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BIOCHEMICAL PARAMETERS FOR *Piaractus mesopotamicus*, *Colossoma macropomum* (Characidae) AND HYBRID TAMBACU (*P. mesopotamicus* X *C. macropomum*)

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ABSTRACT

A study for investigating the values of plasma glucose, serum total protein and serum ions (calcium, potassium, magnesium, sodium and chloride) was carried out in young *Colossomoma macropomum* Cuvier, 1818, *Piaractus mesopotamicus* Holmberg, 1887 and the hybrid tambacu (*P. mesopotamicus x C. macropomum*), kept in intensive system. Glucose concentrations were higher in tambacu than in *P. mesopotamicus* and *C. macropomum*. Total protein levels were higher in *P. mesopotamicus* than both *C. macropomum* and tambacu. *C. macropomum* presented higher concentrations of sodium and chloride, while *P. mesopotamicus* had higher concentrations of potassium and magnesium. However, levels of calcium were similar for the species studied. The hybrid tambacu showed the smallest levels of total protein, and intermediate levels of sodium, potassium, magnesium and chloride, compared to both *P. mesopotamicus* and *C. macropomum*. Baseline values for healthy fish in aquaculture were established, and they may be used for further comparisons in studies with wild populations of *P. mesopotamicus* and *C. macropomum*.

KEYWORDS: Biochemistry, blood, *Colossoma macropomum*, freshwater fish, *Piaractus mesopotamicus*.

RESUMO

PARÂMETROS BIOQUÍMICOS DE *Piaractus mesopotamicus* E *Colossoma macropomum* (CHARACIDAE) E HÍBRIDO TAMBACU (*P. mesopotamicus x C. macropomum*)

Estudaram-se os valores de glicose plasmática, proteína total sérica e íons séricos (cálcio, potássio, magnésio, sódio e cloreto) em jovens de *Colossomoma macropomum* Cuvier 1818, *Piaractus mesopotamicus* Holmberg 1887 e híbrido tambacu (*P. mesopotamicus* x *C. macropomum*), mantidos em cultivo intensivo. As concentrações plasmáticas de glicose no híbrido tambacu foram maiores que em *P. mesopotamicus* e *C. macropomum*. Os níveis de proteínas totais em *P. mesopotamicus* foram maiores em *C. macropomum* e híbrido tambacu. O C. *macropomum* apresentou maior concentração de sódio e cloreto, ao passo que o *P. mesopotamicus* mostrou maior concentração de potássio e magnésio. Porém, os níveis de cálcio mostraram-se similares nas três espécies estudadas. O híbrido tambacu apresentou os menores níveis de proteínas totais e níveis intermediários de potássio, sódio, magnésio e cloreto quando comparado ao *P. mesopotamicus* e C. *macropomum*. Foram estabelecidos valores basais para peixes sadios criados em cativeiro, os quais poderão ser usados em estudos de comparação futura em populações selvagens de *P. mesopotamicus* e *C. macropomum*.

PALAVRAS-CHAVES: Bioquímica, *Colossoma macropomum*, peixes de água doce, *Piaractus mesopotamicus,* sangue.

INTRODUCTION

Interspecific crossing has been used in several fish species to increase genetic and phenotypic variation for both commercial and research purposes (CNAANI et al., 2004). In Brazil, *Piaractus mesopotamicus* Holmberg, 1887 and *Colossoma macropomum* Cuvier, 1818, both species of great economical importance originated the hybrid tambacu. These fish are widely appreciated in fish farming due to quick growth and weight gain, in addition to sportive and pleasure fishing. However, there is little knowledge of the blood biochemical profile of these fish.

The assessments of blood biochemical parameters are important to evaluate the health of many vertebrates, including fish (CNAANI et al., 2004; PEDRO et al., 2005; TAVARES-DIAS & MORAES, 2007). Since blood and biochemical parameters in fish may vary with ambient and other factors (BENTINCK-SMITH et al., 1987; TAVARES-DIAS, 2004; TAVARES-DIAS & MORAES, 2004; TAVARES-DIAS et al., 2004; PEDRO et al., 2005; TAVARES-DIAS & MORAES, 2007), they have been used by fish biologists for a variety of purposes, such as detecting cellular damage caused by toxicant exposure, infection by pathogenic agents, and traumatic handling. In addition, the blood biochemical assessments can also be used to evaluate the effects of the diet on liver function, and the osmoregulatory and ionoregulatory functions, effects of sex and maturation cycle and responses to stressors.

