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Mixes of plant oils as fish oil substitutes for Nile tilapia at optimal and cold suboptimal temperature

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ABSTRACT

Nile tilapia production worldwide is concentrated in the subtropics, where lower winter temperatures is a challenge for the high productivity of tropical fish species. Adequate fatty acid nutrition of fish is a way to offset the lower temperature, as this nutrient is responsible for the structure, fluidity, and functionality of all cell membranes. However, there are still many gaps in Nile tilapia fatty acid nutrition, particularly at lower temperatures. In this study, three plant oil mixes and fish oil (FO) were fed to juvenile Nile tilapia at optimal (28 °C) or suboptimal (22 °C) temperatures for 9 or 12 weeks, respectively. The mixes of plant oils (coconut, olive, sunflower and linseed oils) were formulated to mimic fatty acid groups in FO, only varying the proportions between n-3 and n-6 polyunsaturated fatty acid (PUFA). They were named MIX-S, MIX-SL, and MIX-L since they contained an increasing amount of linseed oil (L) and n-3 PUFA as well as a decreasing amount of sunflower oil (S) and n-6 PUFA. Tilapia fed plant oil mixes exhibited similar growth at each rearing temperature. However, plant oil mixes led to lower daily weight gain than FO, except for MIX-S, which was comparable to FO when tilapia were fed at 22 °C. Feed efficiency and protein utilization were similar at 28 °C, but at 22 °C, the highest values were obtained in fish fed the FO diet. As expected, apparent net utilization of n-3 and n-6 PUFAs was higher when these groups of fatty acids were scarce in the diets. The body fatty acid profile of tilapia was influenced by dietary oil sources, but among the fish fed plant oils, total PUFA and total LC-PUFA were similar within each rearing temperature. Additionally, PUFA retention and conversion to higher chain length homologues in Nile tilapia seemed to be a strategy of adapting to lower temperatures. Our main finding is that changes in the n-3/n-6 PUFA ratio in diets with plant oil mixes do not alter Nile tilapia growth or feed efficiency at either 28 °C or 22 °C rearing temperatures. However, when compared to the diet with fish oil that was rich in LC-PUFA, plant oil diets resulted in lower growth. Plant oil mixes led to similar feed efficiencies as fish oil at 28 °C but not at 22 °C.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is a tropical fish with optimum growth when raised at temperatures between 26 and 30 °C (Azaza et al., 2008; Ma et al., 2015). One of the challenges of tilapia farming is to maintain productivity in subtropical regions, where temperature exhibits large differences between summer and winter, and where tilapia production is more concentrated.

In addition to a genetic selection of more tolerant tilapia strains (Charo-Karisa et al., 2005; Sifa et al., 2002), fatty acid nutrition may be a valuable tool to increase productivity at lower temperatures, as fatty acids are responsible for cell membrane structure, fluidity, and functionality. The dietary fatty acid requirements for tilapia grown at cold suboptimal temperature are unknown and not stated in reviews about tilapia nutrition (Lim et al., 2011; Ng and Romano, 2013; NRC, 2011). However, several studies report the importance of dietary unsaturated fatty acids in tilapia and other fish species when acclimating to lower temperatures (Corrêa et al., 2017; Hsieh et al., 2007; Skalli et al., 2006; Zerai et al., 2010).

All living organisms can synthesize saturated or monounsaturated fatty acids, but most animals are unable to produce polyunsaturated fatty acids (PUFA); therefore, these must be supplied in the diet (Glencross, 2009; Tocher, 2003). It is considered that for omnivorous freshwater fish, such as the Nile tilapia, the essential fatty acids are only

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linoleic acid (LOA, 18:2n-6) and alpha-linolenic acid (α-LNA, 18:3n-3), as these fish can convert them partially to their long-chain polyunsaturated fatty acid (LC-PUFA) homologues: arachidonic (ARA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3), and docosahexaenoic (DHA, 22:6n-3) fatty acids (Olsen et al., 1990; Tocher, 2010).

Tilapia seems to have a PUFA requirement of n-6 and n-3 series (NRC, 2011); however, the establishment of requirement values for optimal growth and feed utilization is difficult since an excess of these fatty acids in the diet can meet the need of each other (Li et al., 2013; Lim et al., 2011). For hybrid tilapia, *O. niloticus* × *O. aureus*, raised at an optimal temperature, the requirement of LOA is 1.14% of the diet dry weight, and this requirement can be met by LOA alone or in combination with α-LNA (Li et al., 2013). At first sight, tilapia would be unaffected by the substitution of fish meal and fish oil by plant oils in the diet or by the substitution of 20 and 22-carbon PUFAs by 18-carbon PUFA, as demonstrated by Al-Souti et al. (2012). However, there are studies indicating that dietary LC-PUFA may have beneficial effects on tilapia growth and feed utilization (Chou and Shiau, 1999; Chou et al., 2001).

Production of fishmeal and fish oil from fisheries has been stagnant since 2005, but the overall demand for aquaculture has increased, raising the prices and the use of plant oils (FAO, 2016). Therefore, in addition to establishing fatty acid requirements, a large challenge exists in understanding the substitution of marine ingredients with plant ingredients in aquafeeds. For a plant oil to be considered a good substitute to fish oil in aquafeeds, it must contain high amounts of saturated and monounsaturated fatty acids as energy sources, thus saving PUFA for other biological roles (Turchini et al., 2009). Additionally, there is evidence that dietary monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) are efficient at sparing LC-PUFA from oxidation (Salini et al., 2017) and that dietary plant oil blends provide similar or higher growth of tilapia than that obtained by the supplementation of a sole plant oil (Lim et al., 2011).

