Applied Animal Behaviour Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Research Paper

Oil sources administered to tambaqui (*Colossoma macropomum*): growth, body composition and effect of masking organoleptic properties and fasting on diet preference

Raquel Tatiane Pereira[a], Renan Rosa Paulino[a], Charlle Anderson Lima de Almeida[b], Priscila Vieira Rosa[a], Tamira Maria Orlando[a], Rodrigo Fortes-Silva[b,*]

[a] Department of Animal Science, University of Lavras UFLA, 37200-000, Lavras, Brazil
[b] Laboratory of Fish Nutrition and Feeding Behavior (AquaUFRB), Faculty of Fish Engineering (NEPA), Center of Agricultural Sciences, Environmental and Biological (CCAAB), Campus Cruz das Almas, Federal University of Bahia (UFRB), 44380-000, Bahia, Brazil

ARTICLE INFO

Keywords:
Fatty acids profile
Feeding behaviour
Nutritional challenge
Nutritional value
Post-absorptive signals
Diet selection

ABSTRACT

Tropical fish feeding behaviour and food intake are regulated by a number of environmental factors (e.g.: stress in intensive aquaculture; type or seasonality of food), and also by complex homeostatic mechanisms that involve nutritional requirements. When access to food is not restricted, e.g. controlled laboratory conditions, fish growth and behaviour remain relatively unaffected. Moreover, fish need dietary fatty acids and, while some are remarkably beneficial, such as long-chain omega-3 oils, other fats can be associated with poor health, mainly under adverse conditions. This study aimed to evaluate the performance and body composition of tambaqui juveniles fed different oil sources (fish, linseed, and corn) (Experiment 1) and the influence of post-absorptive signals or orosensorial properties of the dietary oil source on diet preference (Experiment 2). For Experiment 1, juvenile tambaqui (42.79 ± 0.92 g) were placed into eighteen 100 L tanks (10 fish per tank). Three diets (treatments) were formulated with three oil sources (corn, linseed and fish oil). Three groups of fish were fed each separate diets twice a day for 7 weeks. Experiment 2 assessed tambaqui's ability to select oil sources without coming into contact with the sensorial properties of diet. The same diets of Experiment 1 were used, but diets were encapsulated to isolate sensorial properties. Forty-eight fish (41.26 ± 1.00 g, mean ± SD) were distributed into six 250 L tanks (eight fish per tank). In experiment 1, we found no effects of diet on growth parameters, such as final body weight, weight gain, feed intake, feed efficiency ratio, specific growth ratio and protein efficiency ($p \geq 0.05$). However, body crude protein was higher in the fish fed the corn oil diet ($p \leq 0.05$), but this value did not affect the protein efficiency ratio ($p \geq 0.05$). No differences among treatments were observed for moisture, lipid and ash ($p \geq 0.05$). The fatty acid composition of muscle reflected the dietary oil source. However, the tambaqui fed vegetable oil displayed high levels of polyunsaturated fatty acids, while the linseed oil diet resulted in a greater incorporation of n-3 highly unsaturated fatty acids. In Experiment 2, at first fish did not show the ability to select an oil source without orosensorial properties, but a nutritional challenge of food deprivation (10 days) enabled fish to select among the diets that contained fish oil sources ($p = 0.002$) by using post-ingestive signals. Our findings contribute to the understanding of the mechanisms that relate to lipid metabolism and feeding behaviour in freshwater Amazon species, which goes beyond growth parameters.

1. Introduction

Fish oil (FO) has been used in feeding studies for farmed marine fish and some freshwater species, but it represents a finite resource (Rombenso et al., 2016). The use of alternative oils in freshwater fish feedstuff is increasing due to the rising cost of FO (Ng et al., 2013). Sustainable sources to substitute FO in fish feeds include vegetable oils, such as rapeseed oil and linseed oil, which are commonly used for nutritional studies (Costa et al., 2014; Ferreira et al., 2015). As the substitution of dietary FO for vegetable oils would lower the content of health beneficial long-chain polyunsaturated fatty acids (LC-PUFAs) in these fish, research into finishing diets to enhance their nutritional value prior to harvest is increasing (Ng et al., 2013).

