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ANIMAL SCIENCE

Glucose tolerance in six fish species reared in Brazil: Differences between carnivorous and omnivorous

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Abstract: The objective of this study was to evaluate the effect of pure glucose, glucose plus fructose, and fructose on the blood glucose of omnivorous fish tambaqui (*Colossoma macropomum*), Nile tilapia (*Oreochromis niloticus*), piau (*Leporinus elongatus*), and carnivorous fish hybrid Amazon catfish (*Pseudoplatystoma fasciatum* × *Leiarius marmoratus*), pacamã (*Lophiosilurus alexandri*), and traíra (*Hoplias malabaricus*). In each species, the dose 1 mL per fish with 1,000 mg kg of body weight^{-1} of glucose, fructose or glucose plus fructose were tested intraperitoneally. Blood glucose was measured at times 0 (control), 0.5, 1, 2, 4, 8, 16, and 24 h. The administration of 1,000 mg of glucose or glucose plus fructose per kg of live weight causes hyperglycemia in the omnivorous and carnivorous species studied. In the omnivorous species, glycemic levels were reduced from 2 to 4 h, and the regulation to baseline occurred from 4 to 8 h. In the carnivores fish, blood glucose levels declined between 1 and 8 h, and return to baseline was observed from 8 to 16 h. Tambaqui was also intolerant to high concentrations of fructose. Blood glucose levels are regulated in a shorter time in Nile tilapia (mainly), piau and pacamã.

Key words: fructose, hyperglycemia, intraperitoneal, Nile tilapia, tambaqui.

INTRODUCTION

The incorporation of carbohydrates in fish diets may reduce the amount of protein in the formulation, which has benefits for fish farmers, as it reduces feed costs because protein is one of the most expensive items in the fish diet (Felix e Silva et al. 2020). In addition, appropriate carbohydrate supplement in diet can improve the fish growth and reduce ammonia nitrogen excretion to water (Liu et al. 2018). Therefore, it has been widely used in practical fish diets.

Among the important nutrients that provide energy to be used for the maintenance of the cell vital processes, glucose stands out as a source of carbohydrates. Studies of blood glucose can contribute to the identification of the fish species most and least able to utilize carbohydrates in their diet, through responses to glycemic rhythms (Sánchez-Vázquez & Madrid 2001), and of the biochemical adaptation of this metabolite for the synthesis of glycogen or lipids in fish grown in tropical environments (Melo et al. 2006, Souza et al. 2019). In this sense, an important tool for understanding carbohydrate metabolism is the application of the glucose tolerance test, which can be intraperitoneally administered (Takahashi et al. 2018). A glucose-tolerance test is undertaken to evaluate the ability of fish to use glucose, indicating the period during which glucose remains in the blood or even its inability to be mobilized to tissues (Enes et al. 2012).

The regulation of glycemic response in teleosts is different than in other vertebrates (Polakof et al. 2012). Fish may remain a long time with prolonged postprandial hyperglycemia above the normal values for the species, which could lead to negative growth performance (Moon 2001). In addition, the use of carbohydrates is different among herbivorous, omnivorous and carnivorous fish. Digestive organs in fish vary in size, where, as is generally known, herbivorous fish have the largest intestinal tracts, followed by omnivores and carnivores, respectively. Herbivorous fish use carbohydrates better than other fish (Polakof et al. 2012). Carnivorous fish have little ability to use glucose as a source of energy (Enes et al. 2009). The use of carbohydrates is also different among omnivorous fish. Fish with this eating habit have great variation in morphology and physiology in digestive tracts (Rodrigues et al. 2012), which may affect their ability to synthesize carbohydrates.

The species utilized in this study have economic, environmental, and animal-production importance that may contribute to aquaculture production in South America region, mainly in Brazil. Omnivorous species like the exotic Nile tilapia (*Oreochromis niloticus*), and natives tambaqui (*Colossoma macropomum*), and piau (*Leporinus elongatus*), and carnivore species like the natives pacamã (*Lophiosilurus alexandri*), and traíra (*Hoplias malabaricus*) and hybrid Amazon catfish (*Pseudoplatystoma fasciatum* × *Leiarius marmoratus*) are produced in fish farms on a considerable scale in Brazil (Baldisserotto & Gomes 2020).

