INFLUENCE OF THE ANTIECTOPARASITARY TREATMENT ON CELLULAR RESPIRATION OF SOME CULTURED CYPRINID SPECIES

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Key words: cultured cyprinids, preventive antiectoparasitary treatment, cellular respiration, muscles, gills
Abstract: The study discusses the influence of a preventive treatment against ectoparasites on the intensity of cellular respiration in three, 3 summer-old cultured cyprinid species, namely: common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). The cellular consumption of oxygen (μLO_2/g fresh tissue) in muscles and gills was determined by the Warburg microrespirometric method, on two batches of fish from each species, namely: a reference (untreated) batch and a batch treated against ectoparasites. The results obtained show that the intensity of the cellular consumption of oxygen differs - as a function of the treatment applied, fish species, cellular type and duration of recordings. Therefore, generally speaking, cellular respiration is more intense in gills than in muscles, in both types of batches: treated and untreated. Lower values are registered in common carp, followed, in increasing order, by those recorded in silver carp and bighead carp. The antiectoparasitary treatment has positive effects, intensifying cellular respiration, along with metabolism stimulation and an improved general physiological condition of fish.

INTRODUCTION

Respiration is a complex phenomenon, including a series of processes which assure the energy required by all living organisms; it assumes two categories of processes, closely correlated between them, namely: external (extracellular) processes, which assure the exchange of respiration gases (O_2 and CO_2) at the level of lungs, gills, tracheae, and internal (intracellular) processes. The latter ones require oxygen’s transport by the blood (as a circulating liquid) up to tissues and cells, where cellular respiration occurs, involving oxidation of the metabolic substrate, with participation of NAD and FAD, known as releasing an electron transport up to the O_2 taken over by cells, CO_2 (eliminated), water and energy - in the form of ATP - thus resulting (Lehninger, 1987; Perry and Tufts, 1998).

The general picture of the respiration processes includes two phases, namely: an aerobic and an anaerobic one, the result being the cellular energy represented by 38 molecules of ATP, which reflects the energetic balance of the intracellular metabolization of glucose, as a metabolic substrate, during the development of the two above-mentioned phases.

In fish, aquatic organisms, the exchange of respiration gases occurs through the gills (representing the ventilating system) and through the sanguine circulating system, while the processes of cellular respiration are developed inside the various types of cells, with the participation of mitochondria and of some specific enzymatic systems. The intensity of the processes of cellular respiration is influenced, on one side, by a series of internal factors, among which special mention should be made of: species, age, sex, health condition and energetic requirements of the animals, as well as, on the other side, by some external factors: temperature, water’s degree of purity/pollution, osmotic characteristics, aquatic streams etc.

Cellular respiration of fish has been investigated by several authors yet, on other species and under different experimental conditions, the interest being focused on other aspects, such as: the influence of temperature, of chemical mediators, of some inhibitors or pollutants etc. (Ekberg, 1958; Perry and Tufts, 1998; Brauner and Berenbrink, 2007; Fernandes *et al*., 2007; Neacşu *et al*., 2008).

The purpose of this paper is to evidence the influence of the antiectoparasitary treatment in three cultured cyprinid species upon the cellular respiration intensity, as a parameter of general physiological state of the organism.

MATHERIALS AND METHOD

The present study follows the influence of a preventive antiectoparasitary treatment on the intensity cellular respiration in three, 3 summer-old cyprinid species: common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*).

The experiments were made on two categories of batches: reference (untreated) fish and fish subjected to an antiectoparasitary treatment. In the reference batches, the experiments involved no antiparasitary treatments while, in the

treated ones, treatments were applied, both prophylactically, in the moment of basin's filling with fish, and during the growing period, preventive doses of 0.1 mg/L trichlorfon being applied between April 2007 and April 2008, as well as Calcium chloride (2kg/ha) - two times a week.

The *in vitro* cellular consumption of oxygen in the striated muscular tissue (homogenized) and in the branchial cells (the spangles detached from the branchial arches) was determined by the Warburg microrespirometric method, on two batches, each one formed of five representatives of each fish species here under study, as follows: a reference (untreated) batch and a batch preventively treated against ectoparasites. The cellular consumption of oxygen was recorded at constant temperature (24°C), for one hour, according to the above-mentioned method, for observing the dynamics of cellular respiration intensity over a determined time interval. The values, read on pressure gauges (manometers), at 15 minutes intervals, were expressed as μLO_2/ g fresh tissue, and estimated both statistically (Student test) and as percent, in a comparative analysis of the data obtained on the treated and, respectively, reference batch.