Freshwater fish are better able to desaturate and elongate the fatty

* Corresponding author.
E-mail addresses: fortes@ufrb.edu.br, fortesrs@yahoo.com.br (R. Fortes-Silva).

http://dx.doi.org/10.1016/j.applanim.2017.10.004
Received 23 September 2016; Received in revised form 28 September 2017; Accepted 4 October 2017

acids (FAs) synthesised by plants into EPA and DHA than marine fish (Tocher, 2015). However, the use of various oil sources in tropical freshwater nutrition could have controversial effects. According to Bahurmiz and Ng (2007), the inclusion of dietary palm-origin oils compared to FO did not suffice to cause an effect on final body weight or blood parameters, such as haematocrit, but significantly diminished the total lipid digestibility of diets, due mainly to the reduced digestibility of saturated FAs by red tilapia (*Oreochromis* sp.). Nile tilapia brood fish reared in brackish water require a source of dietary *n*-3 highly unsaturated fatty acids (HUFAs) for optimum spawning performance, while plant oil (soya bean oil) may meet the requirements of brood fish reared in freshwater (El-Sayed et al., 2005). The effects of various dietary oil sources on tropical fish are poorly known. Moreover, some essential FA sources could provide animals with benefits by contributing to their health (Ferreira et al., 2015) and enhancing their FA profile, which is important for human nutrition (Ng et al., 2013). Part of the controversial results obtained in freshwater fish can be explained by the lack of a nutritional challenge, which is normally present under natural conditions. Under controlled laboratory conditions, fatty acids need not be necessarily limiting for growth to take place as freshwater fish can endogenously synthesise fatty acids, even a small amount, to recover their requirements, and generally less than saltwater fish can.

In behavioural terms, fish can express nutritional needs under environmental challenging conditions. Fish feeding preferences are influenced by a set of species-specific factors that include food itself (taste and smell) and animals' physiological requirements (Raubenheimer et al., 2012). The mechanisms by which fish regulate their intake and use oil sources are particularly important to aquaculture because this knowledge enables aquaculture practitioners to avoid inefficient feeding regimes. It has been reported that animal uses inner mechanisms, such as physiological differential absorption of nutrients or overeating some sources and undereating others, to reach its nutrient target. This inherited auto-regulation ability is called 'nutritional wisdom' (Fortes-Silva et al., 2016). The feed intake regulation involves multiple mechanisms that interact to control both physiology and behaviour. Among these control mechanisms, the pre-ingestive and post-ingestive signal provides the animal with an anticipatory behavioural response to food. Methods that evaluate the nutrition and feeding behaviour of fish have been developed. The diet encapsulation method was initially validated in European seabass (*Dicentrarchus labrax*) (Rubio et al., 2003) and subsequently in other species, such as sharpsnout seabream (*Diplodus puntazzo*) (Almaida-Pagán et al., 2006, 2008) and tilapia (*Oreochromis niloticus*) (Fortes-Silva et al., 2011, 2012). These studies strongly suggest that in such species, diet preferences are a direct result of post-ingestive influences rather than the of the orosensorial characteristics of food, and these influences also regulate feed intake.

A flexible diet is a striking aspect of tropical riverine ichthyofauna. Most species respond to seasonal and spatial environmental cues by switching from one food item to another when the relative abundance of the available food resource fluctuates. These considerations highlight the difficulty of establishing reliable species-specific feeding patterns in tropical teleosts (Abelha et al., 2001). Tambaqui (*Colossoma macropomum*) is an omnivorous species from the Amazon and Orinoco rivers that feeds on fruit and seeds (Vidal et al., 1998), and is the most extensively cultivated native species in Brazil and several other South and Central American countries (Fiúza et al., 2015). Tambaqui carcasses present high lipid accumulation, which reflects the peculiar feeding behaviour associated with seasonal food variability. Nutrient deprivation periods are common in many wild fish life cycles and appear to be tolerated by many species as cultured fish can also endure such situations (Tian et al., 2013). Food deprivation can trigger behavioural and physical changes in juvenile and adult fish, but does not impair their capacity to grow (Souza et al., 1997). Under intensive culture conditions, fish are subject to increased stress owing to water quality and health conditions, which can lead to lack of appetite. Thus research into lipid metabolism during fasting and re-feeding is important, and nutritional challenges have been designed to assess the nutritional needs of fish behavioural responses, as has a wellness component.