The objective of the present study was to evaluate the glycemic response (return to baseline levels verified at time 0 h) of six fish species reared in Brazil during 24 h after challenge with intraperitoneal administration (IPA) of pure glucose (GLU), glucose plus fructose (GLU+FRU), or pure fructose (FRU).

MATERIALS AND METHODS

Experimental design

The omnivorous fish tambaqui (32.83 ± 0.79 g), Nile tilapia (36.83 ± 2.12 g) and piau (35.00 ± 1.37 g), and the carnivorous fish pacamã (37.67 ± 1.20 g), traíra (37.00 ± 1.50 g) and hybrid Amazon catfish (43.50 ± 0.80 g) were selected for the current study. The experimental protocol was approved by the Animal Ethics Committee of the Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil (number 0004/180917).

The water physical–chemical parameters remained stable throughout the adaptation and experimental period. Temperature (25.80 ± 0.24 °C) and dissolved oxygen (5.21 ± 0.11 mg O_2 L^{-1}) (Oximeter; Linelab DO Eco, Esteio, Brazil), pH (7.43 ± 0.07) (pH meter; Hanna HI 98130, Barueri, SP, Brazil), total ammonia (0.04 ± 0.02 mg NH_3 L^{-1}) and alkalinity (50.00 ± 0.00 mg $CaCO_3$ L^{-1}) (kit; Alfatecnoquímica, Florianópolis, Brazil) were monitored.

For 30 days before the experiments began, the fish were fed a commercial diet containing 36% crude protein for tilapia, tambaqui, and piau; and 40% crude protein for pacamã and hybrid Amazon catfish; traíra received a natural diet composed of frozen fish (*Astyanax* spp.; crude protein of 16.86-19.45% and 67.44-77.80% in organic and dry matter, respectively; Signor et al. 2008). The diet used for the acclimatization period was chosen according to fish species in order to satisfy its protein requirements. The fish were fasted for 24 h before the experiments. Forty-eight individuals of each species were housed in 500-L tanks (n = 6 fish per tank).

Glucose tolerance test

For the glucose tolerance test, at time 0 h, all fish were anaesthetized with benzocaine (0.1 g L^{-1}), which causes no oxidative stress (Stringhetta et al. 2017) or increase in plasma glucose levels (Gomes et al. 2001). After, they were weighed and administered an IP injection at a volume of 1 mL per fish with 1,000 mg kg of body weight^{-1} of GLU (dextrose; 180.16 g mol molecular weight^{-1}; Biotec®, São José dos Pinhais, Brazil), FRU (levulose, 180.16 g mol molecular weight^{-1}; Biotec®) or GLU+FRU. The dose used was based on study of Chen et al. (2018). Glucose and fructose were dissolved in distilled water. Control fish (n = 6 per species) were injected with equivalent volumes of 0.9% saline solution and samples were taken from these at baseline only (0 h).

After the challenge, blood samples were drawn from the caudal vein at the following times: 0 (control), 0.5; 1; 2; 4; 8; 16 and 24 h to determine blood glucose. Glucose was measured using a digital glucometer (Accu-Chek Roche Diagnosis®, São Paulo, Brazil) immediately after blood collection. The experiment started at 11 a.m. Natural lighting was used (approximately 12:12 h light/dark photoperiod, sunrise at 6 a.m.). Only for blood collection at the times of 8 and 16 h, it was necessary to use artificial light, as during these times the blood collection occurred at night.

Statistical analysis

All data are expressed as the mean ± standard error of the mean. Data were subjected to Levene's test to verify the homogeneity of the variances, and the Shapiro–Wilk test to verify the normality. Comparisons between different treatments were made using two-way analysis of variance (treatments × time), followed by Tukey's test. Comparison with the control group was made using Dunnett's method. Significance was set at $p < 0.05$.

RESULTS

In tambaqui, blood glucose levels were significantly higher at 2 and 4 h in the GLU group and at 2 h in the GLU+FRU group than at other times in the same treatment or in other treatments at the same time ($p < 0.05$). After IP injection, blood glucose levels were significantly lower in the GLU than in the GLU+FRU group at 0.5 h and lower than in the GLU group at 1 h ($p < 0.05$). After 8 h, the blood glucose levels had decreased in almost all treatments ($p < 0.05$) and had returned to baseline levels (except in the GLU group at 8 h) (Figure 1a).

