Acta Veterinaria Hungarica 50 (4), pp. 459–467 (2002)

HAEMATOLOGY AND SOME BLOOD CHEMICAL PARAMETERS OF YOUNG CARP TILL THE AGE OF THREE YEARS

A. SVETINA[1*], Željka MATAŠIN[2], Alenka TOFANT[3], Marija VUČEMILO[3] and N. FIJAN[2]

[1]Department of Pathophysiology, [2]Department for Biology and Pathology of Fish and Bees, [3]Department of Animal Hygiene, Environment and Ethology, Faculty of Veterinary Medicine, Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

(Received May 7, 2002; accepted September 11, 2002)

Haematological and biochemical analyses of blood were performed in carp (*Cyprinus carpio* L.) kept in small ponds. Caught and anaesthetised carp were clinically examined and blood samples were taken at regular intervals during the three years. In the first year of examinations, the haemoglobin and haematocrit values of carp fry significantly increased ($P < 0.01$) from June to September. The intensive growth of carp in the summer period in the second year was accompanied by adequate erythropoiesis. During hibernation haematocrit and haemoglobin significantly decreased ($P < 0.05$) and mean corpuscular haemoglobin concentration (MCHC) increased ($P < 0.01$) in both scaly and mirror carp. MCHC increased also with the age and increasing body weight of the fish. Mirror carp had lower haematocrit and haemoglobin values than scaly carp ($P < 0.01$). Comparative haematological analyses between carp of normal and poor body condition showed that moderate anaemia appeared in those with poor body condition. The results indicate that there is marked seasonal and age-dependent variation in the values of haematocrit and haemoglobin. Pond water quality investigations indicated good environmental conditions. A 50% increase ($P < 0.05$) of glucose concentration was found from June to September in the blood plasma of carp in the third year, accompanied by an even more increased (80%; $P < 0.01$) concentration of total lipids. At the same time, considerable changes of cholesterol and total protein concentrations were not observed. The results suggest that the investigated haematological and biochemical variables could be successfully utilised in monitoring the metabolic balance and health status of fish in intensive culture.

Key words: Carp (*Cyprinus carpio*), haematology, blood biochemical values, age, season, body weight, water quality

The intensive methods used in aquaculture require a good correlation between the environment and the organism, and between the rearing technology and the physiological status of the fish. The water quality of the pond is an important factor as it can affect fish health. The occasional decrease of oxygen con-

*Corresponding author: Prof. Ante Svetina; E-mail: mbelic@vef.hr; Fax: +385 (1) 2390 184

centration in summer or in winter, as well as the high pH and ammonia values encountered with increasing frequency, are the main environmental stress factors influencing the health, growth rate and survival of fish. Inadequate catching methods (particularly removal from the original environment) as well as starvation during hibernation are some of the common stress factors exerting an effect on fish (McDonald and Millicam, 1992). Improved health protection of fish kept in ponds is, therefore, one of the factors most important for high production and economic progress in aquaculture.

As a part of veterinary inspection, haematological and blood biochemical parameters could be important for controlling and assessing the physiological status and condition of fish at an optimal level.

It is known that haematological parameters in fish vary with factors such as species and age of the fish and season (Lehmann and Stürenberg, 1981). An influence of various anaesthetics and bleeding methods on haematological parameters in fish has also been reported (Hoffman et al., 1982). In the study of Fašaič and Adamek (1993) special attention was paid to some haematological parameters in fish living at high stocking density during the winter period. Such fish are usually much more sensitive to diseases in the spring.

Total protein level of the blood plasma is an important parameter in evaluating the condition of fish, a reliable indicator of physiological status and an important diagnostic aid (Adamek, 1977). Decreased concentration of plasma protein was reported in starving fish (Sauer and Heider, 1979, cit. Prihoda and Bienek, 1993).

There are also some data about changes in haematological parameters and biochemical values in parasitised carp. Thus, there was a reduction of serum protein in carp with coccidiosis (Kent and Hedrick, 1985; Jendrysek et al., 1994). Hilge (1980) presented a lot of data about haematological and blood chemical values of carp (body weight from 2858 g to 5305 g) kept in a closed warm-water recycling system, i.e. in artificial environment.

The purpose of the present study was to get more information about haematological and blood biochemical values in carp (*Cyprinus carpio* L.; body weight from 14.33 to 829.00 g) kept in natural environment, particularly in relation to age of the fish. The influence of rearing as well as health condition on the investigated blood parameters was also considered.

