INFLUENCE OF PROPHYLACTIC ANTIPARASITARY TREATMENTS ON SOME HEMATOLOGICAL INDICES VALUES IN CULTURED CYPRINIDS

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Abstract

The paper analyzes the hematological response of some one year-old cultured cyprinids, namely: common carp (Cyprinus carpio), silver carp (Hypophthalmichthys molitrix) and bighead carp (Aristichthys nobilis), grown in polyculture in 0.5 ha ponds, as part of an experiment (including some antiparasitary treatments) developed between April 2007 - April 2008. The ponds have been prophylactically treated in both the moment of their filling (April 2007) and during the fish growing period with preventive doses of 0.1 mg/L trichlorfon, in two steps, and 2 kg/ha calcium hypochlorite, two times/week. The mean concentration of hemoglobin (g Hb/dL blood), the ratio of figurative elements - i.e., the hematocryte (% Ht) and the number of erythrocytes (mil./µL), as well as the values of the erythrocytary constants (MCV, MCH, MCHC) - were determined both in November 2007 and in April 2008. In the end of wintering, the treated fish evidenced a more prosperous physiological condition, comparatively with the reference, the hemoglobin values recorded being higher with 17% in common carp and 19% in silver carp, as well as with 16% upper hematocryte values in common carp, 13% in silver carp and 10% in bighead carp. In the case of common carp, the number of erythrocytes registered in spring is 15-18% higher than the autumn values, 17-48% higher in bighead carp and 32% higher, respectively, in the treated silver carp.

Key words: cyprinids, antiparasitary preventive treatments, hematological indices

INTRODUCTION

The condition of chronic stress, as well as that of malady, appears as almost inevitable during growing of some culture fish species under semi-intensive and intensive conditions. In the case of fish grown in ponds, any perturbation of the environmental conditions - the ones referring to the quantity and quality of the available food and to the proliferation of certain parasites - may become - as a function of its seriousness and duration of action - a factor of stress [1, 4, 5, 6, 7, 8]. The manifestation, in the living environment of fish, of such elements, favourizing the installation of stress - the parasitary-type one, included - is faithfully reflected in the structure of the hematological picture by a multiple insufficiency, marked by the decrease of both hemoglobin and hematocryte concentration, and of the red cells.

Consequently, the condition of stress may be quite easily diagnosed on the basis of the main hematological indices. The behavior of the animal organisms subjected to stress begins with the alert phase, followed by a period along which the fish tries to get adapted to the atypical environmental conditions. Such adaptation of the fish to persistent stress is extremely complex, involving a physiological plane, as well. Some of the mechanisms of physiological adaptation, such as an increased efficiency of the breathing function, may be elucidated by the values of the hematological indices or of the derived erythrocytary constants [2, 3].

The present paper analyzes the hematological response of some one year-old cyprinids: common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), grown in polyculture in ponds, subjected to some preventive antiparasitary treatments. The hematological response of the fish to such treatments was followed by comparative analyzes on the blood collected both in the end of the warm season and in the end of the 2007-2008 winter.

MATERIALS AND METHOD

The investigations were performed at the Research and Development Station for Aquaculture and Aquatic Ecology of Iaşi, between April 2007 and April 2008. The fish batches were put in 0.5 ha ponds, each basin being populated with a mixture of 79% common carp (245 g/piece), 11% silver carp (475 g/piece) and 10% bighead carp (425 g/piece).

In the reference, the experiment involved no antiparasitary treatments while, in the experimental variant, the basin was prophylactically treated both in the moment of its filling (April 2007) and during the growing period, with 0.1 mg/L preventive doses of trichlorfon, in two stages, and 2 kg calcium chloride, twice a week.

Fish feeding was made with concentrated granulated fodder, according to the SAPROFISH 32/SA-1 receipt, the daily administered ratio representing 3-5% of the existing piscicultural biomass. The fodder contained 32% brute protein, 7% cellulose, 13% humidity and 8% fats.

The ratios were periodically updated, on the basis of control weightings, performed monthly by the polling method.

In the end of the experimental period, five fish were taken over from each species, both from the reference and from the experimental variant, for blood samples collecting, by oblique resection of the caudal peduncle, in the region situated between the anal and the caudal fin.

