Rev. Bras. Saúde Prod. Anim., Salvador, v.22, 01 - 09, e2122232021, 2021
http://dx.doi.org/10.1590/S1519-99402122232021

Fishing Resources / Aquaculture
Received on: 27/04/2021
Accepted on: 28/07/2021

Acute response of refeeding in tambaqui submitted to long-term fasting

Respostas agudas da realimentação em tambaquis submetidos ao jejum prolongado

[1]SANTOS, Renata Franco dos
https://orcid.org/0000-0002-8433-9022

[1]LÔBO, Maria Vitória Marialva da Silva
https://orcid.org/0000-0003-0902-2727

[1]SILVA, Shamila Évellem Magalhães da
https://orcid.org/0000-0002-3810-2112

[2]PAZ, Deborah Jacob Freire da
https://orcid.org/0000-0002-8585-6064

[1]BRANDÃO, Monique Ferreira
https://orcid.org/0000-0002-6797-9554

[1*]GIMBO, Rodrigo Yukihiro
https://orcid.org/0000-0001-8913-3165

[1] Programa de Pós-graduação em Aquicultura, Universidade Nilton Lins. Avenida Nilton Lins, 3259, 69058-030, Manaus-AM, Brasil.
[2] Graduação, Universidade Nilton Lins. Avenida Nilton Lins, 3259, 69058-030, Manaus-AM, Brasil.
*Mail for correspondence: rodrigo.gimbo@uniniltonlind.edu.br

ABSTRACT

This study aimed evaluate energetic mobilization in tambaqui submitted to fasting, after a one-day refeeding. 120 tambaqui juveniles were distributed in 12 310L polyethylene boxes. Three treatments were evaluated: Control (14 days of feeding); Fasting for 14 days; and Refeeding (13 days fasting and one day of feedback). After the experimental period, the fish were anesthetized with eugenol for blood collection and serum and plasma were used to measure glucose, triglycerides, cholesterol and serum protein. Subsequently, fish were euthanized to remove liver and mesenteric fat and were used to determine hepatic glycogen and lipid and mesenteric fat index. The results were submitted to ANOVA and the means compared by Tukey test when statistical significance was observed ($P < 0.05$). Glucose, triglycerides and serum protein decreased after fasting, differing statistically with the control. Refeeding resulted in the recovery of three blood indicators. Liver analysis shows glycogen was consumed intensely during fasting and partially recovered after refeeding, when compared to control group. The results obtained in this study suggest that the 14-day fast was not harmful to the fish and the tambaqui are able to quickly adjust their metabolism according to their nutritional status.
Keyword: amazon fish, energy mobilization, feed deprivation, metabolism

RESUMO

Este estudo objetivou avaliar a mobilização energética em tambaqui submetido ao jejum, após a realimentação de um dia. 120 juvenis de tambaqui foram distribuídos em 12 caixas

de polietileno de 310L. Foram avaliados três tratamentos: grupo controle (14 dias de alimentação); Jejum de 14 dias; e realimentado (13 dias em jejum e um dia de realimentação). Após o período experimental, os peixes foram anestesiados com eugenol para coleta de sangue e o soro e o plasma foram utilizados para dosagem de glicose, triglicerídeos, colesterol e proteína sérica. Posteriormente, os peixes foram eutanasiados para remoção de fígado e gordura mesentérica e foram usados na determinação de glicogênio e lipídio hepático e índice de gordura mesentérica. Os resultados foram submetidos à ANOVA e as médias comparadas pelo teste de Tukey quando observada significância estatística (P <0,05). Glicose, triglicérides e proteína sérica reduziram após o jejum, diferindo estatisticamente com o controle. Já a realimentação resultou na recuperação dos três indicadores sanguíneos. A análise no fígado mostra que o glicogênio foi consumido intensamente durante o jejum e recuperou parcialmente após a realimentação, quando comparados ao grupo controle. Os resultados obtidos neste estudo sugerem que o jejum de 14 dias não foi prejudicial aos peixes e os tambaqui são capazes de ajustar rapidamente seu metabolismo de acordo com seu estado nutricional.
Palavras-chave: peixe amazônico, mobilização de energia, restrição alimentar, metabolismo

