

INFLUENCE OF THERMAL AND PARASITARY STRESS ON THE ERYTHROCYTARY HEMOGLOBIN (INDEX M) IN SOME CULTURE CYPRINIDS

C. Misăilă, Elena Rada Misăilă, Gabriela Dumitru

„Alexandru Ioan Cuza” University, Faculty of Biology, Iași, Romania
e-mail: cmisaila@uaic.ro

Abstract

The paper analyzes the variation of the *M* index values ($Hb \times 10^6/E$) in common carp, silver carp, bighead carp and grass carp during the winter season, as well as a function of the anti-parasitary treatments applied. The results of the polyculture growth experiments on 1 year-old common carp, silver carp and bighead carp (C_{1+}) in preventively antiparasitary-treated ponds are discussed, along with those referring to wintering under severe hypothermal and over-density conditions of some 2 year-old common carp, silver carp and grass carp (C_{2+}), respectively wintering under over-density conditions (15 t/ha) of some batches of 1 summer-old common carp, silver carp and bighead carp (C_{0+}) under mild hypothermal conditions.

In the first experiment, the ponds were prophylactically treated, both during populating operations (April), and during growing with 0.1 mg/l trichlorphon, in two stages, and with 2 kg/ha calcium chloride, twice a week, while the hematological evaluation of the effects was made on samples taken over (6 ex./batch) both in the end of the growing season (November) and in the following spring (April).

The results obtained show that the values of index *M* are higher in the autochthonous species - the common carp (6.43 - 5.59), comparatively with the grass carp (6.18), bighead carp (4.83 - 6.04) and silver carp (4.43 - 4.37). The “echo” beneficial effect of the anti-parasitary treatments is more clearly evidenced by index *M*, comparatively with the hemoglobin values. Thus, in April, the *Hb* values of the treated batch were 16.7% higher than those of the reference, while index *M* was 35.5% higher in common carp and 4.8 - 12.8% higher in the case of silver carp and bighead carp, respectively.

Under severe wintering conditions, the erythropoiesis yield visibly decreases, so that the value of index *M* in April is 41.5% higher in common carp and 18.1% higher in silver carp - comparatively with the reference moment (autumn) -, unlike the values obtained under mild wintering conditions, when the levels of index *M* are comparable with the autumn ones in common carp (104.8%) and silver carp (101.8%) while, in the case of bighead carp and, respectively, grass carp, the values remain inferior to those of the reference.

Key words: hemoglobin, Index M, cyprinids, hypothermy, antiparasitary treatments

INTRODUCTION

Similarly with the case of higher vertebrates, the structural and functional integrity of the sanguine tissue in fish is viewed as a criterion which may estimate both the abundance and quality of the available food and the extent of physiological comfort, or even the level of stress in the fish from an aquatic basin [3, 4, 6, 7]. From this perspective, of special significance for the evaluation of the extent of functional prosperity of the organisms and of the

severity of anemia, respectively, appear both the values of sanguine hemoglobin and the mean number of erythrocytes, and the values of the hematocyte, viewed as bearer of a stress-related hematological message.

As early as the second half of the last century, some authors [5] assessed a direct correlation between the values of hemoglobin and the number of erythrocytes and the specific alimentary regime of fish. Consequently, in the case of omnivorous fish, the authors observed that index *M* ($Hb \times 10^6 /$

E) takes higher values than in predatory fish. Previous researches of ours, performed on some fish species from the natural fauna of the Ezăreni accumulation lake, district of Iași [2], showed that the values of index M are 58-63% higher in the representatives of some omnivorous species (Rudd, roach, common carp, Prussian carp), comparatively with the carnivorous ones (pike and perch).

The present paper analyzes the values of index M recorded in three separate experiments, developed on culture cyprinids of different ages (common carp, silver carp, bighead carp and grass carp), under different wintering conditions, and also as a function of the applied prophylactic antiparasitary treatments.

MATERIALS AND METHOD

In the experiment performed at the Research - Development Station for Aquaculture and Aquatic Ecology of Iași, the groups of 1 year-old common carp, bighead carp and silver carp (C_{1+}) were placed in ponds and grown in polyculture, preventive antiparasitary treatments being applied. Thus, in the "treated variant", the pond had been prophylactically treated, both in the moment of its filling (April) and during the growing season, with 0.1 mg/l trichlorphon, in two stages, and with 2kg/ha calcium hypochlorite, twice a week, while hematological evaluation of the samples was made on the samples taken over (6 ex. / species / variant), both in the end of the growing season (November) and in the following spring (April).