If the hypothesis of interesting intensive aquaculture and environmental feeding conditions is supported by the way animals choose nutrients, it must surely provide a new perspective as to how oil sources should change throughout the cultivation period. These nutritional-behavioural requirements may have a welfare component, but may not necessarily influence fish performance. This work aimed to evaluate the effect of three oil sources (fish, corn and linseed) on the growth parameters, body composition and behavioural capability of tambaqui (*C. macropomum*) to detect oil sources after nutritional challenges and to isolate the sensory properties of diet.

2. Material and methods

The animals used in those studies were approved by the Ethics Committee of Animal Welfare of the Federal University of Lavras, protocol number 036/2015.

2.1. Experiment 1 (growth and body composition): experimental design, animals, housing and diets

This experiment was conducted at the Lavras University (Lavras, Minas Gerais, Brazil). Juvenile tambaqui, obtained from the Aquaculture Center-UNESP-Jaboticabal-Brazil, with an average initial weight of 42.79 ± 0.92 g were randomly placed into eighteen 100 L tanks at a density of 10 fish per tank, which provided a total of 180 fish. The feeding trial was conducted in an indoor recirculation system, with 10% water replenished daily. Firstly, fish were fed a commercial diet (Pirá 36, Guabi, Brazil, 36% crude protein) for 2 weeks to acclimate to the experimental condition. During the experimental period, fish were fed *ad libitum* experimental diets twice a day (at 08:00 h and 16:00 h) for 7 weeks.

The experimental diets (divided into three treatments and six replicates: diets made with corn, linseed and fish oil) were formulated to meet tambaqui's nutritional requirements according to Fracalossi and Cyrino (2013). Diets were formulated to contain 7% total lipid with three oil sources, corn oil, linseed oil and fish oil (Table 1), and were manufactured at the Laboratory of Aquaculture of the Federal University of Minas Gerais, Brazil. All the ingredients were initially ground through an 800-mm mesh and then mixed with an automatic paddle mixer. This was followed by extrusion in a single-screen extruder (Inbramaq, São Paulo, Brazil) and ingredients were finally oven-dried at 50 °C for 12 h. The average temperature during the extrusion process was 80 °C at the preconditioned and 120 °C at the single-screen, and 4-mm kibbles were produced. After extrusion, oil was incorporated into the dried kibble by a manual spraying method.

Throughout the trials, the average water temperature was 28.0 ± 0.41 °C, dissolved oxygen was 3.70 ± 0.60 mg L^{-1}, pH was 6.31 ± 0.32, and ammonia was 0.24 ± 0.20 mg L^{-1}. The light:dark cycle was 12:12LD. The mean observed values were in the fish comfort zone (Silva et al., 2007).

At the end of the experiment, fish were weighed, humanely killed by immersion in an overdose of benzocaine (300 mg/L), and then decapitated at the skull insertion point. Fish muscles were frozen (−80 °C) for subsequent analyses. Diets and the body (gutted) composition of fish were analysed according to AOAC methods (AOAC International, 2005). Crude lipid was determined by petroleum ether extraction in a Soxhlet system. Crude protein (nitrogen × 6.25) was determined on a Kjeldahl apparatus after acid digestion. Moisture was determined after drying at 105 °C for 24 h. Ash content was determined by incineration in a muffle furnace at 550 °C for 24 h. The energy content of diets was determined by direct combustion in an adiabatic bomb calorimeter.