For Nile tilapia, a freshwater omnivore, there has been a drastic reduction or complete exclusion of marine ingredients in commercial feeds due to cost constraints. Therefore, it is imperative to investigate alternatives to marine fish oil, especially when tilapia is raised in a subtropical climate, where an adequate body fatty acid profile will help fish to offset temperature fluctuations. In this study, we carried out two trials with Nile tilapia juveniles, at optimal and cold-suboptimal temperature, to compare growth and fatty acid deposition among fish-fed diets containing fish oil or three mixes of plant oils.

2. Materials and methods

2.1. Experimental design

Nile tilapia juveniles were fed four diets containing different lipid sources in two distinct trials (not simultaneous): one trial at optimal (28 °C) and the other at suboptimal (22 °C) temperatures for 9 and 12 weeks, respectively. The temperatures were set to simulate summer and winter of subtropical regions. At the lower temperature, the experimental period was longer to allow for sufficient growth of fish to assess the effects of the diets. Both trials were run in a completely randomized design, with four dietary treatments in triplicate.

Dietary lipid sources were three mixes of plant oils (MIX-S, MIX-SL, and MIX-L) as well as fish oil (FO) (Table 1). Plant oil mixes were formulated to mimic the fatty acid groups of FO, varying the proportion of n-3/n-6 PUFA (Table 2). A similar base of coconut oil and olive oil was added to 1) sunflower oil (MIX-S), 2) sunflower and linseed oil (MIX-SL) or 3) linseed oil (MIX-L). Fish oil provided mainly PUFA of 20 and 22 carbons, and plant-oil mixes provided PUFA of 18 carbons.

Table 1
Composition of experimental diets (g kg^{-1} dry weight).

	Diets[c]			
	FO	MIX-S	MIX-SL	MIX-L
Ingredients				
Cod liver oil[a]	50.0	–	–	–
Coconut oil	–	7.5	7.5	7.5
Olive oil	–	27.5	27.5	27.5
Sunflower oil	–	15.0	7.5	–
Linseed oil	–	–	7.5	15.0
Other ingredients[b]	950.0	950.0	950.0	950.0
Composition				
Dry matter	907.9	905.8	906.3	912.8
Protein	370.5	367.2	370.1	366.4
Lipids	58.6	58.0	58.1	57.9
Ash	43.3	43.7	43.9	44.0
Gross energy (MJ kg $^{-1}$)	18.27	18.23	18.24	18.21
n-3 PUFA	13.1	0.2	3.9	7.4
n-6 PUFA	2.0	11.1	8.1	5.0

[a] Cod liver oil distributed by Química Delaware (Porto Alegre, Brazil) and produced by Berg Lipidtech (Aalesund, Norway); linseed oil from Vital Atman (Uchoa, Brazil); sunflower oil from Cargill Agrícola (Mairinque, Brazil); olive oil distributed by Cargill Agrícola (Mairinque, Brazil) and produced by Victor Guedes (Abrantes, Portugal); coconut oil from Copra (Maceió, Brazil).

[b] Other ingredients (g kg^{-1} dry weight): casein 300.0; gelatin 80.0; corn starch 410.0; cellulose 113.4; macromineral mix 40.0; vitamin-micromineral mix 5.0; coline chloride 0.5; butyl hydroxy toluene (BHT) 0.2. Macromineral mix (Vaccinar, Belo Horizonte; Tortuga, Mairinque; Brazil), composition kg^{-1} product: 720 g dicalcium phosphate; 150 g potassium chloride; 90 g sodium chloride; 40 g magnesium sulphate. Vitamin-micromineral mix (Vaccinar, Belo Horizonte, Brazil), composition kg^{-1} product: 3500 mg copper sulphate; 160 mg calcium iodate; 20,000 mg iron sulphate; 10,000 mg manganese sulphate; 24,000 mg zinc sulphate; 100 mg sodium selenite; 80 mg cobalt sulphate; 2,400,000 IU vit. A; 600,000 IU vit. D_3; 30,000 IU vit. E; 3.000 mg vit. K_3; 4000 mg vit. B_2; 10,000 mg pantothenic acid; 20,000 mg niacin; 8000 μg vit. B_{12}; 100,000 mg choline chloride; 200 mg biotin; 1200 mg folic acid; 4000 mg vit. B_1; 3500 mg vit. B_6; 25,000 mg inositol; 60,000 mg vit. C; 5000 mg BHT.

[c] FO = diet with fish oil; MIX-S, MIX-SL, and MIX-L = diets with mixes of plant oils (coconut, olive, sunflower and linseed) differing only in the inclusion of sunflower oil (S) and linseed oil (L).

Diets were semi-purified and formulated to meet the nutritional requirements of the Nile tilapia (Furuya et al., 2012; NRC, 2011); they differed only in the lipid source (Table 1). Ingredients were mixed, extruded into 2 to 3 mm pellets, dried at 55 °C, and then top-coated with oils. Diets were stored at −20 °C until use.

2.2. Experimental units

In each feeding trial, a total of 240 juvenile Nile tilapia were assigned to 12 experimental units (20 fish per experimental unit), consisting of 80-L tanks connected to a closed recirculating freshwater system with temperature control and 12-h photoperiod. Fish were acclimated over two weeks to the experimental conditions prior feeding of experimental diets. In the first week of acclimation, the water temperature was kept at 28 °C. In the second week, fish used in the optimal temperature trial were continuously acclimated to 28 °C. Fish used in the suboptimal temperature trial were gradually acclimated to the lower temperature in the second week, at the rate of one degree per day, until the temperature reached 22 °C. During the two-week acclimation period, all fish received a basal diet, which contained coconut oil as the single lipid source.

After acclimation, juvenile male Nile tilapia of genetic improved