized with a high degree of significance ($P < 0.01$) for carp and grass carp and with a low degree of significance ($P < 0.05$) for big-head carp.

As to the contents of proteins in the fish body (Table 2) it was registered that in the autumn samples the values for carp are significantly higher in comparison with those registered for big-head carp and grass carp ($P < 0.001$). The difference between the latter is with low degree of significance ($P < 0.05$). In the spring values a significant difference was registered between carp and grass carp, and grass carp and big-head carp. The difference between the values of one-year-old carp and big-head carp is insignificant ($P > 0.05$). The autumn values for carp and grass carp are significantly higher compared to those registered in spring ($P < 0.01$), while for big-head carp the difference is insignificant.

Table 2. Average values of biochemical composition of the tissue of carp fish up to one-year-old age.

Fish spesies , age	Protein , %			Fats , %		
	x	Sx	Cv	x	Sx	Cv
K_{0+}	18.65	0.118	3.69	3.12	0.166	31.12
T_{0+}	15.94	0.261	6.14	2.72	0.119	16.36
A_{0+}	17.08	0.263	5.76	3.59	0.086	8.98
K_1	16.08	0.142	2.65	1.30	0.113	26.07
T_1	16.01	0.142	2.67	1.67	0.089	16.26
A_1	14.88	0.189	3.82	1.64	0.166	30.32

K_{0+} -one-summer-old carp (n=10); T_{0+} - one-summer-old big-head carp (n=10); A_{0+} - one-summer-old grass carp (n=10); K_1 – one-year-old carp (n=10); T_1 – one-year-old big-head carp (n=10); A_1 – one-year-old grass carp (n=10);

The contents of fats in one-summer-old grass carp is significantly higher than that of carp and big-head carp ($P < 0.05$; $P < 0.001$). A mathematically significant difference between autumn and spring values for the three examined types of fish has been established. ($P < 0.001$).

The results obtained indicate a clearly expressed seasonal variability of the investigated parameters with higher autumn and lower spring values and type specifics registered for some of them. The high degree of significance between the differences for autumn and spring values characterize carp, grass carp and big-head carp as species which dur-

ing the period of wintering are more intensively utilizing the stocked organic substances to cover their energetic demands. And it is this that is categorizing them to the group of fish with weak unstable physiological state which is to a great extent dependent on the environmental conditions.

In this respect the examination of the correlations between the biochemical parameters of the blood serum and the body of fish is a prerequisite to get a more objective estimation of the degree of exhaustion of plastic and energetic substances in the period of wintering. Taking into consideration the above the correlation coefficients between the examined parameters were determined.

Table 3, Table 4, Table 5 show the results obtained for the size of the correlation coefficients for each pair of the examined parameters of the blood serum - bactericidal activity (**BA**), total proteins (**TP**) and blood glucose (**BG**) and of proteins and fats in the body of carp, grass carp and big-head carp prior and after wintering up to one-year-old age.

Table 3. A correlation between Total proteins , Blood glucose, Protein, Fats and Bactericidal activity of carp fish.

Fish spesies, age	Total proteins ($g.l^{-1}$)		Blood glucose ($mg.100\ ml^{-1}$)		Protein , %		Fats , %	
	Bactericidal activity, (BA, %)							
	r	p	r	p	r	p	r	p
K_{0+}	0.397	0.018*	0.190	0.274	0.325	0.057	0.014	0.938
T_{0+}	-0.045	0.872	-0.639	0.010**	0.466	0.079	0.396	0.144
A_{0+}	0.473	0.087	0.024	0.934	0.714	0.004**	0.072	0.807
K_1	-0.633	0.049*	0.073	0.840	0.742	0.014*	-0.813	0.004**
T_1	0.146	0.688	-0.460	0.180	-0.488	0.153	0.575	0.082
A_1	-0.634	0.048*	-0.30	0.405	-0.440	0.203	-0.307	0.379

There was established a moderate positive correlation between total proteins and bactericidal activity in one-summer-old carp (K_{0+}) and grass carp (A_{0+}) on the one hand, and considerable in value, negative and significant correlation in one-year-old carp (K_1) and grass carp (A_1) on the other.

The relation between blood glucose and bactericidal activity for the separate types and ages of fish is not simple, both in terms of size and direction, considerable and significant the relation being solely for one-summer-old big-head carp.

The correlation coefficients between proteins in the body and bactericidal activity of the blood serum for one-summer-old fish are positive, while for one-year-old those are negative in direction, the values varying from moderate to considerable (r=0.32 – r= 0.74) and being significant. Varying from moderate to great, significant and negative is the correlation between the fats in the body and the bactericidal activity in one-year-old fish.

Results point out that the correlation between the parameters of the protein exchange and blood glucose are not simple both in terms of size and direction as well, being primarily insignificant.