The IP injection of GLU or GLU+FRU elevated blood glucose levels in Nile tilapia between 0.5 and 2 h and in piau between 0.5 and 4 h ($p < 0.05$). In addition, in Nile tilapia, blood glucose levels were significantly higher at 0.5 h in the GLU and GLU+FRU groups than at other times and at 1 h in the FLU group than at 8 and 16 h ($p < 0.05$). In piau, at 4 h after IP injection, blood glucose levels were significantly lower in the GLU group than at the times between 0.5 and 2 h ($p < 0.05$). Nile tilapia and piau in the FLU group did not present hyperglycemia and in the other groups they had returned to baseline blood glucose levels by 4 and 8 h, respectively (Figure 1b, c).

In pacamã, blood glucose levels were significantly higher in the GLU than in the FRU group between 0.5 and 4 h and, and higher than in the GLU+FRU group at 0.5 and 1 h ($p < 0.05$). Blood glucose levels were significantly higher in the GLU+FRU than the FRU group at 1 and 2 h after IP injection ($p < 0.05$). In general, blood glucose levels were significantly higher in the GLU and GLU+FRU groups at 0.5 and 1 h and at 1 and 2 h, respectively, than at other times in the same groups ($p < 0.05$) (Figure 2a).

The IP injection of GLU or GLU+FRU elevated blood glucose levels in traíra and in hybrid

