Seasonal Changes in Blood Parameters in Juvenile *Cyprinus carpio*

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Abstract

Temporal variations in the biochemical and haematological parameters of blood must be taken into account as it is a useful indicator for assessing the nutritional status, non-specific immunity and health of fish in general.

The aim of this study was to analyse changes in the biochemical and haematological profile of blood in common carp (*Cyprinus carpio*) depending on the environmental changes characteristic for each season. The lower mean haematocrit, identified in the spring, which correlates with the lower erythrocyte count, implies a more pronounced anaemia in carp after the winter period. The specimens analysed in spring and summer show an increase in the percentage of neutrophils by 60%, compared to the specimens analysed in autumn. Lower values for albumin in spring (0.65±0.22 g/dL) suggest a disruption of protein synthesis function in liver, which is not true for biological material during summer (0.90±0.60 g/dL), and autumn (0.95±0.25 g/dL). CK and CRE values are within normal limits throughout the study period, indicating no injury or muscle atrophy for the analysed specimens. These results suggest that seasonal changes in the environment cause temporary changes in the indicators of the biochemical and haematological profile of the blood of *Cyprinus carpio*.

Keywords: Biochemical indices of blood plasma, *Cypinus carpio;* haematological indices

1. Introduction

Scientists are increasingly interested in investigating the state of stress, defined as a modified behaviour of a fish that requires increased energy, triggers suffering, and occurs when a factor promotes physiological changes in an organism that may compromise the integrity of the body [1].

The response to stress in fish is achieved by stimulating the hypothalamus which results in the activation of the neuroendocrine system and a subsequent cascade of metabolic and physiological changes [2].

The responses appear as a consequence to the release of stress hormones [3], also causing changes in blood chemistry.

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The common carp, one of the freshwater fish with a significant economic value, widely cultivated, faces attacks disease that can occur and due to the modified environmental conditions besides the infestation with bacteria, fungi or parasites.

Haematological and biochemical indices provide information about oxygen transport capacity in fish, immune potential, stress level, disease, intoxication, nutritional status etc.

Temporal variations of blood biochemical parameters should be considered when these parameters are used to assess the health of fish.

The aim of this study was to analyse changes in the biochemical and haematological profile of blood in common carp (*Cyprinus carpio*), depending on the environmental changes characteristic for each season.

Such studies could provide predictions for the evolution of current populations and the establishment of rules for the conservation of native fish fauna.

2. Materials and methods

The study was carried out over one year, on fish material of the species *Cyprinus carpio* obtained in the Experimental Laboratory of Agro-Fisheries Research Brateș, Galați, in an earthen pond with an area of 5,000 m^2, with an average depth of 1.5 m, devoid of vegetation and populated with one year old fish. The population of the pond was achieved at the end of April 2020, at a water temperature of 10.2°C. According to the management in the fish ponds, the fish was harvested in September 2020 and introduced into a winter pond until April 2021.

Physical and chemical parameters of technological water

Portable multiparameter, model HQ40D - Hach, was used to measure pH, temperature and dissolved oxygen.

Nitrogen compounds (ammonia, ammonium ions, nitrite ions and nitrate ions), calcium, magnesium, total hardness, carbonates, bicarbonates, alkalinity and chloride were determined spectrophotometrically according to Standard Methods for the Examination of Water and Wastewater, 2005, with a spectrophotometer type DR 1900 from Hach Lange using LANGE kits.

The determination of chemical oxygen consumption (CCO-Mn) was performed by the method with potassium permanganate, expressed in mg $KMnO_4$/L, according to the standard SR ISO 6060: 1996. A mineralizer, model LT 200 from Hach Lange, was used to determine the organic matter.

Haematological analysis

To study the haematological parameters, blood was collected on anticoagulant (EDTA - etilen-diamino-tetraacetic-acid), through puncturing the caudal vein.

To determine the number of erythrocytes, which were expressed as millions of cells in one µl blood (mil/µL), a Newbauer haemocytometer by direct microscope numbering was used, haemoglobin concentration (Hb, g/dl) was determined by Sahli colorimetric method, and haematocrit value (PCV, %) was determined by the microhaematocrit method with a Hettich Haematockit 210 centrifuge.

Mean Corpuscular Volume (MCV, μm^3), Mean Corpuscular Haemoglobin (MCH, pg) and Mean Corpuscular Haemoglobin Concentration (MCHC, g/dL) were determined as follows:

MCV=Hct×10/no. erythrocyte;

MCH=Hb×10/ no. erythrocyte;

MCHC=Hb×100/Hct.

