

BLOOD GLUCOSE, SERUM PROTEINS LEVELS AND SUPEROXIDE DISMUTASE ACTIVITY IN *CYPRINUS CARPIO*, *HYPOPHthalmichthys MOLITRIX* AND *ARISTICHthys NOBILIS* FOLLOWING THERMAL STRESS

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Abstract: The study develops a comparative analysis of the mean levels of blood glucose and of total serum proteins, as well as of the activity of superoxide dismutase in one summer-old common carp (*Common carp*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), both prior to and following the wild wintering conditions of the 2006 - 2007 season. Such parameters represent important biochemical markers in stress evaluation, once known that a constantly increasing stress results in higher values of glycemy and in superoxide dismutase activity, although decreasing proteinemy. In the one summer-old fry taken into study, significantly higher post-hibernal values of glycemy - with 84.9% in bighead carp, 139.1% in common carp and 198.1% in silver carp - were recorded. Thermal stress reduces proteinemy with 11% in bighead carp and with 14% in silver carp while, in the case of common carp, spring viremia reduces proteinemy with 13%, comparatively with the mean pre-hibernal values.

INTRODUCTION

During the cold season, pond fish interrupts active feeding, while the intensity of metabolism and of the necessary oxygen supply decreases with the decrease of water temperature. As a general rule, piscicultural effectives are accommodated in 5 up to 10 times higher densities than in the active growing season.

Sometimes, during harsh and prolonged winter seasons, such severe wintering conditions come to be more and more difficultly supported by fish, which results in the manifestation of hypothermal and over-density stress symptoms. In fish breeding practice, such discomfort conditions are anticipated by adequate measures, involving an adequate water aeration - through hooks made in the ice or over the surface of the basins - and some other specific technological works.

For a correct qualitative estimation of the populating fish and, implicitly, of the productive performances expected, a thorough knowledge on the extent of homeostatic perturbation occurring during cold seasons is essential.

As generally known, the intensity of the populational (thermal or parasitary) stress conditions may be usually evaluated through the values of - among other parameters - glycemy, proteinemy, hematocyte, mean erythrocytic volume, or through the activity of some enzymes characteristic to oxidative stress, such as: catalase, peroxidase, superoxide dismutase etc. Previous investigations have shown that, under conditions of severe chronic stress of hypothermy and over-density (for 100 days in 1996 - 1997 winter, for example), two year-old culture cyprinids (common carp, silver carp and grass carp) respond biochemically by 27.7 - 94.8% higher glycemy values - compared with the initial ones - and by 2.8 - 27% lower proteinemy, respectively.

In the present study, a comparative analysis of the mean levels of blood glucose and total serum proteins, as well as of the superoxide dismutase activity of one summer-old common carp, silver carp and bighead carp, both before and after the mild wintering conditions of the 2006 - 2007, is performed.

MATERIALS AND METHOD

The investigations, developed at the Fish Breeding Farm of Țigănași, district of Iași, followed the evaluation of the general physiological condition of cyprinids fry - both before and after wintering. The working material was represented by one summer-old fish (common carp, silver carp and bighead carp) taken over from a 800 m² hibernation basin, which accommodated 12 tons of fry, corresponding to a wintering density of 15t/ha. Two sampling operations were performed, one prior to wintering (December 11 - 12, 2006) and the other after the cold season (March 25 - 26, 2007). 7 - 8 individuals with mean individual weight, for avoiding extreme values of weight, were taken over from each species.

Glycemy was determined by the photocolorimetric method with ortho-toluidine (Artenie *et al.*, 2008), proteinemy by the refractometric method on an Abbé type refractometer (Artenie and Tănase, 1981), while the activity of superoxide dismutase was evaluated spectrophotometrically measuring the percent of the superoxide dismutase induced inhibition of Nitro Blue Tetrazolium (NBT) reduction by the superoxide radicals resulted in the medium of reaction by riboflavin photoreduction (Artenie *et al.*, 2008). The NBT reduction was followed at 560 nm using Metertek SP830 spectrophotometer. The rate of NBT reduction in the enzyme absence was taken as the reference value. One unit of superoxide dismutase represents the quantity of enzyme which produces 50% inhibition in the standard conditions.

Statistical analysis of the biochemical parameters taken into study was performed using the Student test (Văleanu and Hâncu, 1990), at the 0.05 level of significance.

RESULTS AND DISCUSSIONS

Glycemy. The glucose level in the fish blood is equally nervously and hormonally controlled, being influenced by several internal and external factors. A strong influence is exercised on glycemy by the concrete growth conditions (density of fish populations in the basin, temperature, type of food etc.).

In the case of fish, glycemy represents an especially important marker for putting into evidence the stress condition (Sorvaceev, 1982; Kebus *et al.*, 1992; De Dominis *et al.*, 1993; Barry *et al.*, 1993; Bau *et al.*, 1994; Rehulka, 1996; Misăilă *et al.*, 2005; Patriche, 2007).

