HEPATIC GLYCOGEN AND GLUCOSE IN EIGHT TROPICAL FRESH WATER TELEOST FISH: A PROCEDURE FOR FIELD DETERMINATIONS OF MICRO SAMPLES.

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ABSTRACT

The present paper reports a method adapted by the authors for glycogen quantification aiming to answer some questions. It was reliable and the results are reproducible. It consists in the precipitation of glycogen by ethanol followed by direct determination of glucose through phenol-sulfuric hydrolysis. The hepatic content of glycogen is described for eight fresh water teleost species and the values are compared to hypoxia resistance. The value of [hepatic glycogen]/[plasma glucose] rate is also analyzed for the first time as a reference index to characterize hypoxia resistance in fresh water teleost fishes.

Key words: fish, methodology, glycogen and glucose determination.

RESUMO

Glicogênio hepático e glicose em oito teleósteos tropicias de água doce: método para determinação de micro-amostras.

Este trabalho descreve um método para quantificação do glicogênio hepático, adaptado pelos autores, para responder às questões em pauta. Este método consiste na separação alcoólica do glicogênio seguida da determinação direta de glicose através do método hidrolítico por fenol-ácido sulfúrico. Está descrito ainda o conteúdo de glicogênio hepático de oito espécies de teleósteos de água doce, comparando-o com a resistência à hipóxia apresentada pela espécie. O índice [glicogênio hepático]/[glicose plasmática] é relatado pela primeira vez como um possível índice de referência para a caracterização de resistência à hipóxia em teleósteos de água doce.

Palavras chaves: peixe, metodologia, determinação de glicogênio e glicose

INTRODUCTION

Glycogen is the main energetic store to supply metabolic demands. Such polysaccharide is characteristic of animal cells and particularly abundant

in liver reaching 7% of the wet tissue (Lehninger, 1971). This macromolecule may be stored under different chemical forms. Among that we may highlight the granular forms (α-particles), the rosettes or β-particles; the cell membrane associated forms and the glycogen seas (Hochachka, 1980). Granular α-particles is distributed over the cytoplasm and constitutes smaller forms. It is assumed that β-particles constitute the largest stocks reaching values five times higher as compared to other granular forms. Besides, α-particles seems to be the most efficient way of glucose store. To the glycogen seas it is attributed the emergencial role providing glucose under extreme hypoxia (Hochachka, 1980).

Glycogen contents may reflect some biochemical adaptation to any kind of environmental stresses. Among these, pH, oxygen level, salinity and hard exercise may result into glycogen store (Soengas *et al* 1995; Moraes *et al* 1996). Fishes living in rivers and ponds, in which dry seasons transform the environment into muddy places submitting them to low oxygen levels, ought to express such type of adaptation. This was reported in muscle and liver of *Lepidosiren paradoxa* and *Synbranchus marmoratus* in which large stocks of glycogen were found (Hochachka and Hubert, 1978). The common carp (*Cyprinius carpio*) exhibited a decrease into glycogen content when starved. Such response, followed by increase of glucemia, suggests a way of supplying the energetic demand under the absence of feeding (Blasco *et al.*,1995).

Age and/or size seem to be correlated to adaptations concerning to glycogen contents and salinity in rainbow trout, *Oncorhynchus mykiss*. This species, when transferred to environments of gradually increased salinity, shows glucolytic and glyconeogenesis changes as adaptive response (Soengas *et al* 1995).

In the present paper eight species of freshwater teleost fishes were studied in regard to correlation among glycogen store and blood glucose level versus habitat conditions and oxidative metabolic capacity.

MATERIAL AND METHODS

Four wild species reported in the present paper were collected in Mogi Guaçu River, Pirassununga, SP, Brazil and their size, weight and number of specimens are expressed in Table I. Cultured species of commercial importance, also expressed in Table I, were kindly supplied by CEPTA-IBAMA, Pirassununga-SP. All the animals were collected on October (springtime) and kept under aerated conditions (normoxia) for twenty-four hours in water directly piped from local reservoir. After such period blood was collected by caudal puncture through heparinized syringe transferred to Eppendorf tubes and centrifuged for collecting plasma. Afterwards, the animals were killed by head blow and samples of liver and white muscle from anterior dorsal side were promptly excised and kept under liquid nitrogen for succeeding homogenization. All operations spent no more than five minutes.