In order to determine the biochemical indicators, the serum samples were passed through the fully automatic On-site analyser type Celercare VS.

For the calculation of Viscerosomatic index (VSI) and Hepatosomatic index (HSI) we used the following equations:

Viscerosomatic index (VSI)=total wet viscera (g) / body weight (g) × 100

Hepatosomatic index (HSI)=wet hepatopancreas weight (g)/wet body weight (g) × 100

Statistical analysis

All haematological determinations were performed on samples from 10 specimens. Data are presented as mean±standard deviation (SD). The comparison of several samples was done using the ANOVA test - Single factor followed by T test. The differences were considered significant at $p<0.05$.

3. Results and discussion

The average weight of the biological material at the population of the pond (W) was 15.37±7.55 g/specimen with a dominance of 76.5% for specimens with minimum weight.

The nutrient concentrations in the fodder administered during the period when the temperature allowed the development and consumption of food, are in accordance with the recommendations from literature for the carp species (*Cyprinus carpio*) and the age of one year, respectively 31.38% protein, 11.61% fat, 9.89% moisture and 6.25% total ash [4-5].

Physico-chemical analysis of water

The evaluation of the physico-chemical parameters of water was done weekly, in order to monitor the aquatic environment and the possibility to intervene in case of deviation of the water quality conditions from the allowed limits (Table 1).

Throughout the year, the water temperature, which is influenced by the air temperature, was higher than the normal values for each season. The average values of water pH recorded values

between 8.22±2.15 UpH and 8.83±0.95 UpH, imprinting its alkaline character in the water. Organic substances recorded values above the optimal limit of 60 mg $KMnO_4$/L, in the summer season (79.52±5.25 mg $KMnO_4$/L). Even in the winter season, the organic substances remained at a high value due to higher-than-normal temperatures during the hibernation season. Dissolved oxygen concentration decreases during the summer to 5.20±0.85 mg/L.

Table 1. Evolution of physico-chemical parameters of water in the growing and wintering pond, throughout the study period (values are expressed as Average±SD*)

Chemical parameter	UM	Year 2020			Year 2021
		June, July, August	Sept., Oct., November	Winter period	After wintering
Water temperature	˚C	27.20±5.38	20.33±4.25	2.6±5.85	12.83±3.79
Ph	uPh	8.22±2.15	8.50±2.02	8.83±0.95	8.40±1.45
Dissolved oxygen	mg/l	5.20±0.85	6.3±1.11	5.80±1.15	6.69±2.05
Organic matter	mg $KMnO_4$/l	79.52±5.25	64.62±6.64	50.5±9.85	63.2±11.33
Nitrites, ($N\text{-}NO_2$)	mg/l	0.11±0.02	0.03±0.02	0.035±0.01	traces
Nitrates, ($N\text{-}NO_3$)	mg/l	1.18±0.15	1.12±0.20	0.95±0.09	0.085±0.001
Ammonia	mg/l	0.021±0.002	0.02±0.001	0.02±0.001	0.05±0.002
Ammonium, ($N\text{-}NH_{4+}$)	mg/l	0.29±0.03	0.77±0.025	0.55±0.02	0.15±0.03
Calcium (Ca^{2+})	mg/l	36.00±2.56	50.00±3.50	55.00±2.85	70.00±6.15
Magnesium (Mg^{2+})	mg/l	39.60±2.25	43.20±3.65	45.00±4.71	45.60±3.08
Ca^{2+}/Mg^{2+}		0.91±0.05	1.16±0.40	1.22±0.30	1.54±0.25
Total hardness	dGH	14.90±2.20	17.10±3.63	17.50±4.00	19.07±3.76
Carbonates $(CO_3)^{2-}$	mg/l	251.03±58.76	214.00±24.25	251.00±25.25	287.33±51.86
Bicarbonates $(HCO_3)^-$	mg/l	-	-	-	-
Alkalinity	ml	4.18±0.96	3.72±0.19	3.55±0.25	4.90±0.95
Chloride (Cl^-)	mg/l	118.08±22.31	127.65±19.52	130.25±26.33	47.87±11.65

* Standard deviation

For the rest of the parameters, there were no deviations of the physico-chemical parameters of the water outside the optimal ecart.

The water quality was within the limits allowed and recommended for the waters used in fish farming, being in the second and third quality class, in accordance with the provisions of Ord. MMGA no. 161/2006.