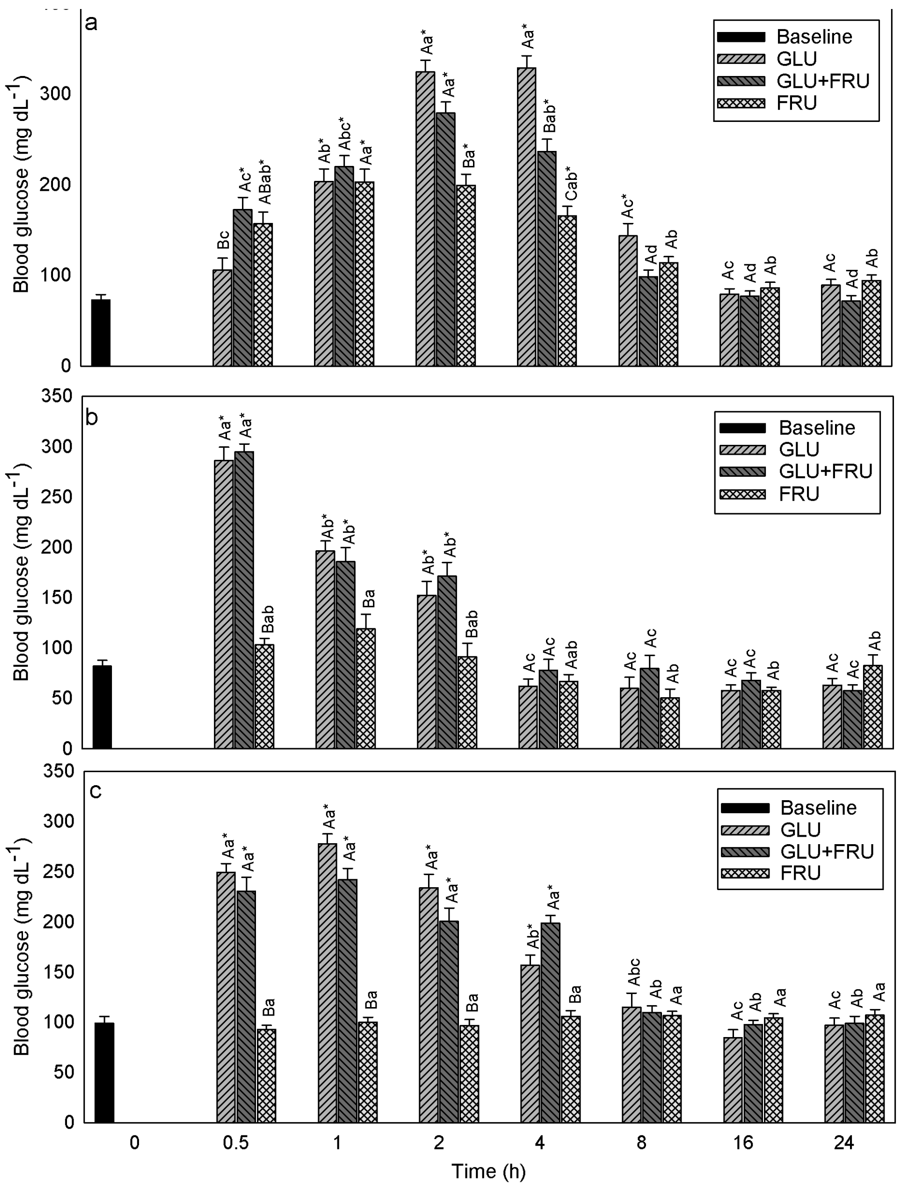

Figure 1. Blood glucose levels over a 24-h period for omnivorous fish tambaqui (a), Nile tilapia (b) and piau (c) submitted to intraperitoneal administration of glucose (GLU), fructose (FRU) or glucose + fructose (GLU+FRU) (1000 mg kg body weight^{-1}). Values are the means ± SEM (n = 6). Different upper-case letters indicate significant differences between treatments, while lowercase letters indicate significant differences between sampling times (Tukey's test: $p <$ 0.05). *indicates significant difference from baseline concentration (Dunnett's test: $p < 0.05$).

Amazon catfish between 0.5 and 8 h (except at 8 h in hybrid Amazon catfish administered GLU+FRU) ($p < 0.05$). In hybrid Amazon catfish, blood glucose levels were significantly higher in the GLU and GLU+FRU groups between 1 and 4 h, and at 0.5 and 8 h, respectively in relation to control group ($p < 0.05$). In traíra, blood glucose levels were significantly higher at 4 h in the GLU group in relation to other times (except 2 h) and at 2 and 4 h in the GLU+FRU group in relation to times between 8 and 24 h ($p < 0.05$). In addition, at 8 h, blood glucose levels were significantly higher in the GLU than the GLU+FRU group ($p <$ 0.05). In general, traíra and hybrid Amazon catfish administered FLU did not present hyperglycemia and fish in the GLU and GLU+FRU groups had returned to baseline glucose levels after 16 h (Figure 2b, c).

For IPA of GLU or FRU, the fastest times to reach blood glucose peak and return to baseline levels were achieved by Nile tilapia (0.5 and 4 h, respectively), pacamã (0.5-2 and