The experiments made at the Piscicultural Farm of Țigănași involved a comparative analysis of the behavior of cyprinid batches subjected to different wintering conditions. On one hand, wintering of some groups of 2 year-old common carp, silver carp and grass carp (C_{2+}), under severe hypothermal (ice bridge thicker than 25 cm, for about 100 days) and over-density conditions (20 t/ha) was followed and, on the other, wintering in over density (15t/ha) of some batches of 1 summer-old common carp, silver carp and bighead carp (C_{0+}), under mild hypothermal

conditions. Both experiments investigated the hematological behavior of fish, on samples of peripheric blood (6 ex. / species / variant) taken over both prior to and after the wintering season.

Hemoglobin concentration (g Hb/dl blood) was dosed by the usual colorimetric method, with the Sahli hemoglobinmeter, while the mean number of erythrocytes (E) was determined by reading of the diluted blood samples on a ML-4 microscope, with the Bürker-Türk hemocytometer, the results obtained being expressed in millions of erythrocytes/ μ l blood [1].

RESULTS AND DISCUSSION

Application of the prophylactic antiparasitary treatments had beneficial effects, both upon the survival levels of fish and upon the growth rhythm and yield of food bioconversion (aspects not approached in the present study). As to the functional response of fish to the applied treatments, mention should be made of both an immediate effect, expressed by the tendency of additional hematological prosperity in the treated fish - comparatively with the non-treated ones - during sampling performed in the end of the growth season (November), and of an "echo" effect, evidenced during sampling in the following spring (April).

Both the values of hemoglobin and of the erythrocyte number put into evidence this tendency, even if some differences may be observed from one species to another (Table 1). Thus, the spring Hb level in the treated variant is 17% higher than in the reference (non-treated) in the case of common carp, and 19% higher in silver carp. On the other hand, in treated fish, the "echo" values of April are higher than the autumn ones - with 14% in common carp and 5% in silver carp, respectively while, in the non-treated variant, the values are lower than the November ones with 15% in common carp and 18% in silver carp, respectively, the values recorded for the bighead carp evidencing a reverse evolution (comparatively with common carp and silver carp).

Table. 1. Hemoglobin and erythrocytes number under conditions of antiparasitary treatments application

Species	Month	Variant	Hemoglobin			Erythrocytes number		
			g/dl	%		x 10 ⁶ /μl	%	
Common carp	November	Treated	7.76	89	100	1.3	88	100
		Non-treated	8.68	100	100	1.47	100	100
	April	Treated	8.82	117	114	1.5	86	115
		Non-treated	7.56	100	85	1.74	100	118
Silver carp	November	Treated	8.62	93	100	1.58	61	100
		Non-treated	9.32	100	100	2.61	100	100
	April	Treated	9.04	119	105	2.09	113	132
		Non-treated	7.62	100	82	1.85	100	71
Bighead carp	November	Treated	9.44	133	100	1.67	111	100
		Non-treated	7.12	100	100	1.51	100	100
	April	Treated	9.21	98	98	1.94	87	117
		Non-treated	9.44	100	133	2.24	100	148

As to the number of erythrocytes, the recorded values show some deviations from the tendency observed for hemoglobin, as follows: in both variants, the spring values are higher than the autumn ones, with 15-18% in common carp and 17-48% in bighead carp, respectively while, in silver carp, a 32% “echo” increase occurs only in treated fish.

As index M includes both the prosperity tendency and hemoglobinemic insufficiency, and the tendency referring to the erythrocyte number, this parameter is expected to express more faithfully the effects of the experimental variables under analysis, comparatively with the extent of expressivity of the two indices, considered separately.

Indeed, according to the values of index M (Fig. 1), the additional vigour of common

carp, as an autochthonous species, comparatively with the Asian cyprinids, results from the fact that the “eco” values of Hb in spring are 16.7% higher in treated fish, comparatively with the non-treated ones, while those of index M are 35.5% higher, *i.e.* much higher than those of silver carp (+4.8%) or bighead carp (+12.8%). More than that, the April Hb values in treated silver carp are 19% higher than those of the reference (Table 1), however index M is only 5% higher than the reference (Fig. 1) and, in the bighead carp, the Hb value is comparable, even 2% lower than in the reference, while, in the treated fish, index M is 13% higher than in the non-treated ones.

