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Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend

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ABSTRACT

A 2×2 factorial 14-week feeding trial was conducted to evaluate the fatty acid metabolism in two different tilapia genotypes [Nile tilapia (*Oreochromis niloticus*, GIFT strain) and red hybrid tilapia (*Oreochromis sp.*)] fed a fish oil (FO)- or blended vegetable oil (BVO)-based semipurified diet. The BVO was formulated using olive oil (15%), sunflower oil (15%), linseed oil (30%) and refined, bleached, deodorized palm olein (40%) to mimic the major fatty acid classes of FO. In general, no significant effect ($P>0.05$) of tilapia genotype or lipid source on fatty acid digestibility was observed. The fatty acid composition of tilapia whole-body, irrespective of genotype, was significantly ($P<0.05$) affected by the dietary lipid source, but interestingly, tilapia fed the BVO diet which contained no polyunsaturated fatty acids (PUFA) longer than C18, recorded significant amounts of both n-6 and n-3 long chain (LC)-PUFA. The present study clearly indicated that tilapia farming can be a net producer of n-3 LC-PUFA. Using the whole-body fatty acid balance method, total fatty acid β-oxidation, Δ-5 and Δ-6 desaturation were observed to be significantly higher in fish fed the BVO diet compared to fish on the FO diet. No apparent Δ-5 desaturase activity on n-3 PUFA was observed in fish fed the FO diet. Both dietary lipid source and tilapia genotype elicited significant effects on the elongase activity on 18:4n-3, 18:2n-6 and 18:3n-6. Tilapia fed the BVO diet exhibited efficient bioconversion of 18:2n-6 to n-6 LC-PUFA indicating that the fatty acid metabolism of tilapia is able to fully compensate for the lack of dietary n-6 LC-PUFA when fed a vegetable oil-based diet. All fish showed active liponeogenesis suggesting that the addition of higher dietary levels of SFA and MUFA may be beneficial to tilapia. Irrespective of diet, GIFT tilapia showed higher rates of fatty acid neogenesis along with higher rates of elongation, Δ-5 and Δ-6 desaturation of both the n-6 PUFA and n-3 PUFA. The farming of improved strains of Nile tilapia may rely less heavily on marine-derived raw materials for aquafeed production.

1. Introduction

Tilapia aquaculture is rapidly expanding with a global production of about 2.8 million metric tons in 2008 (FAO, 2010) and estimated to increase to 8.89 million metric tons by the year 2020 (Tacon and Metian, 2008). This rapid global production of tilapia is due in part to the increasing use of commercial pelleted feeds and the introduction of improved strains of Nile tilapia (*Oreochromis niloticus*) which is the major farmed tilapia species. The Genetically Improved Farmed Tilapia (GIFT) strain developed by the WorldFish Center is one of the more successfully introduced farmed Nile tilapia. The F_7 generation of the GIFT strain was established in Malaysia through natural genetic selection in 2002 (Ponzoni et al., 2005). Despite the improved growth performance of the GIFT strain (Ng and Hanim, 2007), the red hybrid tilapia (*Oreochromis* sp.) is still the dominant species farmed in Malaysia due to its preferred red coloration by local consumers.

Tilapia feeding costs can account for more than 60% of the production costs in intensive culture systems. There is currently a paucity of information concerning the nutrient requirements of high performing tilapia strains compared to non-improved strains. Such information is crucial to feed formulators and fish farmers to allow them to optimize feed formulation and management in order to maximize cost competitiveness. Liebert et al. (2006) reported that different growth performance showed by different genotypes of juvenile tilapia might partly be related to their different daily protein deposition capabilities. Ng and Hanim (2007) reported that GIFT tilapia exhibited slightly increased dietary lipid absorption compared to red hybrid tilapia which might partly explain their higher growth performance. In contrast, Hakin et al. (2006) were unable to correlate the increased digestive enzyme activity observed in genotypes showing higher growth even though the activities of some digestive enzymes in tilapia were found to be affected by both the genotype and protein level in their diet. Mamun et al. (2007) reported no significant

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differences in metabolic efficiency between the improved and non-improved Nile tilapia strains when fed a similar diet.