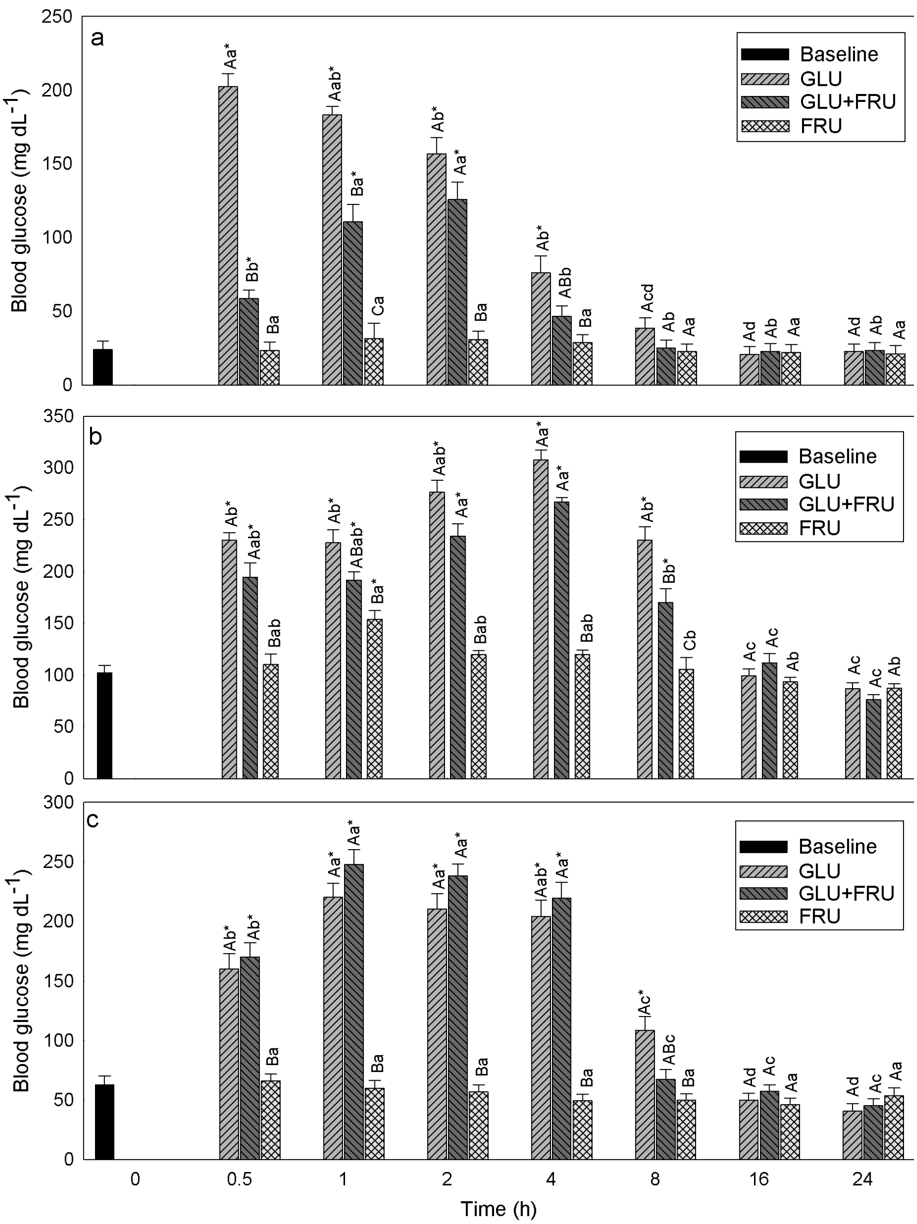

Figure 2. Blood glucose levels over a 24-h period for carnivorous fish pacamã (a), traíra (b) and hybrid Amazon catfish (c) submitted to intraperitoneal administration of glucose (GLU), fructose (FRU) or glucose + fructose (GLU+FRU) (1000 mg kg body weight^{-1}). Values are the means ± SEM (n = 6). Different upper-case letters indicate significant differences between treatments, while lowercase letters indicate significant differences between sampling times (Tukey's test: $p < 0.05$). *indicates significant difference from baseline concentration (Dunnett's test: $p < 0.05$).

4-8 h, respectively) and piau (0.5-4 and 8 h, respectively). The other species (tambaqui, traíra and hybrid Amazon catfish) varied peak times between 1 and 4 h, but recovery times were at least 16 h for one of the treatments (GLU or FRU). However, for IPA of GLU+FRU, only traíra and tambaqui had blood glucose peak in up to 2 h after IPA, returning to baseline levels at 2 and 8 h, respectively (Table I).

DISCUSSION

In the present study, the IPA of GLU or GLU+FRU load increased blood glucose levels 0.5 or 1 h later in fish, showing that glucose is quickly absorbed and transported by the bloodstream. The reduction in blood glucose levels 4 h after IP injection of glucose in Nile tilapia load reinforces the good ability of this specie to cope with glucose loading (Chen et al. 2018). Piau and pacamã showed a reduction in blood glucose

Table I. Time of blood glucose peak and retorn to baseline levels over a 24-h period for six fish species submitted to intraperitoneal administration (IPA) of glucose (GLU), fructose (FRU) or glucose + fructose (GLU+FRU) (1000 mg kg body weight^{-1}).

Fish species	IPA	Peak (h)	Return (h)
	GLU	2-4	16
Tambaqui	FRU	2	8
	GLU+FRU	1-2	8
	GLU	0.5	4
Nile tilapia	FRU	0.5	4
	GLU+FRU	-	-
	GLU	0.5-2	8
Piau	FRU	0.5-4	8
	GLU+FRU	-	-
	GLU	0.5	8
Pacamã	FRU	1-2	4
	GLU+FRU	-	-
	GLU	4	16
Traíra	FRU	2-4	16
	GLU+FRU	1	2
	GLU	1-2	16
Amazon catfish	FRU	1-4	8
	GLU+FRU	-	-

The peak time was only considered when blood glucose levels differed significantly from baseline time (0 h) (Dunnett's test: $p < 0.05$).

levels 8 h after glucose IP injection and they were found to cope with glucose loading more rapidly than tambaqui, traíra and hybrid Amazon catfish.