The samples were taken over alternatively from the fish of the two variants, so that a comparable "stand by" time should be obtained, while the sacrifice of the fish pair occurred at approximately the same time. There have been determined both the mean hemoglobin concentration (g Hb/dL blood) and the ratio of figurative elements, that is the hematocryte (% Ht) and the number of erythrocytes (mil./µL), as well as the values of the derived erythrocytary constants (MCV, MCH, MCHC).

Hemoglobin (g Hb/dL blood) was dosed on a Sahli hemoglobinometer, the microhematocryte (Ht %) was determined by centrifugation of the capillaries, at 12, 000 rpm, for 1 min, while the number of erythrocytes (EN mil./µL) was read on a ML-4 microscope, on using the Bürker-Türk hemocytometer. The erythrocytary constants were determined by calculating: MCV (μm^3/erythrocyte) = Ht x 10/E; MCH (pg Hb/ erythrocyte) = Hb x 10/E and MCHC (g Hb/100 dL erythrocyte amount) = Hb x 100/Ht.

RESULTS AND DISCUSSION

As blood is directly involved in the normal occurrence of the main functions of any organism, it may be viewed as a highly faithful messenger for evidencing the general physiological condition of fish. The main objective of the present investigation was the comparative study of the hematological indices in the fish of the two variants, *versus* the application or non-application of some preventive antiparasitary treatments during the previous growing season, as well as the evolution of such values from their autumn levels towards those of the next spring. From this perspective, one may appreciate that the autumn values of the indices under analysis reflect the short-term effects of the treatments, while the values of the following spring provide information on the "echo" or on the long-term effects of such treatments.

As to the mean hemoglobin concentration in the blood of the third cyprinids species here under investigation (Fig. 1), the observation may be made that, in the end of winter, the mean values recorded in the treated variant exceed the ones of the untreated reference with 17% in the case of common carp and with 19% in silver carp, respectively. The third species, represented by bighead carp, shows a different behavior from that of both common carp and silver carp, the values recorded in this case being comparable in the two variants (9.21 g Hb/dL in the treated batch *versus* 9.44 g Hb/dL in the reference). Such a high hemoglobinic prosperity in the fish of the experimental variant might be interpreted as a long-term effect, namely as the "echo" effect of organism's strengthening, which is the result of the treatments applied during the previous growing season.

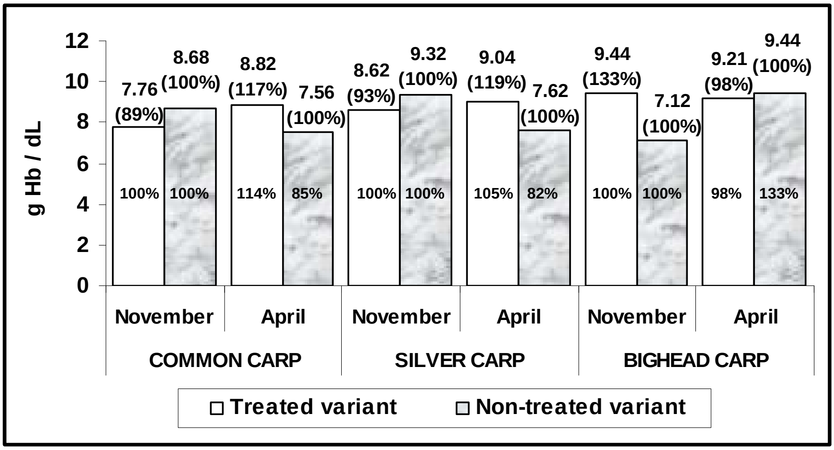

Fig.1. Hemoglobin

A comparison between the pairs of values obtained prior to and after wintering evidence the maintenance of some differences between the average hemoglobin values in the two experimental variants. Thus, on taking the autumn values as reference, one may observe that the fish treated in 2007 shows in April 2008 higher hemoglobinic levels than the untreated one. Figure 1 illustrates that, in the non-treated common carp, the mean hemoglobin value of April is 15% lower than in November one while, in the treated fish, this value increases with 14%. In a similar manner, in the silver carp from the non-treated variant, the mean values of the April hemoglobin are 18% lower than the November ones while, in the fish of the treated variant, the hemoglobin values increase with 5%. This time again, the bighead carp evidences a different behavior from that of the common carp and silver carp, which is an aspect still to be investigated in further studies.