INTRODUCTION

Feeding managements increase food efficiency and guarantee economic benefits for producers (KABIR et al., 2019; LUNA et al., 2019) and improve water quality to provide fish growth (CYRINO et al., 2010). An alternative to traditional feeding methods is fasting and refeeding protocol to stimulate better growth and use of dietary nutrients (ALI, 2003; URBINATI et al., 2014; HERRERA, 2016).

In natural environment fish are able to survive after being subjected to long-term fasting (NAVARRO; GUTIÉRREZ, 1995; BAR, 2014). During this period, energy reserves are mobilized due to the low food supply. It involves a series of endocrine, metabolic, and behavioral responses, which result in a decrease in the metabolic rate and mobilizes energy reserves to maintain the vital functions of the body, such as brain function, breathing, and regulation of the hydro-mineral balance, and others. (FURNÉ et al., 2012), and after the return to food, it is common to observe compensatory growth (MOMMSEN et al., 1999), but metabolic adjusts of refeeding are still unclear, meanly about acute responses son after firsts feeds. We hypothesized that as faster fish recover their energy reserves, faster and more efficient will be growth after refeeding.

Generally, glycogen is first substrate catabolized during fasting in most fish species, such as *Piaractus mesopotamicus* (SOUZA et al., 2003; TAKAHASHI et al., 2011; GIMBO et al., 2015), *Ictalurus punctatus* (PETERSON; SMALL, 2004), *Dicentrarchus labrax* (PÉREZ-JIMENÉZ et al., 2007; CHATZIFOTIS et al., 2011), *Rhamdia quelen* (BARCELLOS et al., 2010), *Prochilodus lineatus* (RIOS et al., 2011), *Dentex dentex* (PÉREZ- JIMENÉZ et al., 2012) and *Brycon amazonicus* (URBINATI et al., 2014), followed by lipid and protein reserves.

However, results after refeeding the recovery of energy reserves pattern may

vary depending on fish species, fasting intensity, quality of the food (FURNÉ et al., 2012; FAVERO et al., 2017; GIMBO et al., 2015). In addition, strategy adopted by fish regarding recovery of energy reserves after first hours of refeeding is still unknown. Such information is extremely important to determine intensity of fasting to possibility correct choose of fasting/re-feeding protocols.

Thus, we aimed to evaluate acute responses of refeeding in tambaqui (*Colossoma macropomum*), an omnivorous fish, with great ability in adjust to captive conditions, accepting formulated diets and also because it has interesting productive characteristics, which positions it as the most produced native species of continental waters in South America (GOMES et al. 2020; VALLADÃO et al., 2018).

MATERIAL AND METHODS

All procedures were performed at Laboratory of Fish Physiology and Metabolism of Nilton Lins University, under previous evaluation and approval of proposal by the Ethics Committee on the Use of Animals (CEUA - Registration number 003/2018) and fish were supplied by Institute National Research Institute (INPA, Manaus – Amazonas - Brazil).

120 tambaqui juveniles with an average weight of 118.6 ± 6.1 g were used, kept in 12 polyethylene tanks with 310 L (10 fish per tank) for two weeks to acclimatize to experimental conditions. All tanks were kept in a recirculation water system, containing aeration and individual biological filter, and heaters.

After this period, three treatments were randomly distributed into 12 tanks, which are: Control, fish fed every day; Fasting, fasting fish throughout the experiment and Refeeding, fish were not fed for 13 days and fed on the last day. Regarding management, animals were observed daily, and water quality was monitored by temperature (31.2 ± 0.5 ° C) and dissolved oxygen (5.4 ± 0.3 mg / L), and food were offered until apparent satiety, with commercial diet (6 mm Presence NUTRIPISCIS® 28% of crude protein, 4% of lipid), twice a day (9:00 am and 5 pm).