Materials and methods

Animals, feeding and handling

The investigations were performed on carp fry stocked in ponds with a surface of 0.5 ha and an average depth of 3 m on a fish farm in Croatia. The rearing ponds were stocked with 45 days old carp fry at the beginning of the investigations.

Before stocking 200 kg/ha of lime was used in the ponds. The ponds were supplied with semi-automatic feeder and with aeration. During the summer (growing period) fish were fed with pelleted complete food for carp.

Average body weight was initially 14.33 g for carp fry (at the beginning of the study) and it changed during the experiment to 829.00 g (at the end of investigations). The average body weights (in g), body lengths and heights (in cm) of fish during the experiment are presented in Table 1. There were a lot of differences in body weight in two-year-old carp in April. Therefore, these fish were divided into two groups, one with lower and the other with higher body weight than the calculated average value. Feeding-up coefficient (for each fish) was calculated using the formula $Q = (\text{body weight in g} \times 100) : (\text{body length in cm})^3$. Based on calculated feeding-up coefficient the carp were divided into a group of normal and another one of poor body condition.

Carp were caught gently from ponds in a small net, with maximum avoidance of stress, and immediately anaesthetised with MS 222 (Sandoz) in a concentration of 1:15,000. The time taken for the fish to be anaesthetised was usually about 2–3 min, as shown by loss of equilibrium and by immobility when touched. All fish were examined clinically. The skin and gills were examined microscopically. Gills, livers and kidneys were examined histopathologically and the kidneys bacteriologically.

Sample collection and analyses

Blood samples were taken from fish caught by the random selection method between June of the first year and February of the third year of investigation.

Haematological analyses were carried out on the following groups: carp fry (first year), carp of normal and poor body condition in April, only carp of normal body condition in June and September (of the second year), and healthy scaly and mirror carp in the third year of the experiment. Blood samples of 30–36 carp were analysed in each group.

For all determinations blood samples were taken by puncture of the caudal blood vessels puncture into sterile plastic syringes containing heparin as anticoagulant. The following haematological parameters were determined: haematocrit (PCV) in vol. %, haemoglobin (Hb) in g/L and mean corpuscular haemoglobin concentration (MCHC) in %. Haematological tests were used for fish as described by Svobodova (1986).

After an aliquot of blood samples was taken for haematological analyses, the rest of samples were centrifuged at 3,000 rpm for 5 min and plasma was separated for determination of glucose (Gl) concentrations. Total proteins (TP), total lipids (TL) and total cholesterol (Ch) were analysed in the blood serum. All constituents were determined spectrophotometrically using standard tests (Boehringer-Mannheim GmbH, Mannheim, Germany).

Table 1

The values of haematocrit (PCV), haemoglobin (Hb) and the mean corpuscular haemoglobin concentration (MCHC) in the blood of carp with regard to the age, body weight, length and height of fish

Parameters	Age of carp							
	1-year		2-year		3-year			
	June	September	April		June	September	February	
			Normal condition	Bad condition			Scaly	Mirror
Body								
Weight, g	14.33	57.25	97.57	81.67**	211.33	829.00		
Length, cm			17.89	17.03	22.57	34.80		
Height, cm			5.64	5.00	7.40	12.28		
Haematological values								
PCV, vol%	36.54 ± 1.08	43.63 ± 0.18**	45.36 ± 1.17	38.04 ± 1.49**	39.50 ± 0.55	40.67 ± 0.89	29.00±1.60**	22.60 ± 4.06**
Hb, g/L	68.55 ± 1.29	78.33 ± 2.07**	86.73 ± 2.44**	74.37 ± 2.77**	79.33 ± 1.26	87.33 ± 1.98**	67.60 ± 2.54**	51.20 ± 3.85**
MCHC, %	18.90 ± 0.59	17.70 ± 0.55	18.71 ± 0.28*	19.74 ± 0.54*	20.07 ± 0.33	21.33 ± 0.71*	23.60 ± 0.87	25.10 ± 3.68

Asterisks indicate statistical differences from the preceding value (the value in younger group): $^{*}P < 0.05$; $^{**}P < 0.01$

Water quality

Water sampling was performed at the same time when carp were caught.