As to the mean values of the hematocryte (Fig. 2), they also support hypothesis of a long-term effect of the preventive antiparasitary treatments. The results recorded in April evidence the superiority of the hemoglobin values in the treated fish, comparatively with the reference ones, present both in the common carp and in the two Asian planktonophagous species, silver carp and bighead carp. For example, in the common carp treated in 2007, the hematocryte values registered in April 2008 are 16% higher than those of the reference, while they are 13% higher in silver carp and 10% higher in bighead carp, respectively.

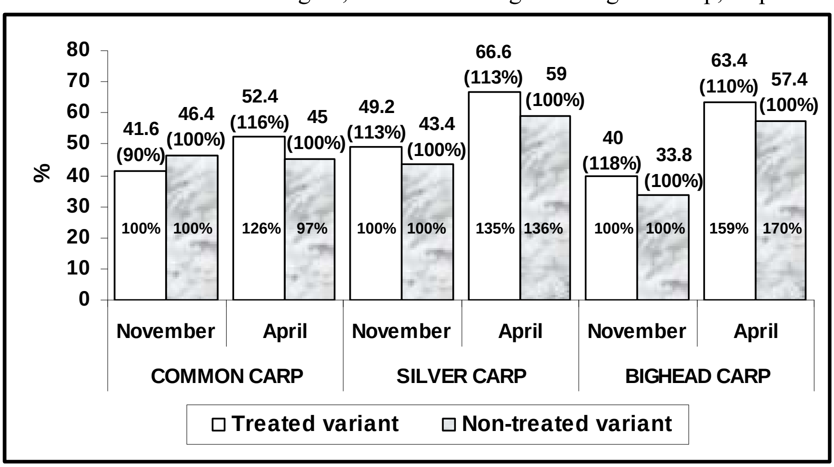

Fig.2. Hematocryte

A comparison between the pair of values obtained prior to and after fish wintering shows that, in the treated variant, the hematocryte values increase in April - *versus* November - with 26% in common carp, 35% in silver carp and 59% in bighead carp. A possible explanation might involve the long-term effect of the preventive antiparasitary treatments applied, resulting in a more efficient counteracting - from the part of the fish - of the effect of the parasitary attacks, which is due to a higher hematological prosperity. Another possible explanation is also available, namely: as the hematocryte is known as a hematological index with a special significance for stress condition, its higher values are usually associated with a more ample level of stress to which the organism is subjected. Consequently, under conditions of hypothermy, over-density and starvation during the cold season, the fish evidences quite different levels of stress, more moderate in the common carp, where the increase is of only 26%, and more intense in the two Asian species (*i.e.*, a 35% increase of the mean hematocryte values in silver carp and a 59% increase in bighead carp, respectively), which consume more energy for adapting themselves to the unfavorable winter conditions, comparatively with the common carp. An argument supporting this hypothesis is that, in the non-treated variant, the hematocryte value increases in April, comparatively with November, the increases being higher than in the treated fish. For example, in the non-treated common carp, the mean hematocryte values in April are 36% higher than in November, while the increase recorded in bighead carp is of 70%.

The values of the erythrocytes number (Fig. 3) are quite well correlated with those of the already analyzed indices, as well as with those of the derived erythrocitary constants (MCV, MCH and MCHC). As to the April values, the erythrocytes levels in the treated common carp are 14% lower than in the reference, the difference in the case of bighead carp is of 13% while - this time - the silver carp records higher values of the erythrocytes number in the treated variant, comparatively with the reference.