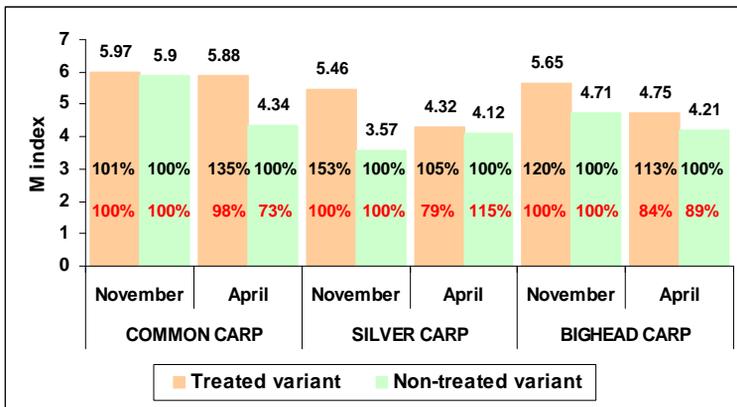


Fig.1. M index under conditions of antiparasitary treatments application

The data illustrated in Figure 1 permit the conclusion that, in all three species, the values of index M are - in both autumn and spring time - constantly higher in the variant subjected to antiparasitary treatments, comparatively with those recorded in non-treated fish, while the values of hemoglobin and the number of erythrocytes (Table 1) are fluctuating.

On the average, the values of index M resulted from all our determinations are higher in grass carp (6.18) and common carp (6.01), followed by bighead carp (5.43) and

silver carp (4.4), the maximum value being recorded - in the spring following the severe wintering - for common carp (7.54).

The evolution of index M in the groups of cyprinids analyzed during wintering shows that starvation, thermal stress and over-density induce a pronounced hematological insufficiency (Table 2), especially under severe hypothermal conditions, when the average hemoglobin level decreased - comparatively with the autumn one - with 26.4% in common carp, 11.8% in silver carp and 16.7% in grass carp, respectively.

Table.2. Hemoglobin and erythrocytes number under severe wintering conditions

Species	Season	Hemoglobin		Erythrocytes number	
		g/dl	%	$\times 10^6/\mu\text{l}$	%
Common carp	Autumn	10.6	100	1.99	100
	Spring	7.8	73.6	1.035	52
Silver carp	Autumn	9.3	100	2.34	100
	Spring	8.2	88.2	1.75	75
Grass carp	Autumn	10.2	100	1.46	100
	Spring	8.5	83.3	1.585	109

Besides inhibiting hemoglobin-synthesis, the above-mentioned experimental conditions also induce some depletion of erythropoiesis, *i.e.* reduction, in the cold season, of the erythrocyte number with 48% in common

carp and 25% in silver carp while, in the case of grass carp, the mean number of erythrocytes in spring time is 9% higher than in the reference.

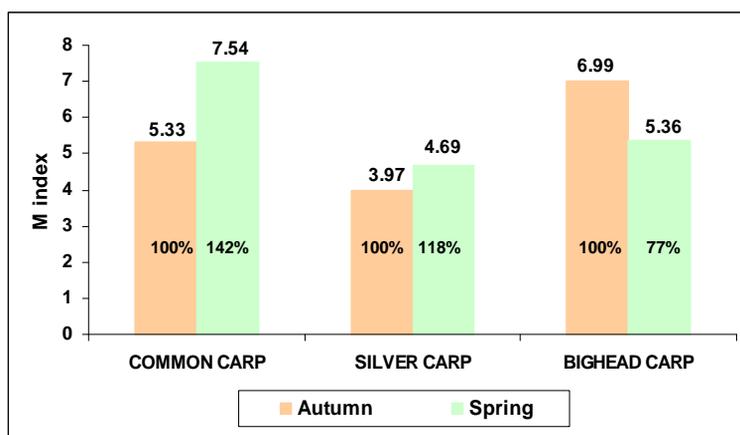


Fig.2. M index under severe wintering conditions

Consequently, the values of index M increase during winter (Fig. 2), exceeding the autumn levels with 42% in common carp and 18% in silver carp, under sever hypothermy, and with 4.8% in common carp and 1.8% in

silver carp, respectively, under mild wintering conditions (Table 3, Fig. 3) while, in the case of bighead carp and grass carp, the values recorded are inferior to those of the reference.