In freshwater fish, transmembrane ion gradients are the key for several physiological processes. Gill cells are exposed to different ion gradients across their apical and basolateral surfaces as a result of their exposure to water and blood, respectively, requiring different membrane transport mechanisms for intracellular and blood ionic balance. Thus, when sodium concentrations are increased, potassium is generally decreased in fish plasma or serum (FURIMSKY et al., 1999). The maintenance of a consistent internal environment is essential for normal cells to function in multicellular organisms. The ionic composition of this internal environment, the extracellular fluid, must remain within narrow limits to maintain the transmembrane electrical potentials of different cell types. Specialized organs and organ systems have evolved in vertebrates to carryout the maintenance of the homeostasis of fluid compartments. Most commonly, the kidneys are the organs that play a major role in controlling fluid and electrolytes (BALDISSEROTTO, 2002; BRAUN, 2003). However, in fish, renal function may be considered of a relatively minor role depending on the external environment. Thereby for many fishes, the gills, bladder and intestine make large contributions to ionic and fluid regulation (BRAUN, 2003).

Calcium taken up from the water in freshwater fish follows a transcellular, hormone-controlled pathway located in the chloride cells of the gills. Magnesium is an essential element for all vertebrates, indispensable for proper functioning of all cells, involved in a variety of enzymic reactions (FLIK et al., 1993) in intermediate metabolism, skeletal tissue metabolism, osmoregulation, and neuromuscular transmission (LIM & KLESIUS, 2003). At least 80% of the magnesium required for growth and homeostasis is absorbed from the food via intestine, while the gills contribute with less than 20% (FLIK et al., 1993). In fish, the sodium and potassium are predominant electrolytes, with the predominance of sodium in the serum and in other fluids and potassium in extracellular fluids. The main function of proteins and electrolytes, mainly the sodium and potassium, is to regulate the acid-basic balance maintaining thereby an ionic adequacy on the tissue functions (DAVIS, 2004; TAVARES-DIAS, 2004; TAVARES-DIAS et al., 2008). The levels of glucose and osmoregulatory disturbances can be used as stress indicators in fish (BENTINCK-SMITH et al., 1987; ZUIM et al., 1988; CNAANI et al., 2004; DAVIS, 2004; TAVARES-DIAS & MORAES, 2007). Thus, many parameters have been used as quantitative indicators of stress.

In the present study it was proposed to determine the baselines values of plasma glucose, total protein and serum ions for *P. mesopotamicus* and *C. macropomum* and also for their hybrid, the tambacu, because biochemical profile of these fish when kept in intensive system has not been reported.

MATERIALS AND METHODS

Fish

Forty clinically healthy specimens of young *Piaractus mesopotamicus* (301.5-839.0 g and 24.5-34.0

cm), Colossoma macropomum (369.5-873.0 g and 26.0-37.0 cm) and hybrid tambacu (199.0-418.5g and 22.0-29.0 cm) were obtained from a commercial fish farm (21º 07'45"S, 48º03'57"W), and transported to the Aquaculture Center (CAUNESP), Jaboticabal, São Paulo, Brazil. These fish were maintained in twelve cement boxes (500 L) and acclimatized for 15 days, with controlled flow-through water system. These fish were fed with pelleted diets (35% of crude protein) once a day. During the acclimatization and study periods the fish were observed daily for any clinical signs of diseases, including lack of appetite, increased opercular movements, or visible lesions of the skin, tail and fins. Signs of disease were not apparent during either period of observation. The water temperature varied from 25.1 to 29.8ºC; pH 6.5 to 7.0; dissolved oxygen 5.2 to 6.3 mg/L; electric conductivity 111.6 to 139.5 µS/cm.