Analogical is the data registered for the correlation coefficients between fats and blood glucose (Table 5) and fats in the body and total serum proteins (Table 4) for carp and grass carp. As far as big-head carp is concerned the correlation is moderate, reverse and significant, the values for r = - 0.54 and P <0.037 for one-summer-old big-head carp and r

= -0.57 and P <0.048 for one-year-old big-head carp, which in its essence is an indicator for its type specifics.

Table 4. A correlation between Total proteins and Blood glucose; Protein and Fats in the tissue of carp, big-head-carp and grass carp and Total proteins.

Fish spesies, age	Total proteins ($g.l^{-1}$)		Protein , %		Fats ,%	
	Blood glucose ($mg.100\ ml^{-1}$)		Total proteins ($g.l^{-1}$)			
	r	p	r	p	r	p
$К_{0+}$	0.167	0.337	0.535	0.0009***	-0.155	0.375
$Т_{0+}$	-0.339	0.216	0.725	0.002**	0.465	0.081
$А_{0+}$	0.301	0.275	0.540	0.047*	0.155	0.582
$К_{1}$	-0.130	0.719	-0.318	0.369	0.451	0.191
$Т_{1}$	0.237	0.509	-0.231	0.521	-0.08	0.824
$А_{1}$	0.252	0.482	-0.120	0.740	0.026	0.942

Table 5. A correlation between Protein and Fats in the tissue of carp, big-head-carp and grass carp and Blood glucose.

Fish spesies, age	Protein , %		Fats , %	
	Blood glucose ($mg.100\ ml^{-1}$)			
	r	p	r	p
$К_{0+}$	-0.314	0.066	0.296	0.084
$Т_{0+}$	-0.712	0.003**	-0.541	0.037*
$А_{0+}$	0.218	0.434	0.316	0.251
$К_{1}$	-0.418	0.229	-0.258	0.472
$Т_{1}$	0.805	0.005**	-0.567	0.048*
$А_{1}$	0.161	0.657	-0.326	0.358

In one-summer-old fish the relation established between proteins in the body and total serum proteins varies from moderate to considerable, is positive and significant (r = 0.54-0.73; P <0.047-0.0009), while in one-year-old fish a relation that is weak, reverse and insignificant has been registered (Table 4).

The equations drawn for some more clearly expressed correlations show that within the limits of the examined correlations the parameters total serum proteins, blood glucose, proteins and fats determine a high relative share (%) of variation of bactericidal activity (R^2 = 0.40 - R^2 = 0.66), which is more clearly expressed in one-year-old fish. From the examined correlations proteins in the body define a higher percentage (%) of variation of serum proteins (R^2 = 0.30-0.50).

For all the remaining correlations that have been established between the biochemical parameters the relative share (%) of variation is low ($R^2 < 0.3$), which is an indicator for the existence of a weak dependence between them. It is probable that the differences in the correlation between the investigated parameters are due to the samples of fish belonging to different types and age.

Higher autumn values of total serum proteins, blood glucose, proteins and fats could be regarded as a result of the intensive summer feeding of the examined species and the accumulation of plastic and energetic material in their organism. This is a prerequisite for

their normal wintering. Feeding, by means of the type of food and the intensity of feeding, is a factor which exerts influence on the biochemical composition of fish. The higher correlation coefficients that were found out for one-summer-old fish point out that the intensive accumulation of plastic and energetic substances in the fish organism also has an influence on the strength of the established correlations, the latter being expressed more clearly. A suitable demonstration of the above is the correlations between the parameters of protein exchange.

The high relative share (%) of variation of the bactericidal activity in correlation with the parameters total serum proteins, blood glucose, proteins and fats could be related to the fact that in the period of wintering the keeping of organism's homeostasis is above all at the expense of the metabolism and the depletion of the stocks of fats and proteins. The data received point out the importance and the effect that non-protein components such as blood glucose and fats have on metabolism. The energy required for the vital processes in the winter period is primarily provided by the disintegration of the stocked fats (S o r v a c h e v, 1982), whereas the basic depot of fats in carp is the muscle tissue.

The presence of correlations between the examined biochemical parameters makes possible their use as an additional criterion to estimate the quality of the stocking material up to one-year-old age, as well as to judge its readiness to spend the winter. Referring to the above the practical importance under the climatic conditions in Bulgaria is related to pond fisheries where the issue of wintering and preserving the stocking material is of primary importance.

CONCLUSIONS

A seasonal variability of the biochemical parameters of the blood serum and the body of carp, grass carp and big-head carp up to one-year-old age was proved, higher autumn and lower spring values and type specifics for some of them being registered.

The correlation that has been established between the examined pairs of parameters: total serum proteins and bactericidal activity for the investigated types and ages of fish varies from moderate to considerable, significant and different in direction.

A moderate, reverse and significant correlation between fats and blood glucose and proteins and blood glucose in big-head-carp was found out.

The correlation that has been established between proteins in the body and the total proteins in one-summer-old carp, big-head carp and grass carp varies from moderate to considerable, being reverse and significant ($r = 0.54$; $r = 0.73$; $P < 0.047$; $P < 0.0009$).

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