In general, carnivorous fish utilize dietary carbohydrates worse than omnivorous and herbivorous ones due to striking morphological and physiological differences in their digestive tracts (Gominho-Rosa et al. 2015). In addition, although carnivorous fish are mostly classified as glucose-intolerant, the current study finds that species showed different degrees of intolerance. Pacamã is a carnivorous fish, but in the present study it rapidly reduced its hyperglycemia similarly to omnivorous fish. This may be due to its hypoglycemic characteristic, as it was the species with the lowest baseline blood glucose levels (24.00 ± 5.63 mg dL^{-1}). Tambaqui, in turn, reduced its glycemia more slowly after IP injection of GLU, with times similar to that observed in the carnivorous fish traíra and hybrid Amazon catfish. So, the time taken for blood glucose to be regulated was moderately long, with the fish remaining hyperglycemic during this period.

It is possible that the period of hyperglycemia in the fish studied here is associated with their degree of glucose tolerance. The findings from our study also cannot be dissociated from the stress of handling and IP injection, which were common to all fish and could contribute to increased blood glucose levels. This degree of tolerance or intolerance that leads to the maintenance of hyperglycemia is determined by several physiological factors, such as insulin secretion, the glycogen storage capacity, the utilization of glucose by the tissues, and nervous and hormonal stimuli generated by the intake of glucose (Polakof et al. 2012). It is likely that many of these factors act together, depending on the conditions to which the fish is subjected. In fact, the mechanisms that would help to maintain glucose homeostasis in fish have not yet been completely elucidated. A reduction in insulin secretion is one of the most commonly referenced causative factors of hyperglycemia (Moon 2001). The glucose homeostasis also involves the coordinated regulation of many metabolic pathways, including gluconeogenesis and glycolysis (Walker et al. 2020).

The severe hyperglycemia observed in tambaqui, traíra and hybrid Amazon catfish could be, in part, a consequence of the failure of glucose utilization in peripheral tissues and/or absence of inhibition of endogenous glucose production (Enes et al. 2009). Fish with specific feeding habits may have lost some of their metabolic capacity to use increased blood glucose after carbohydrate intake, for example, carnivorous species that do not normally ingest carbohydrates in their natural diets (Polakof et al. 2012). In fact, most teleost fish species (mainly carnivorous) have impaired glucose tolerance and they often exhibit prolonged postprandial hyperglycemia after a carbohydrate-rich meal or glucose load (Moon 2001).

The increased blood glucose levels resulting from the IPA of fructose has been described as normal and occurs via gluconeogenesis (Dirlewanger et al. 2000). In the current study, in general, the blood glucose levels were similar in fish IP injected with GLU or GLU+FRU. When ingested excessively with glucose, fructose absorption is reduced, requiring equimolar doses for better transport (Smith et al. 1995). There is a clear interrelationship between the metabolism of fructose and glucose, because the former, when IP administered, is captured by the liver cells and converted to glucose and mainly glycogen. This has been observed during gluconeogenesis, when IPA of FRU leads to an increase in blood glucose and glycogen (Koo et al. 2008).

Finally, in the present study, the application of FRU only caused hyperglycemia in tambaqui. Despite the tambaqui's frugivorous habit, we observed higher blood glucose levels in the FRU than the GLU group only 0.5 h after IPA. In channel catfish (*Ictalurus punctactus*), fructose appeared to be poorly absorbed from the intestine and was not converted to glucose after oral carbohydrate tolerance tests (Wilson & Poe 1987). So, the lower absorption of fructose from the intestinal tract could explain the lower blood glucose levels found in the FRU group at 2 and 4 h compared to the GLU group after IP injection in tambaqui, which could indicate that dietary fructose is not a promising carbohydrate source for tambaqui diets.

CONCLUSIONS

High IPA of GLU or GLU+FRU led to hyperglycemia in the studied fish, which demonstrates that they are glucose-intolerant in these conditions. Furthermore, tambaqui was intolerant to a high concentration of FRU. Glucose elimination ability is species-dependent; blood glucose levels were regulated most rapidly by Nile tilapia, followed by piau and pacamã, and these three fish species are thus the most glucose-tolerant, allowing the administration of diets with cheaper ingredients, such as carbohydrates.

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Author contributions

Anderson M. de Souza: carried out the experiments, physiological analysis and discussion of the results. Carlos E. Copatti: statistical analysis, collaboration on discussion, final text and supervised the findings. Daniela F.B. Campeche: collaboration on data sampling and physiological analysis. Fúlvio V.S.T. Melo: collaboration on data sampling and discussion. José F.B. Melo: conception and design, discussion of the results and supervised the findings.