At the fish farm, the three species lived in similar water conditions. This fact prevented major differences in the maintenance of the fish. The *P. mesopotamicus*, *C. macropomum* and hybrid tambacu fry received pelleted and extruded fish diets with 42% and 35% of crude protein, respectively. After that, fish were stocked in ponds of 1,000 m^2 that received extruded ration with 28% of protein. During this period the water temperature was within 24.5-30.2ºC; pH 6.7-6.9; dissolved oxygen 5.4-6.2 mg/L; electric conductivity 115.6-129.8 µS/cm and pluviometric index 0.0-342.0 mm^3

Blood collection procedure and biochemistry analyses

Two blood samples were collected by puncture of the caudal vessel as follows, one with syringe containing 10%-EDTA and another without any anticoagulant. All these procedures occurred within 0.8-1.2 minutes, to avoid sampling stress. Blood samples collected with anticoagulant were used for the determination of plasma glucose by glucose oxidase. The second blood sample, which was taken without anticoagulants, was left for 10 minutes at room temperature and then centrifuged for 10 minutes at 750 G to obtain the serum that was collected and frozen at -80ºC until analyses were done. Total serum protein concentration was determined by the biuret reaction. Serum ion concentrations were determined in flame photometer (Zeiss M4Q2) by emission (sodium and potassium) and atomic absorption (calcium and magnesium). Serum chloride concentration was determined by the thiocynate method using a commercial kit (Sigma 461).

Statistical analysis

The parameters were statistically analyzed using ANOVA followed by Tukey test, when the differences were significant ($p<0.05$).

RESULTS AND DISCUSSION

Biochemical parameters assessments of blood are important in evaluating the fish health (TAVARES-DIAS & MORAES, 2004; PEDRO et al., 2005; TAVARES-DIAS & MORAES, 2007), in captivity or wild species. In intensive culture, osmoregulatory disturbances can occur due to diseases and several other factors (BENTINCK-SMITH et al., 1987; STOSKOPF, 1993; TAVARES-DIAS, 2004). Hence, aquaculture needs constant accompainment of fish health status to avoid compromising performance parameters and the financial aspects of fish farming. Biochemical baselines values established may allow important clinical decisions about fish species (TRIPATHI et al., 2003; TAVARES-DIAS & MORAES, 2007).

C. macropomum have higher concentration of sodium and chloride than *P. mesopotamicus* and tambacu, but *P. mesopotamicus* presented higher potassium and magnesium concentration and smaller sodium concentration, while calcium level was similar for these three fishes (Table 1). Since these species are originated from the same environment and similar conditions, such variation levels are specific-species. Interspecific variation have been also reported for protein, calcium and magnesium levels in *Oreochromis aureus, O. mossambicus, O. niloticus* and hybrid red tilapia (CNAAI et al., 2004), for sodium, potassium and chloride levels in *Brycon amazonicus* (= *cephaus*) and *B. orbignyanus* (TAVARES-DIAS, 2004), as well as for sodium, calcium, magnesium and chloride in *Leporinus macrocephalus* and *Prochilodus lineatus* (TAVARES-DIAS et al., 2008). Furthermore, minerals in the water, adequate gill function, and alterations in water intake influence biochemical parameters in fish (TRIPATHI et al., 2003).

It has been stated that the glucose concentration depends on the fish life mode, and particularly on its locomotive capacity (CARNEIRO & AMARAL, 1979). Moreover, glucose levels have been attributed to the stress caused by the low oxygen rate, the manipulation and diet, and also to brachial damage. The characids *C. macropomum* (VILLACORTA-CORREA & SAINT-PAUL, 1999) and *P. mesopotamicus* (VAZ et al., 2000) are omnivorous and migratory fishes, which when in natural environment, develop as alimentary strategy the accumulation of reservations during periods of high food offer, to survive thereby in periods of shortage. BALDISSEROTO (2002) reported that fish with omnivorous habits are often exposed to natural food with variable quantity of carbohydrates. They need to be capable to regulate the glucose absorption according to its available quantity in the food. The glucose levels were higher in hybrid tambacu than in *P. mesopotamicus* and the latter one presented higher levels of glucose than *C. macropomum* (Table 1). Interspecific variation in glucose levels was also reported for fish species of different ecological habitat (HRUBEC & SMITH, 1999; RODRIGUES et al., 1999). In contrast, in *L. macrocephalus* and *P. lineatus*, which have different ecological habitats, the values of plasma glucose were similar (TAVARES-DIAS et al., 2008). However, in *Brycon amazonicus* and *B. orbignyanus*, which have similar ecological habitat, the values of plasma glucose were similar (TAVARES-DIAS, 2004), as expected. Biochemical parameters diverge among individuals and among species, too (TAVARES-DIAS & MORAES, 2007), and glucose seasonal changes (BENTINCK-SMITH et al., 1987).