Under normal conditions, the level of glucose in fish blood oscillates between 40 and 90 mg/dl (Reichenbach Klinke, 1973), *versus* 60 - 110 mg/dl, the values recorded in healthy human organism while, under conditions of stress, acceleration of glycogenolysis, increasing the glucose reserves used by the organism as an immediate energy source for counteracting stress, may be noticed.

The results of the present investigation (Fig. 1) show that, in one summer-old common carp, silver carp and bighead carp fry, the glycemy levels recorded in the beginning of winter oscillate between 25 and 43 mg/dl, the highest mean value being registered in common carp, while the minimum one - in silver carp. These values are close to or slightly lower than the minimum levels given in literature for freshwater fish (40 mg/dl).

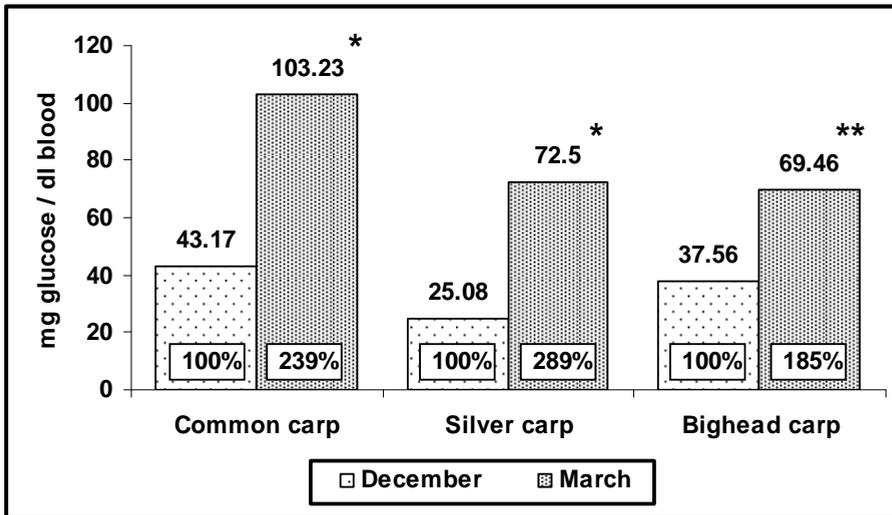


Fig.1. Glycemy

* $p < 0.001$; ** $p < 0.01$

As a response to stress, induced equally by hypothermy and over-density, as well as by some observed parasitary incidences, the mean levels of blood glucose recorded in spring are significantly higher than those registered in the beginning of winter.

From this perspective, common carp evidences a 139% increase of glycemy over the pre-hibernal level, the glycemy values recorded in silver carp and bighead carp being of 189%

and 85%, respectively. The spring values are below the upper limit given in literature (90 mg/dl) in silver carp and bighead carp, and over it in the case of common carp, the mean value of which is 103.12 mg/dl, *i.e.* a 12.8% increase.

Increase of the spring levels of glycyemy in all fish representatives taken into study (common carp, silver carp and bighead carp), comparatively with the pre-hibernal values, indicates the manifestation of stress in all three species of cyprinids fry.

Proteinemy. It is especially important to know the level of serum proteins in culture fish, as this parameter expresses the exact nutritional status of the organism, as well as the fish health condition.

Generally, the normal values of total proteins from the serum of freshwater fish oscillate between 3.5 and 5.5 g/dl (Siwicki and Anderson, 1993). Any reduction in proteinemy, suggesting perturbation of liver's protein synthetic function, may be the result of both spring viremia - in the case of common carp - and erythrodermatite - in cyprinids -, and of the lower temperatures characterizing the cold season (Rehulka, 1996; Parent and Vellas, 1981; Hille, 1982). At the same time, fodders poor in protein and chronic inanition may cause temporary reductions in the amount of total serum proteins. More than that, a less oxygenated water - resulting from over-normal densities - may determine a significant reduction of proteinemy.

The results obtained give mean values of proteinemy oscillating - in the pre-hibernal season - between 3.24 g/dl in common carp, 3.8 g/dl in bighead carp and 4.08 g/dl in silver carp (Fig. 2), all ranging within the normal limits established for fish, with the only exception of the common carp, in which proteinemy decreases below 3.5 g/dl.