Grab-bottle samples were taken out from a depth of 30 cm beneath the water surface, representing the composition of the sample at the time and place. On each occasion, physicochemical parameters were determined in accordance with standard APHA methods (Anonymus, 1975; HACH, 1992) using titration and photometric procedures on HACH Drel 2000 chemistry/apparatus module.

Statistical analyses

The results were analysed by personal computer. The mean and standard deviation of the mean, as well as standard error were calculated for each group. Validity was determined by Student's *t*-test and the differences were accepted as significant when P values were 0.05 or lower. Physicochemical parameters of pond water were expressed as mean values of the samples analysed in duplicate.

Results and discussion

Haematological parameters

In the first year of life, the haematocrit (PCV) and haemoglobin values of carp were higher in September than in June ($P < 0.01$), whereas the MCHC value was slightly lower in September (Table 1). Comparative haematological analysis of carp of normal and poor body condition showed that the haematocrit value, haemoglobin and MCHC concentration as well as the mean body weight were significantly higher ($P < 0.01$; $P < 0.01$; $P < 0.05$ and $P < 0.01$, respectively) in carp of normal body condition. According to the PCV and MCHC values, in carp of poor body condition borderline anaemia appeared which was classified as normocytic normochromic anaemia.

The comparison of haematological parameters in healthy carp between April and June (of the second year) indicates that intensive growth occurring during that period resulted in considerable changes of the parameters of red blood cells, such as a decrease of haematocrit (from 45.36% to 39.50%) and haemoglobin (from 86.73 g/L to 79.33 g/L). However, the haemoglobin concentration markedly increased ($P < 0.01$) from June to September. The mean body weight of carp increased in that period about four times.

Furthermore, the results show that scaly and mirror carp have the lowest haematocrit and haemoglobin values in the period of hibernation (in February). It has been shown that haematological parameters of carp fingerlings were also lower during winter (Černikova, 1974; Adamek, 1977; Palačkova, 1991). The decrease is probably associated with the reduced total activity of the fish. The comparative haematological analyses between scaly and mirror carp of the same age indicate that scaly carp have a markedly higher haematocrit volume ($P < 0.01$) and haemoglobin concentration ($P < 0.01$) in February. A similar difference in the haematocrit between scaly and mirror carp during hibernation was found by Spurny and Mareš (1993). During the present experiment the increase of body weight from 14.33 g to 829 g was followed by a constant increase of MCHC. Hilge (1980) found a similar increase of MCHC during the growth of carp from an average body weight of 2858 g to 5305 g. It can be concluded that MCHC increases parallel with the increase of body weight in all categories of carp.

In conclusion, the results of our research showed that the marked variability of haematocrit and haemoglobin occurred as a result of nutrition, age of fish and seasonal influences. Age-related and seasonal variations in fish haematology have been already reported (Lehmann and Stürenberg, 1981). Haematocrit and haemoglobin in carp from our experiment increased markedly during the first year of life. In the second year haematological parameters were well balanced during the intensive growth of carp (from about 100 to 800 g between April and September). This means that the rapid increase of mean body weight, as well as the increase of blood volume, were accompanied by adequate erythropoiesis.

Kovacheva et al. (1993) found that haematological parameters varied within narrow limits in carp stocking material when held under good ecological conditions. Haemoglobin concentration was 67 to 72 g/L and PCV was between 30 and 35%. They assumed that such carp were well prepared for wintering. In our experiments haemoglobin and haematocrit values were even higher, indicating good conditions in the pond.

Microscopic examination of the gills and skin showed ectoparasitic infestation with *Trichodina* sp., *Apiosoma* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., and *Ichthyophthirius multifiliis* only in three-year-old carp, in February. However, no marked effects of ectoparasites on the haematological values of these carps were observed. In younger experimental groups of carp, some of the ectoparasites were only sporadically present.

The results of water quality investigations (Table 2) indicate that all determined parameters fulfil the conditions set for ponds culturing cyprinids (Boyd, 1982). Namely, the investigated parameters measured in the water of the pond during each fish sample collection were generally within acceptable limits, and some of them (e.g. oxygen) had even better values. Therefore, it may be assumed that the physicochemical constituents of pond water did not have a harmful effect on the investigated haematological and blood chemical parameters of carp.

Fluctuations in the values of ammonia, nitrates and nitrites and phosphorus can be attributed to the ecological interaction between the surrounding fertilised agricultural fields and the water during the winter and springtime.