TABELA 1. Plasma glucose, total serum protein and serum electrolytes for *P. mesopotamicus* (N= 40), *C. macropomum* (N=40) and hybrid tambacu (N=40) in intensive culture. Mean values ± standard deviation. Values among parentheses are range. Different letters indicate significant differences between species by Tukey test ($p < 0.05$)

Species	Glucose (mg/dL)	Total protein (g/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (mmol/L)	Magnesium (mmol/L)	Chloride (mmol/L)
C. macropomum	62.1 ± 16.5a	3.5 ± 0.2b	155.0 ± 3.8a	3.4 ± 0.9b	2.7 ± 0.2a	1.2 ± 0.2b	123.0 ± 6.8a
	(31.7–102.9)	(2.9–4.1)	(146.0–162.0)	(1.8–5.5)	(2.1–3.1)	(0.9–1.8)	(111.0–141.0)
P. mesopotamicus	78.1 ± 33.2c	4.7 ± 1.0a	136.0 ± 3.3c	4.6 ± 0.8a	2.7 ± 0.2a	1.4 ± 0.5a	110.9 ± 5.6b
	(36.1–201.7)	(3.8–8.2)	(129.0–145.0)	(3.0–6.0)	(2.2–3.3)	(0.7–2.8)	(102.0–121.0)
Hybrid tambacu	100.6 ± 31.9b	3.2 ± 0.3c	149.4 ± 8.0b	3.7 ± 1.3b	2.6 ± 0.3a	1.3 ± 0.2b	114.0 ± 11.2b
	(61.1–192.8)	(2.8–3.8)	(127.0–163.0)	(1.8–8.5)	(2.1–3.2)	(0.9–1.6)	(91.0–144.0)

In the present study, total protein levels of tambacu were smaller than in *C. macropomum* and *P. mesopotamicus*, but in this latter it was higher than in *C. macropomum*. Similar interspecific variation in total protein levels has been reported for *L. macrocephalus* and *P. lineatus* of intensive culture (TAVARES-DIAS et al., 2008). However, total protein levels of *P. mesopotamicus* were similar to this same wild specie when in captivity (RANZANI-PAIVA & GODINHO, 1988) and in natural habitat (ZUIM et al., 1988). Hence, the values found here may be considered baselines for cultured *P. mesopotamicus*. On the other hand, total protein levels of *C. macropomum* were higher than of this same specie cultivated in net cages (CHAGAS et al., 2005).

A low level of total protein may occur in consequence of alimentary restriction (CHAGAS et al., 2005), of protein synthesis reduction due to hepatic disfunction, and also in consequence of a permeability increase for plasma proteins or of proteins degradation by the proteolytic enzymes that are released from endothelial cells destroyed by virus or bacteria (STOSKOPF, 1993). On the other hand, increases of total protein levels may be associated to hemolysis (HRUBEC & SMITH, 1999). Thus, it has been reported that captive adults of *P. mesopotamicus* have higher levels of plasma total protein when compared to wild fish due to the reduction in energy expenditure and the reproductive blockage that occurs in lack of migration (ZUIM et al., 1988). Therefore, these results indicate how important it is to establish baseline values, which can be used to further assessment of fish health status.

CONCLUSION

Tambacu hybrid had the smallest levels for total protein and intermediate levels for sodium, potassium, magnesium and chloride when compared to *P. mesopotamicus* and *C. macropomum*. Only calcium levels were similar for the fish studied. It is unlikely that species different in taxonomic and ecological affinities have identical metabolic patterns that result in uniform blood biochemistry values. Since biochemical parameters may be affected by several factors, to avoid these variations, hybrid tambacu, *P. mesopotamicus* and *C. macropomum* were sampled from a defined reference population that was maintained and managed under identical environmental conditions. Furthermore, laboratory procedures to analyze the serum specimens were also performed through standardized techniques and protocols. Hence, the biochemical levels variation found were specific-species. Therefore, the baseline values obtained here may provide general guidelines for the interpretation of laboratory data for these species of fish, which are very important for Brazilian aquaculture.

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