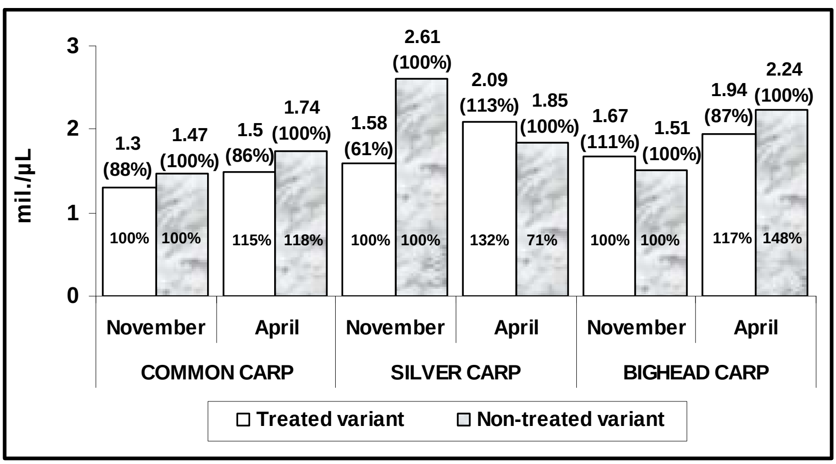

Fig.3. Erythrocyte number

A correlation between these values and the MCV ones (Fig. 4) shows that, in April, the non-treated common carp has a 14% higher number of erythrocytes than the treated one, yet these cells are 35% lower than in the treated fish. A similar situation was also observed in the case of bighead carp, the non-treated fish evidencing 13% higher red cells, which are 28% lower.

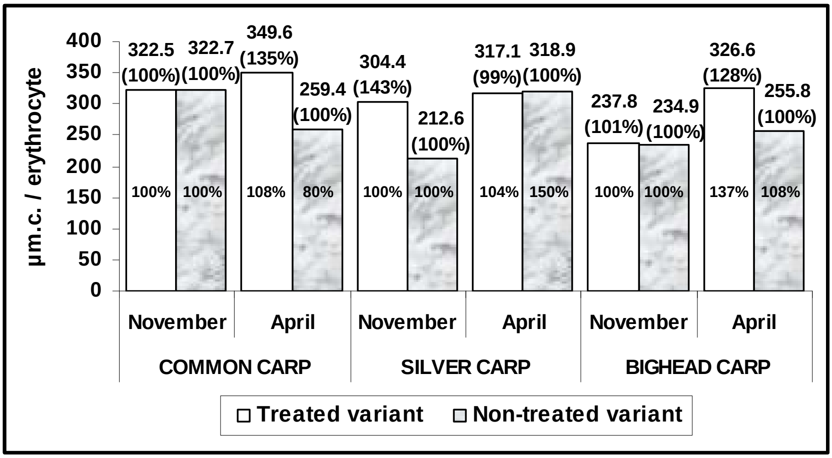

Fig.4. Mean cell volume (MCV)

The occurrence of a higher number of small-sized erythrocytes represents the adaptation response of the fish from the non-treated variant, meant at extending the breathing surface, resulted from the effort made by the organism to counteract the additional parasitary stress - comparatively with the treated fish.

Such an explanation is also supported by the fact that, both in the treated and in the non-treated fish, the values of the erythrocytes number in April are constantly higher than the November ones - with 15-18% in the common carp, with 17-48 % in the bighead carp and with 32% in the treated silver carp, respectively.

The condition of spring hypochrome anemia is also present in the fish under analysis - as evidenced by the MCH and MCHC (Figs. 5 - 6). Such values permit a correct establishment of the type of anemia - if present in the fish population - and also of the adaptation direction of the breathing function of fish to the concrete conditions of water oxygenation. In this way, in all three species, the April data show that the hemoglobin charge of the erythrocytes from the treated variant are 2% higher in the reference - in the silver carp - 13% higher in the bighead carp and 32% higher in the common carp, respectively (Fig. 5). This actually represents the "echo" effect of the treatments, in the treated fish hypochromy being much attenuated, comparatively with the reference. More than that, in all species and in both experimental variants, the MCV values are much lower in April than in November. Thus, in the common carp, a hemoglobin charge of the erythrocytes lower with 11 - 13% was determined in April, comparatively with November, while the values in the bighead carp were 21-41% lower, and in silver carp - 31-40% lower.

The April values of the mean erythrocytary hemoglobin concentration are comparable in the two variants, for all species under study, although - in the case of MCHC - differences between the April and the November values are still maintained.