Table 2

Physicochemical parameters of pond water

Parameters*	Year of investigation 1		2		3
	June	September	April	September	February
Temperature, °C	19.3	15.5	13.5	16.0	10.5
pH	8.2	8.3	8.7	8.2	8.1
Ammonium (NH_4-N), mg/L	0.12	0.09	0.09	0.10	0.11
Nitrite (NO_2-N), mg/L	0.021	0.009	0.008	0.010	0.019
Nitrate (NO_3-N), mg/L	0.7	1.7	1.3	1.8	1.1
COD-Mn, mg/L*	7.02	5.52	9.29	6.12	3.76
Chloride (Cl^-), mg/L	35	32	32	33	31
Phosphorus (P), mg/L	1.38	0.37	1.30	0.55	0.68
Hardness, °dH	8.1	9.3	7.7	9.9	10.4
Dissolved oxygen (O_2), mg/L	5.96	8.90	6.80	7.91	13.3
5-day BOD, mg O_2/L**	3.50	2.70	3.20	2.90	2.50

For each parameter the water sample was analysed in duplicate and the mean value was expressed; *COD-Mn = Chemical Oxygen Demand - potassium permanganate consuming capacity; **Biochemical Oxygen Demand for 5 days in an incubator at 20 °C in the dark

Biochemical parameters

Blood biochemical analyses of two- and three-year-old carp (Table 3) show that the concentration of total lipids in the blood serum was about 80% higher ($P < 0.01$) in September than in June, while glucose concentration in the blood plasma increased about 50% ($P < 0.05$; Table 3). In the same period mean body weight increased about four times (from 211.33 to 829.00 g). After overcoming the period of starvation during hibernation, in February of the next (third) year the serum concentration of total lipids decreased markedly ($P < 0.01$) in carp in our experiment. However, as opposed to total lipids, there were no significant variations in the cholesterol concentrations with regard to age, season and body condition. Considerable changes in total protein concentrations of the serum were not observed during the experiment. The serum values of total protein in our experiments are almost equivalent to those obtained by Červinka (1973), which are regarded as the required physiological values between June and September for successful surviving rates. Similar small changes in total plasma protein were found by Hilge (1980) in carp of higher body weight, except that the higher values were observed during late winter and early spring when normal sexual maturation of carp occurs in the closed systems they lived in. We did not find significant differences in any biochemical parameters between scaly and mirror carp.

According to the extent of biochemical changes in the carp blood in our experiment, it can be concluded that the increase of glucose concentration in the blood of carp in the summer period of the second year is associated with an even more increased concentration of total lipids in the serum. This means that the intensive carbohydrate diets resulted in a higher conversion of carbohydrates to fat in the liver. The stable level of blood cholesterol during the intensive growth of carp can be favourable from the aspect of human nutrition.

Table 3

The concentrations of total lipids (TL), cholesterol (Ch), and total proteins (TP) in the blood serum and glucose (Gl) concentration in the blood plasma of carp aged two/three years

Parameters	Age of carp				
	2–3 years				
	June	September	February		
			Pond A	Pond B	
				Scaly	Mirror
Body					
Weight, g	211.33	829.00			
Length, cm	22.57	34.80			
Height, cm	7.40	12.28			
Biochemical values					
TL, g/L	8.50 ± 1.29	14.35 ± 2.95**	10.65 ± 0.97**	8.75 ± 1.75	7.28 ± 1.04
Ch, mmol/L	4.20 ± 0.65	3.98 ± 0.84	5.27 ± 0.27	4.95 ± 0.36	4.31 ± 0.81
TP, g/L	33.39 ± 6.44	32.76 ± 4.70	29.92 ± 3.29	30.14 ± 3.80	30.69 ± 2.08
Gl, mmol/L	4.70 ± 0.69	6.44 ± 1.15*	6.70 ± 0.64	5.75 ± 0.70	6.29 ± 0.38

Asterisks indicate statistically significant differences from the preceding value (the value in younger group): *$P < 0.05$; **$P < 0.01$

Histopathological findings in liver and kidneys were within normal limits in the majority of animals. Only sporadic cases had initial signs of hydropic and vacuolar degeneration in the liver and kidneys and fat infiltration in the liver.