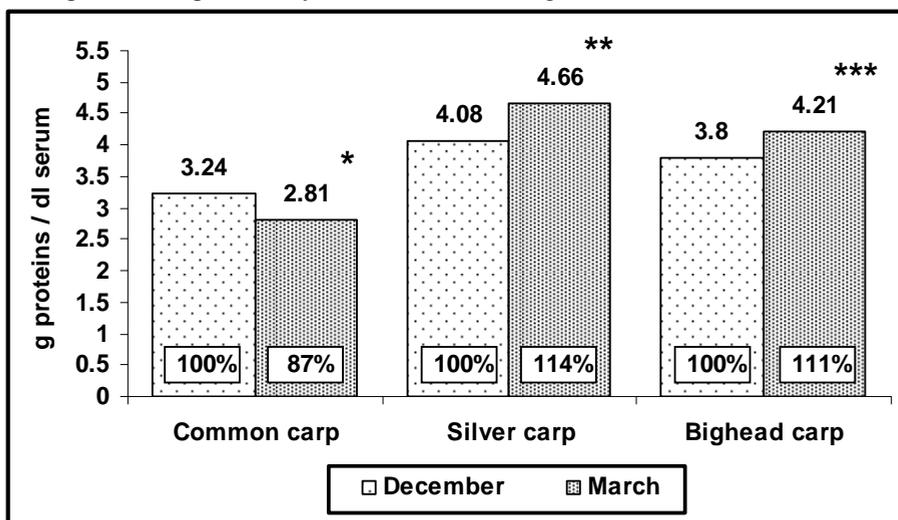


Fig.2. Proteinemy

* $p < 0.001$; ** $p < 0.01$; *** $p < 0.5$

Mention should be made of the fact that, in December, water temperature oscillated between 2 and 3°C, which might cause hypothermy, comparatively with temperature values of 6 - 7°C, registered in the second half of March. This might explain the values of proteinemy recorded in December - namely, 14% lower in silver carp (a statistically significant difference) and 11% lower in bighead carp (a statistically non-supported difference) - compared to the spring

ones. In spite of the fact that common carp hibernated at the same temperatures, we believe that, in this species, the parasitary stress had been more influential than the thermal one, so that, in March - a time when the species is usually affected by spring viremia - the values of proteinemy recorded are up to 13% lower than those of December - which actually represents a statistically significant difference.

Superoxide dismutase (SOD) is a metallo-enzyme playing an important part in the neutralization of the superoxide anion, also evidenced in fish organisms as an extra-cellular CuZn - SOD, with a molecule made of four proteic subunits. The enzyme is utilized as an oxidative stress biomarker, its activity being intensified in various pathological states - in stress states, generally.

The results illustrated in Figure 3 show different values of SOD activity in the three species under investigation, the mean values recorded in common carp being almost two times higher than in silver carp and four times higher than in bighead carp.

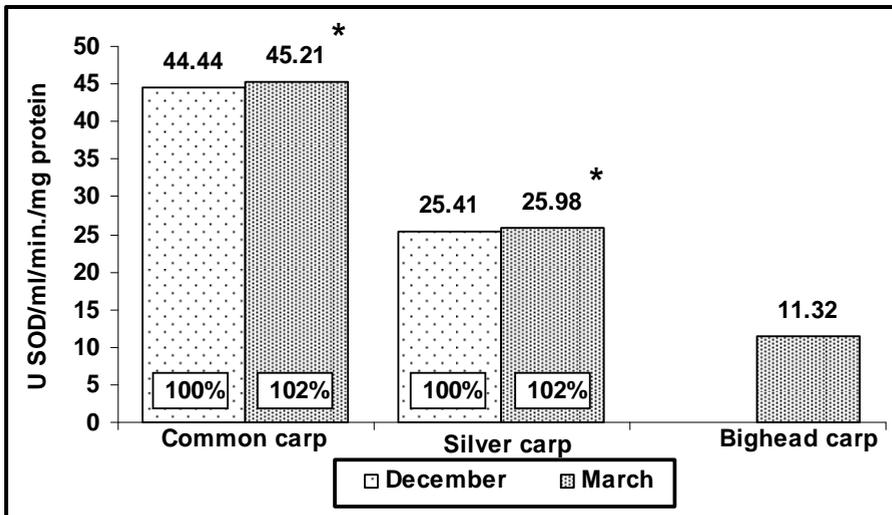


Fig.3. Superoxide dismutase activity

* $p > 0.05$

A comparison between the mean SOD values attained in the two experiments shows that, in the case of both common carp and silver carp, a slight increase occurs in the end of winter, of 1.74% in common carp and 2.22%, respectively, in silver carp, both representing statistically non-significant differences. However, the trend of these modifications suggests the response to stress of the fish under analysis, as correlated, from this perspective, with the values registered for glycemymy.

CONCLUSIONS

The response of the common carp, silver carp and bighead carp fry hibernating under conditions of over-density is typically illustrating the stress condition, *i.e.* higher values of glycemymy in the end of winter - representing up to 184.9 - 289.1% of the pre-hibernal ones.

Proteinemy increases with 11% in bighead carp and 14% in silver carp, in parallel with increasing water temperature while, in the case of common carp, the influence of the parasitary stress in prevailing, so that, during the spring viremia season, the values of proteinemy decrease with 13% comparatively with the reference values.

Occurrence of a low intensity wintering stress is also expressed by the SOD activity values, which - in the end of winter - are slightly higher (1.7 - 2.22%) than the pre-hibernal ones.

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