Haematological profile of the blood

The determination of haematological and serological parameters can give important indications on how to capitalize on food and resistance to medial factors. In this way, prompt and effective measures can be taken to remove the limiting factors that slow down the growth or cause disease and death of fish.

Blood samples were taken from a batch of 10 specimens each month, in summer, before and after the wintering of the biological material (Table 2). The haematocrit varied in the analysed fish between 22.5% (spring) - 50% (autumn), registering in the carp specimens analysed in spring an average value of 26.57%, in those analysed in summer an average value of 30.60%, and in those analysed in autumn an average value of 42.21%. The lower mean haematocrit, identified in spring, which correlates with the lower erythrocyte count, implies a more pronounced anaemia in carp out of winter.

Compared to the literature that mentions for healthy carp values between 36% and 40%, the lower haematocrit in the fish we researched is an additional argument for the existence of anaemia even in the apparently healthy material.

The total number of erythrocytes varied between the minimum value of 0.55 mil./µL, identified in spring and the maximum value of 1.75 mil./µL identified in autumn. The average values being 0.76±0.18 mil./µL for the carp specimens analysed in spring, 0.98±0.27 mil./µL for those analysed in summer and 1.34±0.23 mil./µL for those analysed in autumn.

According to Ghitino, 1983 [6], the values of erythrocytes for healthy carp fall in the range 1.10-2.20 mil./µL. Comparing the data of our research with those in the literature, we can appreciate that the number of erythrocytes in the blood of fish examined in autumn is within limits, those researched in summer are at the lower end of

the range, while the specimens examined in spring have values below the limit identified by Ghitino, 1983 [6], the biological material being characterized by a slight anemia. The haematological examination also includes the analysis of erythrocyte indices, that describe the morphology and properties of erythrocytes, such as MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration).

The increase in MCV after the winter period may be a response to the anaemic condition of *Cyprinus carpio*.

Table 2. Variation of haematological indicators (values are expressed as Average±SD *, n=10)

Parameters	UM	Year 2020		Year 2021
		Summer	Before wintering	After wintering
Haematocrit (Ht)	(%)	30.60±2.59	42.21±4.92	26.57±.21
Haemoglobin (Hb)	(g/dL)	8.09±0.24	9.78±0.82	7.71±0.94
Erythrocyte (RBC)	(mil/µL)	0.98±0.27	1.34±0.23	0.76±0.18
Mean Corpuscular Volume (MCV)	(fl)	334.71±93.48	325.73±77.29	361.42±73.30
Mean Corpuscular Haemoglobin (MCH)	(pg)	88.58±24.82	74.72±12.75	105.69±26.37
Mean Corpuscular Haemoglobin Concentration (MCHC)	(g/dL)	26.58±2.15	23.41±3.11	29.08±3.27

* Standard deviation

The values identified in this study, which defines the haematological profile of blood in 1-year-old carp, are similar to those identified by Bocioc E. et al., 2015 [7], while studying the haematological profile of juvenile carp (*Cyprinus carpio*, L. 1758) reared in a recirculating system and fed probiotic supplements.

In carp after the wintering period, the number of leukocytes varied between 75 and 124 × 10^3/µL, in the summer season the number ranged from 81 to 166 × 10^3/µL and in autumn the number ranged from 66 and 115 × 10^3/µL (Table 3).

There is an increase in the number of leukocytes in the analysed specimens in a summer month.

In the leukocyte formula, in the specimens analysed in spring and summer, compared to the specimens analysed in autumn, there is an increase in the percentage of neutrophils by 60%.

Table 3. Variation of the white blood cell absolute number (WBC) at the start of the experiment (values are expressed as Average±SD*, n=10)

Parameters	UM	Year 2020		Year 2021
		Summer	Before wintering	After wintering
WBC	(×10^3/µL)	110.90±26.23	91.20±15.65	99.60±17.81
Min	(×10^3/µL)	81	66	75
Max	(×10^3/µL)	166	115	124
Lymph	(%)	77.91±6.11	82.71±7.24	79.93±8.39
Mon	(%)	3.56±0.82	4.16±1.00	1.91±0.89
Neu	(%)	16.44±3.57	10.24±2.43	16.12±2.88
Eos	(%)	1.01±0.57	1.54±0.76	0.89±0.45
Baso	(%)	1.08±0.20	1.35±0.31	1.15±0.52

* Standard deviation

There are authors [8] who mention changes in the number of leukocytes in fish depending on sex, season, feeding habits, stress, water pollution and disease.

Our results confirm a seasonal influence on the leukocyte formula.