Blood sampling and analysis

At end of experimental period, three fish from each tank (n=12) were captured and anesthetized with eugenol (60 mg / L) for blood collection by puncturing the caudal vessel, then it was dispensed in two microtubes, one containing Glistab® (EDTA added with potassium fluoride), which was centrifuged at 3000rpm for 10 minutes (Hettich Universal 320R centrifuge) to obtain plasma, used to determine plasma glucose and triglyceride concentration, and another microtube without anticoagulant, to separate serum, used to determine cholesterol and serum protein, using commercial In Vitro test kits enzymes, following manufacturer's recommendations.

Tissue analysis

Fish were euthanized by brain injury to remove liver and used to determine the concentrations of hepatic lipid and glycogen. The methodologies used to obtain results were by MOON et al. (1989) for hepatic glycogen, while for lipids it was by BLIGH AND DYER (1959),

based on the extraction of the lipid with chloroform and methanol. Mesenteric fat was also weighed for calculation of mesenteric fat index (MFI) using the equation: MFI = Mesenteric fat weight / body weight.

Statistical analysis

The results were tested for statistical assumptions of normality by Cramer Von Mises test and homogeneity by Brown Forsythe test, and subjected to analysis of variance (ANOVA) and means compared by Tukey test when statistical significance was observed (P <0, 05) through software R.

RESULTS

To verify acute responses of the refeeding, fish were submitted to continuous feeding, fasting and refeeding and sampled after 14 days to evaluate blood and tissue responses of tambaqui juveniles.

During experiment, mortality was not observed and mean values of glucose, plasma triglycerides and serum protein showed the same response pattern, with higher averages being observed in control group, which were statistically different of fasted fish (P <0.05). Refed fish presented intermediate values, but did not differed from fasting and control fish (P>0.05). Regarding cholesterol, there was a numerical difference, no statistical difference was observed, but the high cholesterol in fish submitted to fasting stands out, in relation to the feed (Figure 1).