RESULTS AND DISCUSSION

The results obtained evidence that the intensity of the cellular consumption of oxygen registers different average values, as a function of fish species, cellular type, presence or absence of the treatment and duration of recordings.

In all animal organisms under analysis - either treated or not and in both types of cells (muscular and branchial) - the cellular consumption of oxygen records increasing values (μLO_2/g fresh tissue), over the whole duration of determinations (60 minutes), while the increase ratio is getting lower and lower, at 15 minutes intervals, when the indications of the pressure gauges from the Warburg installation were read (Figs. 1 - 3).

Such decrease in the intensity of cellular respiration during the *in vitro* determinations may be explained by the fact that the aerobic respiration processes occur in a closed precinct, isolated from the atmospheric air represented by the Warburg respiration vessel in which the tissue sample is placed, so that, during respiration, the oxygen from the closed precinct is consumed, the amount available for respiration getting more and more reduced, as well as the energetic substrate from the cells isolated from the organisms, which get more and more consumed during the recordings. To those phenomena, one should also add the accumulation of some cellular metabolic residues, which contributes to the inhibition of the respiration processes (Lehninger, 1987; Karp, 1996; Hăulică, 2007).

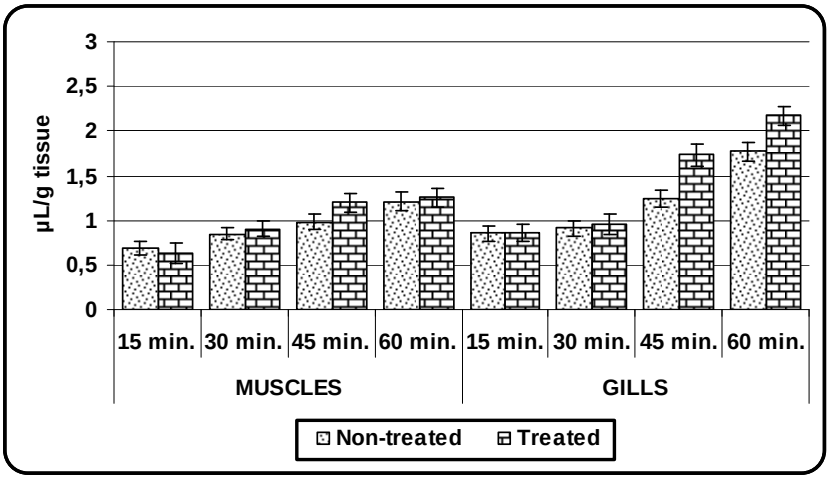

Fig.1. Oxygen respiratory consumption of the muscular and branchial cells in common carp

Consequently, in the reference, non-treated batch, the oxygen consumption of the muscular striated cells records highest values in the common carp (Fig. 1), followed by silver carp and bighead carp, for all four intervals of measurements (15, 30, 45 and 60 minutes), the final values (recorded at 60 minutes) being the following: 1.2118 µL/g fresh tissue in common carp (Fig. 1), 1.1518 µL/g fresh tissue in silver carp (fig. 2) and 1.0849 µL/g fresh tissue in bighead carp (Fig. 3).

As to the fish batches subjected to a preventive antiectoparasitary treatment, the values of oxygen consumption in the muscular cells are generally higher than those of the reference, while the increase recorded differs, as a function of both fish species and moment of measurements.

According to such observations, in the treated common carp batch, after the first 15 minutes of recordings, the average value is slightly lower than in the reference batch (92.18%), after which it increases up to 121.83% at 45 minutes, while the increase recorded after 60 minutes is weaker (103.41%), being non-significant *versus* the corresponding value of the reference (Table 1).

Table 1. Oxygen respiratory consumption (%) of striated muscular cells in the fish under experiment

Species	15 minutes		30 minutes		45 minutes		60 minutes	
	NT	T	NT	T	NT	T	NT	T
Common carp	100%	92.18%	100%	106.3%	100%	121.83%	100%	103.41%
Silver carp	100%	118.92%	100%	108.41%	100%	144.9%	100%	134.74%
Bighead carp	100%	104.76%	100%	111.8%	100%	147.74%	100%	151.3%