However, no significant differences were identified in the specimens examined during the same season and kept under the same conditions in the same experimental laboratory pond.

Biochemical profile of blood plasma

Biochemical parameters are studied to evaluate the physiological systems of biological material.

The results of the biochemical indices from blood plasma are presented in Table 4.

From the examination of the table with biochemical indicators it can be seen that the average value of total serum proteins, which should register values of 2.625 g/dL (normal values according to Ahmed and Ali, 2013) [9],

increases from 2.30 g/dL when the fish is taken out of winter, to 2.85 g/dL in summer and 2.95 g/dL, value characteristic of the autumn season.

Fish, when exposed to stress, can suffer from oxygen deficiency that can affect the protein content in the body [10], which is not the case in our experiment, the temperatures in winter were higher-than-normal, without ice bridges on the surface of the pond. Reducing protein levels over the winter may indicate inhibition of protein synthesis due to hyperactivity of the biological material in the winter season with high temperatures and lack of food.

The two protein components, albumin that prevents the leakage of fluids from blood vessels, and globulin with an extremely important role in maintaining the health of the immune system, have lower values when the biological material comes out of winter (0.65 g/dL, respectively 3.39 g/dL), compared to the values identified in summer (0.90 g/dL, respectively 1.95 g/dL) and in autumn (0.95 g/dL, respectively 2.00 g/dL).

Table 4. The values of biochemical analyses of blood serum (values are expressed as Average± *Standard deviation*)

Parameters		UM	Year 2020		Year 2021
			Summer	Before wintering	After wintering
Total serum protein	TP	g /dL	2.85±1.11	2.95±0.55	2.30±0.32
Serum albumin	ALB	g/dL	0.90±0.60	0.95±0.25	0.65±0.22
Globulin	GLO	g/dL	1.95±0.75	2.00±0.17	1.65±0.31
Albumin / globulin ratio	A/G	-	0.46	0.48	0.39
Calcium	Ca	mmol/l	2.05±0.36	2.15±0.22	1.56±0.43
Serum glucose (blood glucose)	GLU	g/dL	3.70±1.10	3.50±0.35	3.90±0.54
Serum phosphorus	P	g/dL	2.55±0.55	2.45±0.40	2.15±0.35
Total cholesterol	CHOL	g/dL	1.22±0.75	1.20±0.16	1.42±0.74
Alanine aminotransferase	ALT	U/L	50.25±4.01	35.21±2.56	55.34±3.65
Aspartate aminotransferase	AST	U/L	69.42±8.55	64.22±5.74	99.85±7.85
Alkaline phosphatase	ALP	U/L	42.33±4.46	55.12±3.33	33.45±5.50
Serum creatinine	CRE	mg/L	0.85±0.08	0.80±0.05	1.01±0.45
Creatine kinase	CK	U/L	2.15±0.75	2.20±0.45	2.09±0.60

The level of albumin and globulin identified by Mohammad & Bashi, 2020 [11], which included spirulina in common carp food, is twice as high as those identified in this study.

The albumin/globulin ratio is at the limit (0.39) for carp taken out of winter and above the limit of 0.3 which is a significant adaptation of the body to the living conditions provided during the summer until autumn.

Lower spring albumin values (0.65±0.22 g/dL) suggest a disruption of liver protein synthesis function which is not true for biological material in summer (0.90±0.60 g/dL), and in autumn (0.95±0.25 g/dL).

Glucose is an important parameter used to determine normal body function in toxicology studies [12].

There is a declining trend in glucose levels after the wintering period of biological material from 3.90±0.54 g/dL, to 3.70±1.10 g/dL and 3.50±0.35 g/dL, but the differences are insignificant ($p>0.05$). The values in this experiment are lower than those identified by Nazish Iftikhar and Imran Hashmi, 2020 [13], in the control group, studying the haematological and biochemical response of the species *Cyprinus carpio*, which went through prolonged exposure to antibiotics.

The concentration of ALT and AST decreased during the period when the temperature was optimal for food development and consumption, respectively 26-27˚C and increased in the winter season when fish metabolism shows the lowest values due to the body temperature that follows that of the environment.

The values are comparable to those identified by Mohammed Shaker Al-Khshali and Hasan Ali Al Hilali, 2019 [14], studying changes caused by high salinity concentrations in *Cyprinus carpio.*

Temperature rises during winter result in interruption of hibernation and the more or less active search for food.