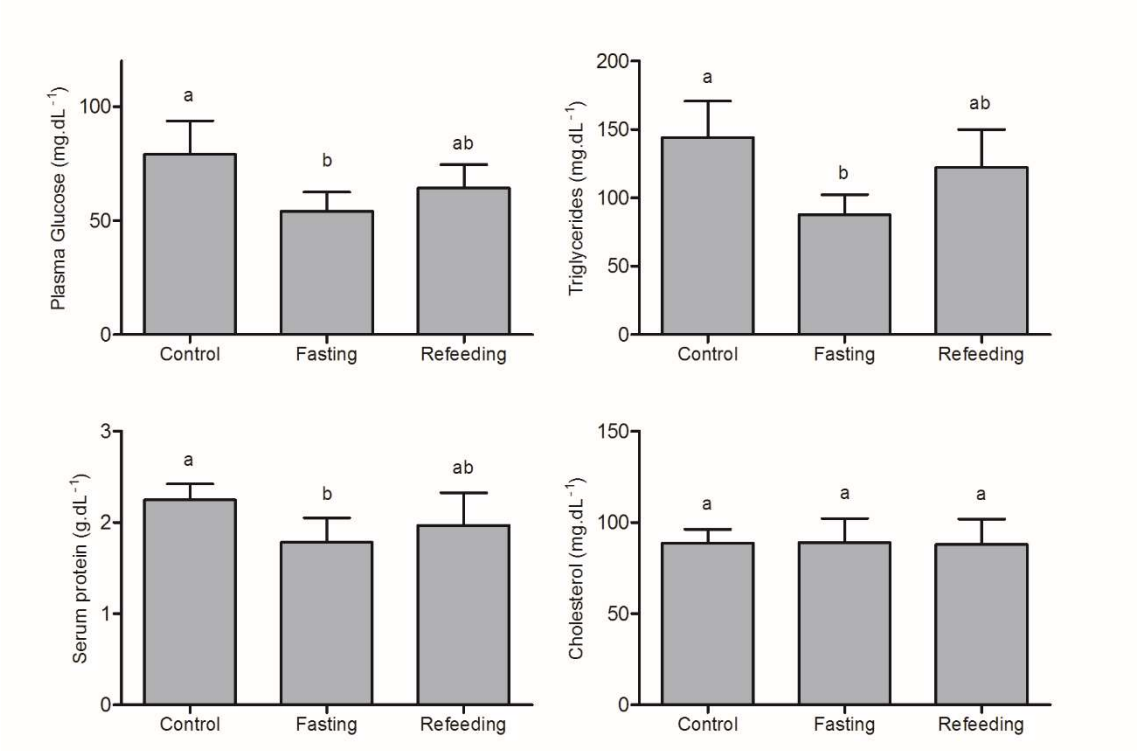

Figure 1. Blood glucose, triglycerides, serum protein and cholesterol in tambaqui submittes to fastind and refeeding. Different letters indicates statistical difference by Tukey test (P<0.05).

Regarding energy reserves of liver, the comparison of means shows reduction in concentration of glycogen after fasting, when compared to continuously fed fish, whereas acute refeeding exposed partial restoration of liver glycogen compared to fasting, but without reaching the values observed in the control group, differing statistically from both groups. Fasting fish showed a significant increase in lipid concentration in the liver, when compared to fish of control group. Refeeding provided a reduction in lipid levels to the same level observed in the continuously fed group ($P<0.05$) (Figure 2). The MFI of the fish in the control group (1.67 ± 0.08%) was statistically higher than that of the fish that were fasted and fed (0.41 ± 0.02% and 0.38 ± 0.05%, respectively), which did not differ from each other.

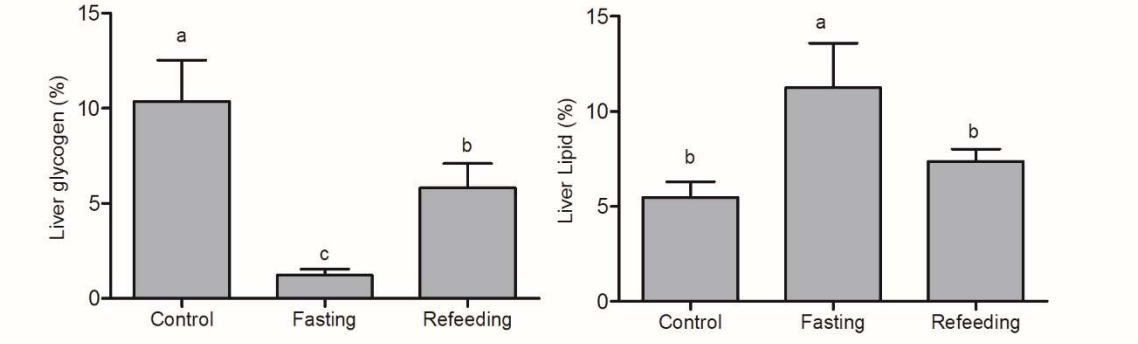

Figure 2. Liver glycogen and lipid in tambaqui submittes to fastind and refeeding. Different letters indicates statistical difference by Tukey test ($P<0.05$).

DISCUSSION

The reduction in blood glucose in fish submitted to fasting when compared to continuously fed fish, was not intense, considering the 14-day period. In the first moment, glycemia was maintained at the cost of glycogen reserves, which reduced until almost depleted, and in the second moment, gluconeogenesis proved to be a secondary source of glucose, based on the consumption of lipid and protein reserves, evidenced by the reduction in MFI, blood triglyceride and serum protein levels. This study is the first to evaluate metabolic response soon after one day refeeding, but fish showed, similar response to observed in *Acipenser baerii* (MORSHEDI et al., 2017) and *Piaractus mesopotamicus* (FAVERO et al., 2017) submitted to food deprivation and refeeding, increased triglyceride values.