NT = non-treated, T = treated

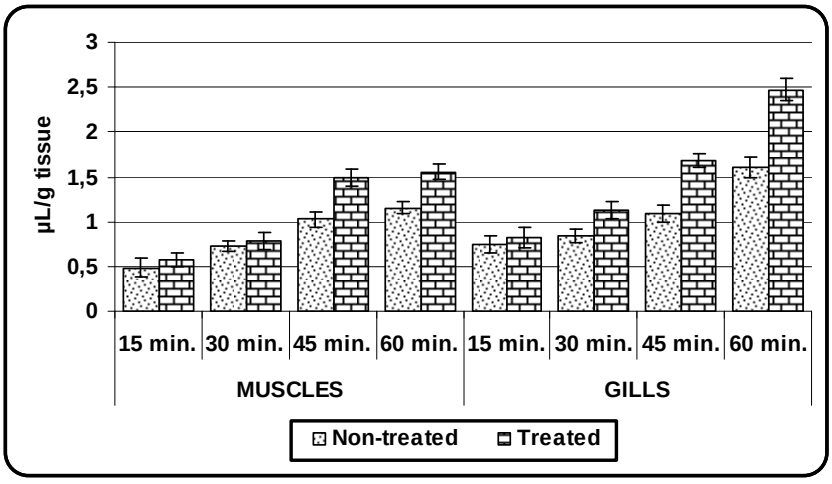

Fig.2. Oxygen respiratory consumption of the muscular and branchial cells in silver carp

The treated silver carp and bighead carp batches register much higher increases in the intensity of muscular cells respiration, comparatively with the reference, than the values recorded in common carp. Thus, in the bighead carp (Fig. 2), the average value recorded after 15 minutes is of 118.92%, comparatively with the reference, the increase recorded after 30 minutes being weaker (108.41%), although, in the end of the measurement period, an ample increase could be noticed, of 144.9% *versus* the reference, at 45 minutes, and of 134.74%, respectively, at 60 minutes, comparatively with the reference values ($p < 0.002$).

Bighead carp registers the highest increase of oxygen consumption in the muscular cells under the action of the treatment, comparatively with the untreated batch, the values recorded being more homogenous along the whole duration of the measurements (Fig. 3), the final value (at 60 minutes) being of 151.3% (Table 1) - quite significant, comparatively with the corresponding one, recorded in the reference batch ($p < 0.001$).

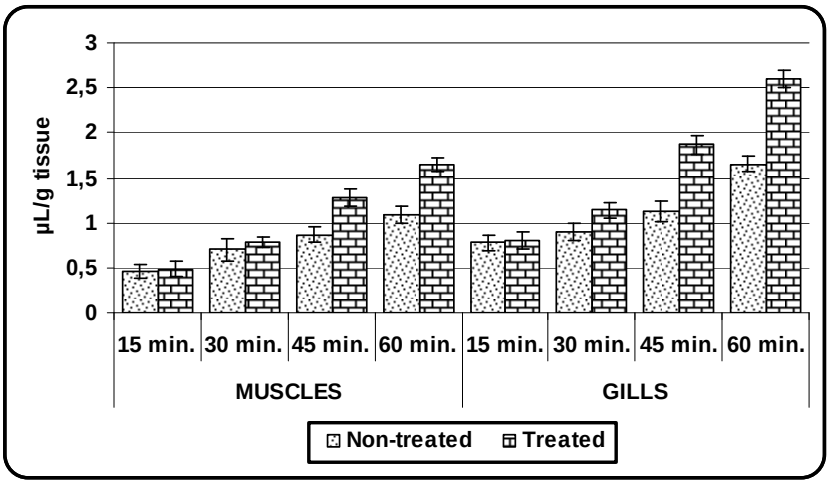

Fig.3. Oxygen respiratory consumption of the muscular and branchial cells in bighead carp

Quite similar aspects with those observed for muscular cells may be evidenced in branchial cellular respiration, as well, although some peculiarities should be here mentioned (Figs. 1 - 3). Thus, the intensity of the respiratory consumption of oxygen in the branchial cells is generally higher than that of the muscular cells, for all three species from both batches. The gills, as specialized organs assuring the exchange of respiratory gases (O_2 and CO_2) in aquatic organisms, evidence an adequate morphological structure, their cells being arranged as spangles on bony arches, so that an as large as possible contact surface should be assured directly with the aqueous environment; at the same time, their intense vascularization should be mentioned so that, as organs with continuous activity, they should assure an intense cellular respiration (Ekberg, 1958; Perry and Tufts, 1998; Brauner and Berenbrink, 2007; Fernandes *et al*., 2007).

The dynamics recorded by the respiratory consumption of oxygen in the branchial cells is generally similar to that of the muscular ones, the results obtained evidencing the fact that the treatment applied stimulates respiration of branchial cells in all three fish species under study.