The results suggest that the investigated haematological and biochemical tests could be successfully utilised for monitoring the metabolic balance and healthy status of fish in intensive culture. Since a sufficiently high correlation exists among haematological parameters on the one side and blood chemical parameters on the other, even at each level of age and body condition, the results show that incomplete blood analyses can be successfully performed as part of the necessary veterinary inspection on fish farms.

References

Adamek, Z. (1977): Changes of selected condition parameters during the overwintering of carp fingerlings in ponds. Manuscript VŠZ Brno. p. 12.

Anonymus (1975): Standard Methods for Examination of Water and Wastewater. 14th edition. APHA-AUWA-WPCF, Springfield.

Boyd, C. (1982): Water Quality Management for Pond Fish Culture. Elsevier, Amsterdam.

Černikova, V. V. (1974): Haematological characteristics of carp fry during the overwintering (in Russian). Leningrad, Izv. Gosniorch. **88,** 109–135.

Červinka, S. (1973): The possibilities of employment of differentiation of blood serum and total protein for physiological and pathological evaluation of fish condition. Manuscript State Fishery, pp. 45–47.

Fašaič, K. and Adamek, Z. (1993): Changes of blood picture in one- and two-year-old carp (*Cyprinus carpio* L.) during wintering. In: Vykusova, B., Svobodova, Z. and Machova, J. (eds) Ichthyohaematology. (Proceedings of the 3rd Ichthyohaematology Conference, Litomysl, Czech Republic, November 30 – December 2, 1993). Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic. pp. 15–18.

HACH (1992): Water Analysis Handbook. HACH Company, Loveland, Colorado.

Hilge, V. (1980): Long-term observation on blood parameters of adult mirror carp (*Cyprinus carpio* L.) held in a closed warm water system. Arch. Fisch Wiss. **31,** 41–50.

Hoffman, R., Lommel, R. and Riedl, M. (1982): Influence of different anaesthetics and bleeding methods on haematological values in fish. Arch. Fisch. Wiss. **33,** 91–103.

Jendrysek, S., Steinhagen, D., Drommer, W. and Körting, W. (1994): Carp coccidiosis: intestinal histo- and cytopathology under *Goussia carpelli* infection. Dis. Aquat. Org. **20,** 171–182.

Kent, M. L. and Hedrick, R. P. (1985): The biology and associated pathology of *Goussia carpelli* (Leger and Stankovitch) in goldfish *Carassius auratus* (Linnaeus). Fish Pathol. **20,** 485–494.

Kovacheva, N. P., Grozev, G. and Loshev, L. (1993): Influence of some ecological conditions on the physiological state of carp stocking material. In: Vykusova, B., Svobodova, Z. and Machova, J. (eds) Ichthyohaematology. (Proceedings of the 3rd Ichthyohaematology Conference, Litomysl, Czech Republic, November 30 – December 2, 1993). Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic. pp. 59–63.

Lehmann, J. and Stürenberg, F. J. (1981): The importance of blood parameters and methods for their determination in fish toxicology (in German). Fisch und Umwelt **9,** 25–39.

McDonald, D. G. and Millicam, C. L. (1992): Chemical properties of the blood. In: Hoar, W. S., Randall, D. J. and Farel, A. P. (eds) Fish Physiology, vol. **11**, Part B. The Cardiovascular System. Academic Press, San Diego. pp. 55–133.

Palačkova, J. (1991): The influence of abiotical factors on carp (*Cyprinus carpio* L.) fingerlings (in Czech). Autoref. kandid. disert. prace, VŠZ Brno. p. 27.

Prihoda, J. and Bienek, P. (1993): Reaction of blood protein to stress. In: Vykusova, B., Svobodova, Z. and Machova, J. (eds) Ichthyohaematology. (Proceedings of the 3rd Ichthyohaematology Conference, Litomysl, Czech Republic, November 30 – December 2, 1993). Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic. pp. 97–104.

Spurny, P. and Mareš, J. (1993): Changes in the haematological indicators of the carp fry of various genotypes during hibernation. In: Vykusova, B., Svobodova, Z., Machova, J. (eds) Ichthyohaematology. (Proceedings of the 3rd Ichthyohaematology Conference, Litomysl, Czech Republic, November 30 – December 2, 1993). Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic. pp. 118–124.

Svobodova, Z. (1986): The use of haematological methods in fishery practice: Sb. 1. Celostatni ichtyohaematol. Konf., Litomyšl 1985, pp. 14–19.