In this study, an accumulation of hepatic lipid was observed in fish submitted to fasting, as observed in *Dentex dentex* (PÉREZ-JIMÉNEZ et al., 2012), is known as a liver esterification process. This process occurs when there is a portion of free fatty acids, resulting from hydrolysis from adipose tissue, carried by lipoproteins and than re-esterified and accumulated in liver (RODWELL et al., 2016). Refeeding reduced lipid accumulation in the liver, which differed from fasting. And compared to the

ISSN 1519 9940

control group, the lipid was almost at the same level.

Reduction in serum protein concentration in this study was due to use of amino acids as an energy source, since glycogen reserves were significantly consumed. Studies show an important reduction in concentration of serum protein in blood in different species subjected to long periods of fasting (PERES et al., 2014; PÉREZ-JIMÉNEZ et al., 2012). Already in a situation in which fasting is not as intense, there were no changes in accumulations of serum protein in blood of *Acipenser baerii*, as well as there was no such intense consumption of hepatic glycogen and lipid reserves that are totally consumed and proteolysis occurs (ASHOURI et al., 2013). Based on this information, the importance of glycogen as a primary source of energy during situations of food deprivation is evident (ALI et al., 2003).

In addition to glycogen, lipid reserves have also been shown to be an important source of energy during long periods of fasting (HUNG et al., 1997), the reduction in concentration of triglycerides in plasma, as well as accumulation of lipids in the liver are reflections of mobilization of lipids from adipose tissue, muscle and liver. Similar responses were also observed with *Piaractus mesopotamicus* (TAKAHASHI et al., 2011; GIMBO et al., 2015; FAVERO et al., 2017) and *Brycon amazonicus* (URBINATI et al., 2014).

Regarding cholesterol, there were no significant responses, however, fasting promotes an increase in circulating cholesterol levels due to endogenous synthesis (BERG et al., 2002) and the effect of this on cholesterol concentrations is uneven, as studies with fish have different responses such as elevation (FAVERO et al., 2017) or cholesterol reduction during food deprivation (KIM et al., 2014), showing that the response of this variable is not only dependent on the intensity of the applied fast, but also it must be taken into account that cholesterol is a precursor to biomolecules, such as cortisol and steroid hormones (GIMBO et al., 2015).

CONCLUSION

Based on the results, this study showed 14 days of fasting were not harmful to tambaqui juveniles, and during refeeding fish can quickly normalize metabolism, evidenced mainly by restoration of glycogen reserves and rapid mobilization of lipids in liver, after a single feeding.

ACKNOWLEDGMENTS

We are grateful to INPA for fish supply. The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a Master's program scholarship.

REFERENCES

ALI, M.; NICIEZA, A; WOOTTON , R.J. Compensatory growth in fishes: a response to growth depression. **Fish and Fisheries**, v. 4, p. 147-190, 2003.

ASHOURI, G.; YAVARI, V.; BAHMANI, M.; YAZDANI, M.A.; KAZEMI, R.; MORSHEDI, V.; FATOLLAHI, M. The effect of short-term starvation on some physiological and morphological parameters in juvenile siberian sturgeon, Acipenser

baerii (Actinopterygii: acipenseriformes: acipenseridae). **Acta ichthyologica et piscatoria**, v. 43, n. 2, p. 145-150, 2013.
BAR, N. Physiological and hormonal changes during prolonged starvation in fish. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 71, p. 1-12, 2014.

BARCELLOS, L.J.G.; MARQUEZE, A.; TRAPP, M.; QUEVEDO, R.M.; FERREIRA D. The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá Rhamdia quelen. **Aquaculture**, v. 300, p. 231-236, 2010.