Table.3. Hemoglobin and erythrocytes number under mild wintering conditions

Species	Season	Hemoglobin		Erythrocytes number	
		g/dl	%	x 10 ⁶ /μl	%
Common carp	Autumn	8.47	100	1.551	100
	Spring	8.6	102	1.503	97
Silver carp	Autumn	11.5	100	2.67	100
	Spring	11.5	100	2.615	98
Grass carp	Autumn	10	100	1.525	100
	Spring	11.5	115	2.086	137

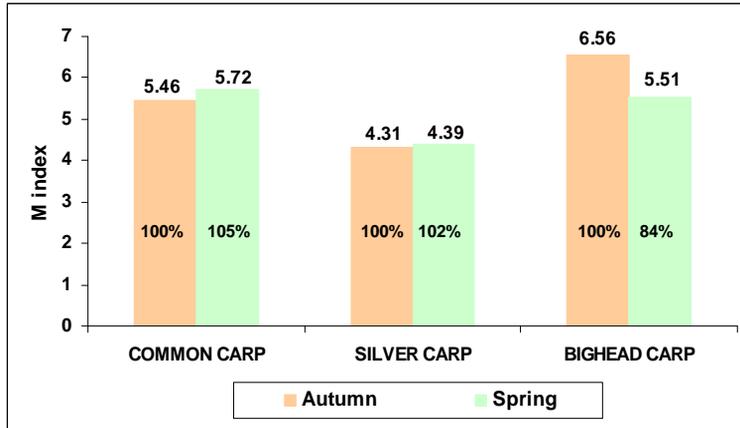


Fig.3. M index under mild wintering conditions

CONCLUSIONS

1. Index M, a hematological constant calculated from the values of hemoglobin concentration and erythrocytes number, expresses faithfully the functional condition of an organism, comparatively with the image provided by the two parameters considered separately.

2. Under the applied experimental conditions, the values of index M oscillated between 6.18 in grass carp and 6.01 in common carp, followed by those of bighead carp (5.43) and silver carp (4.4).

3. Both prior to and after wintering, the values of index M recorded in common carp, silver carp and bighead carp are constantly higher in the group subjected to antiparasitary treatments, comparatively with those registered for non-treated fish.

4. The physiological discomfort caused by the severe wintering conditions is most faithfully expressed by the values of index M, which increase with 42% in common carp and 18% in silver carp while, during mild wintering, such increases are 10 times lower (4.8% in common carp and 1.8% in silver carp).

REFERENCES

- [1] Misăilă C., Comănescu Gianina: Elemente de hematologie generală, Ed. Corson, Iași, 1999.
- [2] Misăilă C., Misăilă Elena Rada, Comănescu Gianina: Conținutul de hemoglobină al eritrocitului (indicele M) la unele specii de pești din lacul de acumulare Ezăreni, USAMV Iași, Lucr. Șt., Seria Zootehnie, 2005, 48: 616-621.
- [3] Misăilă C., Misăilă Elena Rada, Comănescu Gianina: Haematological changes in some farming cyprinids under the hibernation conditions, Scientific Papers Animal Sciences and Biotechnologies, Ed. Agroprint Timișoara, 2008, 41 (2): 81 - 87.
- [4] Misăilă C., Misăilă Elena Rada, Dumitru Gabriela: Influence of prophylactic antiparasitary treatments on some hematological indices values in cultured cyprinids, Lucrări Științifice, Seria Zootehnie, U.S.A.M.V. Iași, Ed. „Ion Ionescu de la Brad” Iași, 2010, 53 (15): 581 - 587.
- [5] Molnar G., Tamassy E.: Study of the haemaglobin content of a single erythrocyte (Mindix) in various cultured fish species, J. Fish. Biol., 1970, 2: 167 - 171.
- [6] Murad A., Houston A. H., Samson L.: Haematological response to reduced oxygen carrying capacity, increased temperature and hypoxia in goldfish (*Carassius auratus* L.), J. Fish. Biol., 1990, 36: 289-305.
- [7] Pop Al., Marcus I., Onețiu O.: Aspecte hematologice și biochimice sanguine la păstrăvii infectați cu *Hexamitus salmonis*, Bul. Univ. Șt. Agric., Seria Zootehnie și Med. Vet. Cluj-Napoca, 1994, 379-383.