Glucose analysis: Tissues were homogenized in 50% trichloroacetic acid (TCA) keeping the proportion of 100 mg per 1.0 ml of TCA. After centrifuge for five minutes at 5000 rpm the contents of glucose was determined in the supernatant. Plasma samples were submitted to the same procedure keeping the same proportions (100μl of plasma/1.0ml of TCA). Glucose was determined

by DuBoie's hydrolytic method (1956). It consists of suitable aliquot of glucose into a final volume of 0.5 ml, added of 0.7 ml of 3% phenol. After shaking, 2 ml of concentrated sulfuric acid (H_2SO_4) was added into one stroke developing strong heat of reaction. The product was determined at 540 nm in a single colorimeter.

Glycogen analysis: Samples of muscle (200 mg) or liver (50-100 mg) were quickly separated from freezed tissues and transferred to essay tubes containing 1.0 ml of 6N potassium hydroxide (KOH). The tubes were transferred to boiling water bath and left along three to five minutes for complete dissolution. Aliquots of the resultant solution (250 µl) were added to 3 ml of 95% ethanol-water and, after mixing, 100 µl of 10% potassium sulphate (K_2SO_4) was appended. A cloudy white precipitate was formed and the supernatant was discharged after centrifuging at 3000 rpm for three minutes. It was added 2.5 ml of distilled water to the precipitate, which was promptly dissolved. Suitable aliquots from such solution were employed to DuBoie reaction. Glycogen is expressed in µmols of glucosil-glucose per gram of wet tissue.

Statistic: All the results were statistically analyzed comparing the means at IC = 0.05 for different variances.

RESULTS AND DISCUSSION

The method employed for glycogen quantification was adapted by authors to the present work. It consists of the classical glycogen precipitation by ethanol. The method was improved by the increase of the ionic strength in the reaction medium by the addition of potassium sulphate salt. Glycogen was estimated through glucose liberated after acid hydrolysis. Such estimation was done by DuBoie's method (DuBoie, *et al.* 1956) which combines polysaccharide hydrolysis followed by glucose determination through the production of furfural derivative in only a single path. Such procedure as a whole has proved to be simple and precise enough for the present purposes.

Hepatic glycogen is considered as the emergencial stock used only at the first moments of critic situations (Christiansen and Klungsoyr, 1987). Nevertheless, muscular glycogen, particularly from white muscle, is responsible for energy supply under exhausting moments as hunting or escaping.

Our results present some teleost species with high levels of hepatic glycogen (Fig. 1) specifically when compared to reported species (Table I). Such results suggest that species as *Hoplias malabaricus* (traira) and *Piaractus mesopotamicus* (pacu) are biochemicaly adapted to endure, for short periods, severe moments as low oxygen levels and or absence of food.

The hepatic role on glycogen catabolism seems to be mainly to provide metabolical intermediates for biosynthetic processes more than supplying energy (Christiansen and Klungsoyr, 1987). Under hypoxia, traira exhibits glycogen mobilization from liver to white muscle (Moraes *et al.*, 1996) suggesting that this is the kind of biochemical strategy used as a response against that stress. Such adaptation confers special ability for colonizing unfavorable environments as swamps, ponds, crowded by aquatic plants in decomposition, etc. Those environments are characterized by low oxygen levels and some times very poor concerning food availability (Godoy, 1975).

Species as *Prochilodus scrofa* (curimata), *Brycon cephalus* (matrinchã), *Salminus maxillosus* (dourado) and *Pimelodus maculatus* (mandi), living in speedy water rivers, showed relatively low hepatic glycogen levels and high values of glucemia (Fig. 1). This data suggest great energetic demand to bear with such habitats. Yet, bearing in mind the feed habit, high levels of blood glucose found in *S. maxillosus* could be explained considering its energetic demand, as a typical greedy predator (Godoy, 1975), and the considerable amounts of energy spent for chasing.

More interesting is the index resulted from [hepatic glycogen/plasma glucose] (Table I). The highest values were observed for *H. malabaricus, P. mesopotamicus* and *Hipostomus regani* (cascudo). In spite of employing different strategies against hypoxia these species resist very low levels of oxygen. The kind of habitats whither they are found, if not the same, are characteristically formed by quiet waters and occasionally hypoxic ones. Species as *P. scrofa, B. cephalus* and *S. maxilosus* have low resistance to hypoxia (personal observation). These species feature very low index when compared to the aforesaid. Yet, they have in common the fact of living in fast waters where oxygen levels are usually high. Species as *Oreochromis niloticus* presented intermediate values.