BERG, J.M.; TYMOCZKO, J.L.; STRYER, L. **The complex regulation of cholesterol biosynthesis takes place at several levels**. In: BERG, J. M.;

TYMOCZKO, J. L.; STRYER, L. Biochemistry, 5th Edition, 2002.
BLIGH, E.; DYER, W. A rapid method of total lipid extraction and purification. **Can J Biochem Physiol,** v. 37(8), p. 911-917, 1959.

CHATZIFOTIS, S.; PAPADAKI, M.; DESPOTI, S.; ROUFIDOU, C.; ANTONOPOULOU, E. Effect of starvation and re-feeding on reproductive índices, body weight, plasma metabolites and oxidative enzymes of sea bass (Dicentrarchus labrax). **Aquaculture**, v. 316, p. 53-59, 2011.

CYRINO, J.E.P.; BICUDO, A.J.A.; SADO, R.Y.; BORGHESI, R.; DAIRIKI, J.K. A piscicultura e o ambiente – o uso de alimentos ambientalmente corretos em piscicultura. **R. Bras. Zootec**., v.39, p.68-87, 2010.
FAVERO, G. C.; GIMBO, R.Y.; MONTOYA, L.N.F.; ZANUZZO, F.S.; URBINATI, E.C. Fasting and refeeding lead to more efficient growth in lean pacu (*Piaractus mesopotamicus*). **Aquaculture Research**. p.1-8, 2017.

FURNÉ, M.; MORALES, A. E.; TRENZADO, C. E.; GARCÍA-GALLEGO, M.; HIDALGO, M. C.; DOMEZAIN, A.; RUS, A. S. The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. **Journal of Comparative Biochemistry and Physiology (Part B)**, v. 182, p. 63-76, 2012.

GIMBO, R.Y.; FAVERO, G.C.; FRANCO MONTOYA, N.L.; URBINATI, E.C. Energy deficit does not affect immune responses of experimentally infected pacu (Piaractus mesopotamicus). **Fish and Shellfish Immunology**, v. 43, p. 295-300, 2015.

GOMES, L.C.; SIMÕES, LN ; ARAUJO-LIMA, C.A.R.M. . **Tambaqui (Colossoma macropomum)**. In: Bernardo Baldisserotto; Levy de Carvalho Gomes. (Org.). Espécies Nativas para piscicultura no Brasil - segunda edição, ampliada e revisada.. 2ed.Santa Maria: Editora UFSM, 2020, p. 175-204.

HERRERA, L.A. Crescimento compensatório e privação alimentar no desempenho produtivo do robalo flecha. **Pesq. agropec. Bras**. v.51, n.6, p.776-779, 2016.

HUNG, S.S.O.; LIU, W.; LI, H.; STOREBAKKEN, T.; CUI, Y. Effect of

starvation on some morphological and biochemical parameters in white sturgeon, Acipenser transmontanus. **Aquaculture**, v. 151, p. 357-363, 1997.

KABIRA, K.A.; VERDEGEM, M.C.J.; VERRETH, J.A.J.; PHILLIPS, M.J.; SCHRAMA, J.W. Effect of dietary protein to energy ratio, stocking density and feeding level on performance of Nile tilapia in pond aquaculture. **Aquaculture**. v. 511, p. 634200, 2019.

KIM, J-H.; JEONG, M-H.; JUN, J-C.; KIM, T-I. Changes in hematological, biochemical and non-specific immune parameters of Olive Flounder, Paralichthys olivaceus, following starvation. **Asian-Australasian Journal of Animal Sciences**, v. 29(9), p. 1360-1367, 2014.

LUNA, M.; LLORENTE, I.; COBO, A. Integration of environmental sustainability and product quality criteria in the decision-making process for feeding strategies in seabream aquaculture companies. **Journal of Cleaner Production**, v. 217, p. 691-701, 2019.