When in such situations, the carp does not have enough food, it loses a lot of weight (5-20%) and is exposed to mortality due to diseases. In general, the warmer the winter, the higher the weight loss of the carp. Higher levels of Alkaline phosphatase (ALP) during the summer and autumn may be due to the higher concentration of chlorides found in the pond during this period, the alkaline

phosphatase (ALP) enzyme being one of the important enzymes in the osmoregulation process.
CK and CARE values are within normal limits, indicating no lesions or muscle atrophy for the specimens analysed.
Viscerosomatic index (VSI) and Hepatosomatic index (HSI) were measured after winter and during the subsequent vegetation period. During hibernation, the Viscerosomatic index (VSI) increased by up to 38.57% and the Hepatosomatic index (HSI) increased by up to 20.00%. It was found that VSI and HSI are influenced by water temperature and season (Table 5). Statistically, the results on the hepatosomatic index (HSI) showed that there were no significant differences over the monitored period ($p>0.05$).
Regarding the viscerosomatic index (VSI) there are at least two averages that differ significantly $p<0.05$. Significant differences are before and after the wintering period of the one year old carp.

Table 5. Viscerosomatic index (VSI) and Hepatosomatic index (HSI), values are expressed as Average±SD*

Parameters (%)	After wintering	Summer	Before wintering	P-value
Viscerosomatic index (VSI)	13.65±2.08	10.25±2.60	9.85±2.47	0.00238
Hepatosomatic index (HSI)	4.60±1.21	4.22±1.58	3.85±0.75	0.366412

* Standard deviation

4. Conclusions

Haematological and biochemical analysis of blood is an important practical and useful tool for assessing the physiological and health status of fish in fish farms.
The observed changes suggest that some parameters are sensitive to environmental changes and provide information on physiological disorders, prior to the onset of their external symptoms.
Results indicate a seasonal influence of haematological parameters on *Cyprinus carpio*.

References

1. Barreto, R. E. & Volpato, G. L., Stress responses of the fish Nile tilapia subjected to electroshock and social stressors. Brazilian Journal of Medical and Biological Research, 2006, 39, 1605-1612
2. Lowe, C. J., Davison, W., Plasma osmolarity, glucose concentration and erythrocyte responses of two Antarctic nototheniid fishes to acute and chronic thermal change, Journal of Fish Biology, 2005, 67, 752-766
3. Bruce, A. Barton, George, K. Iwama, Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids, Annual Review of Fish Diseases, 1991, 1, 3-26
4. Ghittino, P. Tecnologia e Patologia in Aquacoltura, 1983. vol. I, Ed.Bono, Torino
5. Bocioc E., Cristea V., Patriche N., Grecu I., Antache A., Creţu M., Hematological Profile of the Juvenile Carp (Cyprinus carpio, L. 1758) Reared into a Recirculating Aquaculture System with Probiotics Supplement, Bulletin UASVM Animal Science and Biotechnologies, 2015, 72(1), 8-14
6. Ahmed, Z. I. Sheikh, A., Comparative study of hematological parameters of snow trout *Schizopyge plagiostomus* and*Schizopyge niger* inhabiting two different habitats, Eur. Zool. J., 2020, 87(1), 12-19
7. Ahmed, S. M., Ali, A. H., Serum proteins and leucocytes differentialcount in the common carp (*Cyprinus carpio* L.) infested with ectoparasites, Mesopot. J. Mar. Sci., 2013, 28(2), 151–162
8. Ramesh, M., Thilagavathi, T., Rathika, R., Poopal, R. K., Antioxidant status, biochemical, and hematological responses in a cultivable fish Cirrhinus mrigala exposed to an aquaculture antibiotic Sulfamethazine. Aquaculture, 2018, 491, 10–19
9. Mohammad, M. A. Qasab Bashi, W. A., Effect of Partial Substitution Spirulina Instead Ofsoybean Meal in Common Carp *Cyprinus Carpio l.* Diet on Some Bloodpicture Andsome Biochemical, Criteria, Iraqi Journal of Agricultural Sciences, 2020, 51(6), 1740-1746
10. Vutukuru, S. S., Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton), Bulletin of Environmental Contamination and Toxicology, 2003, 70(1), 0118-0123
11 Nazish Iftikhar, Imran Hashmi, Assessment of immunohematological, hematolog cal and biochemical responses in cultivable fish *Cyprinus carpio* exposed to an antibiotic sulfamethoxazole (SMX), J Water Health, 2020, 19(1), 108–119
12. Mohammed Shaker Al-Khshali and Hasan Ali Al Hilali, Some physiological changes of common carp caused by high salinity, Biochem. Cell.Arch., 2019, 19(2), 4605-4610