The present paper reported eight species concerning to glucemia and hepatic glycogen levels employing a simple and accurate chemical field procedure for glycogen determination. Among the studied species is the well known *O. niloticus*, which was employed as reference since it came from similar conditions to the others. Hepatic glycogen values observed in pacu and traira are comparable to turtles (Table II) pointing out their typical resistance to hypoxia, if considered this value as an index (Hochachka and Somero 1984). However, in spite the short number of species the authors propose the rate [glucemia/glycogen] as a possible index to infer hypoxia resistance and environmental preferences. Other species have to be studied to confirm the worth for such proposed index as well as any relationship between it and phylogeny.

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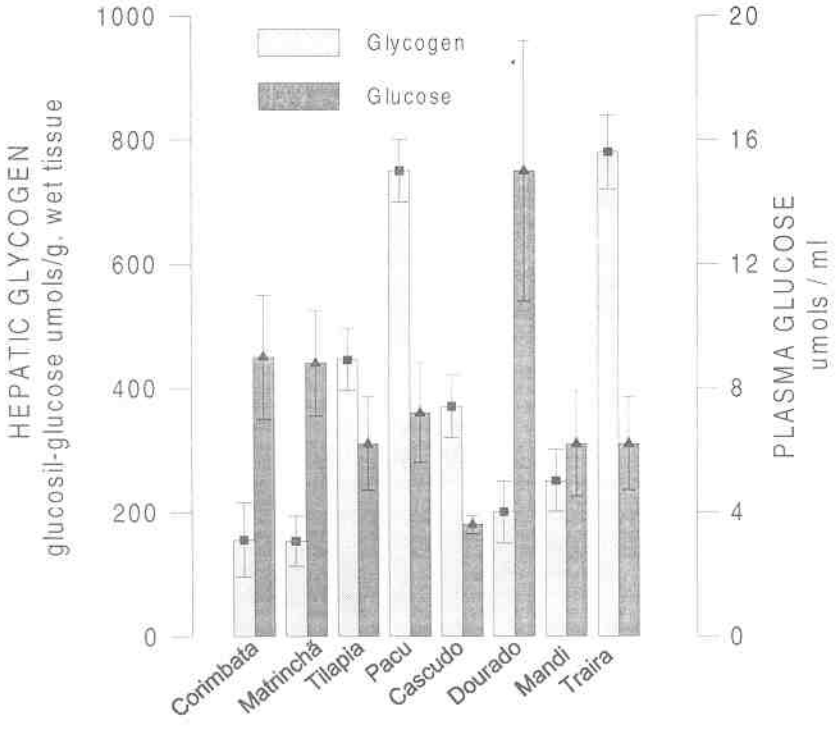

Fig. 1. Hepatic glycogen and plasma glucose contents in eight species of freshwater teleost fish. Glycogen was estimated by DuBoie method after ethanol isolation and adapted by authors to the present pur pose. Results are expressed as the means for eight animals (N=8) and compared at IC = 0.05.

TABLE I Glycogen:Glucose rate for eight studied freshwater teleost species. Weight (g), and size (cm) are expressed as well as the standard deviation. (N) express the number of specimens used. Wild species are pointed out as (*)

Species	Common name	Weight (g)	Size (cm)	[Glycogen] / [Glucose]
Hipostomus regani (*)	Cascudo (N=6)	296±98	30.5±2.4	104.16
Pimelodus maculatus (*)	Mandi (N=5)	546±147	36.6±2.6	40.3
Hoplias malabaricusl (*)	Traira (N=8)	201±63	25.8±3.2	71.7
Prochilodus scrofa	Curimatá (N=6)	813±37	37.6±6.1	17.2
Brycon cephalus	Matrinchã (N=6)	495±34	35.3±0.5	17.4
Piaractus mesopotamicus	Pacu (N=7)	670±198	33.8±3.2	104.16
Oreochromis niloticus	Tilápia (N=7)	145±15	21.7±0.8	71.7
Salminus maxillosus (*)	Dourado (N=6)	1790±749	52.0±8.6	13.3

TABLE II - Hepatic Glycogen from different vertebrates related to hypoxia tolerance (Hochachka and Somero, 1984). (.) Moraes *et al* 1996.

SPECIES	LIVER GLYCOGEN
	µmoles glucosil-glucose/g wet tissue
Anoxia tolerant	
Goldfish	1,300
Turtle	860
Traira *	850
Anoxia non tolerant	
Trout	235
Sunfish	185
Rat	210
Mouse	220