MOMMSEN, T.P.; VIJAYAN, M.M.; MOON, T.W. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. **Reviews in Fish Biology and Fisheries**. v. 9, p. 211-268, 1999.

MOON, T., FOSTER, G., & PLISETSKAYA, E. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. **CAN. J. ZOOL**., 67, 2183-2193, 1989.

MORSHEDI, V.; KOCHANIANB, P.; BAHMANIC, M.; YAZDANIC, M.A.; POURALIC, H.R.; ASHOURIB, GH.; PASHA-ZANOOSIBA, H. Cyclical short-term starvation and refeeding provokescompensatory growth in sub-yearling Siberian sturgeon,Acipenser baerii Brandt, 1869. **Animal Feed Science and Technology**, v. 232, p. 207–214, 2017.

NAVARRO, I.; GUTIERREZ, J. Fasting and starvation. **Biochemistry and Molecular Biology of Fishes**, v. 4, p. 394-434, 1995.

PERES, H.; SANTOS, S.; OLIVA-TELES, A. Blood chemistry profile as indicator of nutritional status in European seabass (Dicentrarchus labrax). **Fish Physiology and Biochemistry**, v. 40, p. 1339-1347, 2014.

PÉREZ-JIMÉNEZ, A.; CARDENETE, G.; HIDALGO, M. C.; GARCÍA-ALCÁZAR, A.; ABELLÁN, E.; MORALES, A. E. "Metabolic adjustments of Dentex dentex to prolonged starvation and refeeding". **Fish Physiology and Biochemistry**, v. 38, p. 1145-1157, 2012.

PÉREZ-JIMÉNEZ, A.; GUEDES, M.J.; MORALES, A.E.; OLIVA-TELES, A. Metabolic responses to short starvation starvation and refeeding in Dicentrarchus labrax. Effect of dietary composition. **Aquaculture**, v. 265, p. 325-335, 2007.

PETERSON, B.C.; SMALL, B.C. Effects of fasting on circulating IGF-binding proteins, glucose, and cortisol in channel catfish (Ictalurus punctatus).

Domestic Animal Endocrinology, 26: 231-240. 2004.

RIOS, F. S. A.; CARVALHO, C. S.; PINHEIRO, G. H.; DONATTI, L.; FERNANDES, M. N.; RANTIN, F. T. Utilization of endogenous reserves and effects of starvation on the health of Prochilodus lineatus (Prochilodontidae). **Environmental biology of fishes**, v. 91(1), p. 87-94, 2011

RODWELL,V. W.; BENDER, D.A.; BOTHAM, K.M.; KENNELY, P.J.; WEIL, P.A. **Bioquimica ilustrada de Harper**. 30ª Ed. 2016.

SOUZA, R., CAMPECHE, D., CAMPOS, R., FIGUEIREDO, R., MELO, J. Frequência de alimentação para juvenis de tambaqui. **Arq. Bras. Med. Vet. Zootec**. v. 66, p. 927-932, 2014.

TAKAHASHI, L. S.; BILLER, J. D.; CRISCUOLO-URBINATI, E.; URBINATI, E. C. Feeding strategy with alternate fasting and refeeding: effects on farmed pacu production. **Journal of Animal Physiology and Animal Nutrition**, v. 95, p. 259-266, 2011.

URBINATI, E.C.; SARMIENTO, S.J.; TAKAHASHI, L.S. Short-term cycles of feed deprivation and refeeding promote full compensatory growth in the Amazon fish matrinxã (*Brycon amazonicus*). **Aquaculture**. v. 433(20), p. 430-433, 2014.

VALLADÃO, G. M. R.; GALLANI, S. U.; PILARSKI, F. South American fish for continental aquaculture. **Reviews in Aquaculture**, v. 10(2), p. 351-369, 2018.