Consequently, in the reference common carp batch, the average value of the oxygen consumption in the branchial cells is of 0.8537 µL/g fresh tissue after 15 minutes, of 0.9087 µL/g

fresh tissue after 30 minutes, of 1.2385 µL/g fresh tissue after 45 minutes and of 1.7692 µL/g fresh tissue after 60 minutes, respectively (Fig. 1). In the antiectoparasitary-treated batch, the increase in oxygen consumption determined after 30 minutes is non-significant (104.87%), comparatively with the reference. Nevertheless, after 45 minutes, cellular oxygen consumption increases significantly (139.65%) comparatively with the reference, a tendency maintained up to 60 minutes, although slightly diminished (122.64%), which represents a significant value, ranging, however, between the normal physiological limits (Fernandes *et al*., 2007).

Table 2. Oxygen respiratory consumption (%) of branchial cells in the fish under experiment

Species	15 minutes		30 minutes		45 minutes		60 minutes	
	NT	T	NT	T	NT	T	NT	T
Common carp	100%	100.84%	100%	104.87%	100%	139.65%	100%	122.64%
Silver carp	100%	109.89%	100%	135.06%	100%	154.3%	100%	154.16%
Bighead carp	100%	103.18%	100%	155.76%	100%	165.84%	100%	157.99%

NT = non-treated, T = treated

The treatment applied to silver carp and bighead carp stimulates a more intense cellular branchial respiration, comparatively with the values registered for common carp.

Consequently, the average values of oxygen consumption in the branchial cells are higher in the treated batch - comparatively with the non-treated one - initially insignificant (109.89% after 15 minutes), then more and more intense, up to a final value of 154.16%, which is highly more significant than that of the reference (Table 2).

In the bighead carp, the average values of cellular branchial respiration in the treated batch are, again, much higher than those of the reference batch, being characterized by an initially non-significant value (103.18% after 15 minutes), followed by a more intense increase, up to 165.84% (comparatively with the reference) at 45 minutes and up to 157.99%, respectively, at 60 minutes of recording (Table 2).

The observation to be therefore made is that, in the case of both gills and muscles, the antiectoparasitary treatment induces the highest stimulation of cellular respiration in the bighead carp, followed by that of silver carp and common carp.

The results thus obtained may be correlated with the general biological characteristics, different in these fish species, as belonging to two categories. For example, the common carp, which is an autochthonous species, with omnivorous feeding, more closely related to the bottom of the aquatic basin, and characterized by relatively limited movements, evidences an energetic and physiological equilibrium generally specific to its mode of living, which is to be reflected in a more reduced influence of the preventive antiectoparasitary treatment applied, comparatively with the other two cyprinid species under investigation.

Silver carp and bighead carp have different biological characteristics, as species of warmer water, coming from South-East Asia. As they consume both phyto- and zooplankton, their movements in water, for its filtration, are more active, which requires a higher energetic level and, consequently, a relatively more intense oxygen consumption.

As a matter of fact, the physiological and biochemical characteristics of both silver carp and bighead carp make more intense the reactivity of their cellular respiration, as a result of the antiectoparasitary treatment applied, which is expressed by a more intense oxygen consumption, especially at the level of branchial cells, which actually illustrates an improvement in their general health condition, manifested by a higher energogenetic level.

All these data permit the conclusion that the antiectoparasitary preventive treatment applied to the three fish species does influence their general physiological condition, which assumes intensification of aerobic cellular respiration, concomitantly with stimulation of oxidative phosphorilation (accompanied by ATP production) and, implicitly, a more intense cellular energogenesis (Ekberg, 1958; Lehninger, 1987; Karp, 1996; Alberts *et al*., 1998).

CONCLUSIONS

The results obtained demonstrate that the intensity of cellular respiration in the three fish species considered for experiments differs as a function of species, cellular type, treatment applied and duration of recordings.

Generally, the respiratory consumption of oxygen is more intense in the branchial cells than in the striated muscular ones, both in the reference batch and in the antiectoparasitary-treated one.

The preventive antiecoparasitary treatment has positive effects upon the general physiological condition of the fish here under study, which is expressed by a more intense cellular respiration, correlated with stimulation of cellular metabolism. The values of cellular respiration recorded - for the three cyprinid species - under such conditions, generally range between the normal variation limits of this physiological parameter, the antiectoparasitary treatment applied inducing no irreversible physiological perturbations.

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