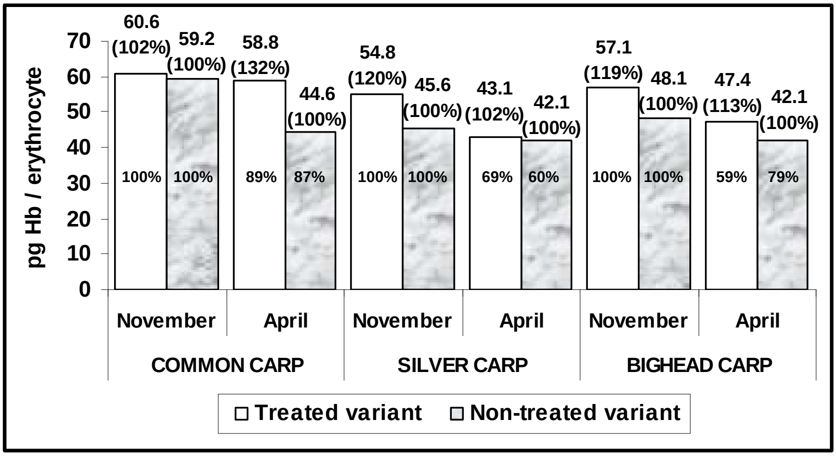

Fig.5. Mean cell hemoglobin (MCH)

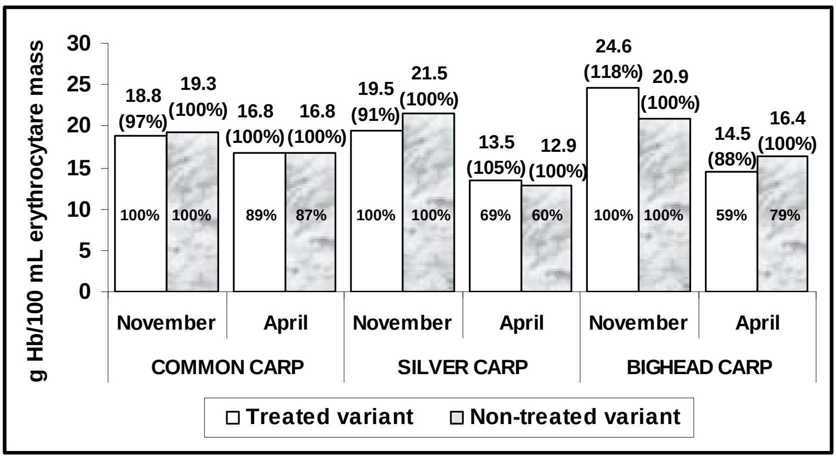

Fig.6. Mean cell hemoglobin concentration (MCHC)

CONCLUSIONS

1. In the end of wintering, the mean value values of the hemoglobin concentration in the treated variant exceed those of the non-treated reference with 17% in common carp and 19% in silver carp. In these two cases, the fish treated in the preceding summer show, in April 2008, hemoglobinic levels higher than in November (14% higher in common carp and 5% in silver carp), while the untreated ones evidence decreasing values, comparatively with those recorded prior to the cold season (15% lower in common carp and 18% lower in silver carp).

2. The values of hematocryte recorded in April 2008 are higher in the treated variant, comparatively with the reference, with 16% in common carp, 13% in silver carp and 10% in bighead carp, respectively. In the treated fish, the hematocryte increases in April - *versus* November - with 26% in common carp, 35% in silver carp and 59% in bighead carp. This increase is even more ample in the non-treated fish (36% in silver carp and 70% in bighead carp), the representatives of these adapted species giving a more expressive response to hypothermal stress.

3. In the common carp of both variants, the spring values of the erythrocytes number

are 15 - 18% higher than the autumn ones, the values recorded in the bighead carp being of 17-48%, and in the treated silver carp - of 32%.In the common carp and bighead carp, the spring values of the erythrocytes number are higher in the treated variant - comparatively with the reference - with 14% and 13%, respectively, being correlated with the MCV ones, seen as lower than the reference with 35% in common carp and 28% in bighead carp, respectively.

4. In the end of wintering, hypochromy is ampler than in autumn, the hemoglobin charge of the erythrocytes being lower in all species, comparatively with November, with 11-13% in the common carp, 21-41% in bighead carp and 31-40% in silver carp, respectively. In all three species, hypochromy is more intense in the non-treated fish, comparatively with the treated batch, as a result of the organism's effort to counteract the additional parasitary stress.

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