Traditionally, fish oil (FO) is the major dietary lipid source used in commercial fish feeds, including tilapia feeds. As global FO production is stagnating, increasing demand has greatly inflated FO prices necessitating the use of alternative lipid resources in aquafeeds (Turchini et al., 2009). Vegetable oils (VO) are viable alternatives as they are readily available, their production is considered to be environmentally and economically more sustainable and their utilization in aquafeeds is more cost-effective than FO. Several studies reported that VO can partially or fully replace FO in fish diets without compromising growth performance in a variety of species as long as their essential fatty acid requirements are met (Caballero et al., 2002; Ng et al., 2003a; Turchini et al., 2003). Nevertheless, at the same time, numerous studies have indicated that dietary fatty acids composition is reflected in fish tissues (Tocher et al., 2003; Turchini et al., 2003; Bahurmiz and Ng, 2007). Thus, the modification of fillet fatty acid make-up of fish fed alternative lipid sources is likely to be the most stringent constraint of FO replacement in aquafeed (Turchini et al., 2009). This is because all vegetable oils do not contain n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) which are known to be beneficial to human health. Furthermore, it has been well established that fatty acid metabolism in fish is affected by dietary fatty acids (Bell et al., 2001a; Turchini and Francis, 2009; Torstensen and Tocher, 2010).

In general, when FO is replaced by terrestrial alternatives such as VO, the resultant decreased supply of dietary LC-PUFA has been reported to stimulate both the transcription rate (Bell et al., 2001a) and the actual activity (Francis et al., 2007a) of the enzymes (fatty acid desaturases and elongases) involved in the fatty acid bioconversion pathway. There is currently great interest in understanding the actual capability of farmed fish in bio-converting the two basic essential fatty acids (C_{18} PUFA, namely linoleic acid, 18:2n-6 and α-linolenic acid, 18:3n-3) which are both commonly present in VO, into the longer and more unsaturated LC-PUFA of the n-6 and the n-3 classes, respectively. Tilapias, similar to other freshwater fish, are capable of biosynthesising $C_{20/22}$ LC-PUFA via the desaturation and elongation of C_{18} PUFA when fed C_{18} PUFA rich diet (Olsen et al., 1990). Nevertheless, Nile tilapia was reported to possess a limited capacity for *de novo* synthesis of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from dietary 18:3n-3 (Karapanagiotidis, et al., 2007).

We have previously shown that the GIFT tilapia was more efficient in utilizing dietary protein compared to red hybrid tilapia (Ng and Hanim, 2007) and lipid digestibility was slightly higher for the GIFT tilapia. The aim of the present study was to determine if the improved growth performance of the GIFT strain is also related to differences in the overall efficiency of their fatty acid metabolism and whether tilapia genotypes play a role in their ability to utilize dietary fatty acids when fed a FO- or a VO-based diet. The present study will also attempt to estimate the actual overall *in vivo* tilapias' capability to produce LC-PUFA from dietary 18:2n-6 and 18:3n-3 so as to provide new fundamental knowledge about fatty acid metabolism in tilapia as it relates to genotypes and diet.

2. Materials and methods

2.1. Experimental diets

Two semi-purified experimental diets were formulated to be isonitrogenous and isoenergetic. The ingredient composition of the diets were the same, with the exception of the added lipid sources (fish oil or a blend of vegetable oils), which were added at a level of 10% of the total diet (Table 1). The blended vegetable oil (BVO) was formulated using four commonly available vegetable oils in the form of olive oil (15%), sunflower oil (15%), linseed oil (30%) and refined, bleached, deodorized palm olein (40%). All oils were purchased from various local suppliers or from grocery stores. The fatty acid composition of BVO was formulated to mimic the major fatty acid classes of FO (Table 2). Casein and gelatin were used as protein sources in diets, whilst dextrin was included as the carbohydrate source. Chromic oxide was included in diet at an inclusion rate of 0.5% as an inert marker for determination of apparent nutrient digestibility. All purified feed ingredients were purchased from Sigma-Aldrich (MO, USA). Dry ingredients were mixed homogenously in a Hobart mixer. Then, the oil was thoroughly mixed with the ingredient mixture and distilled water was added. The moist dough was screw-pressed through a 2-mm die and the feed pellets formed were fan-dried and stored frozen at −20 °C until use.

Table 1
Ingredient and proximate composition (g/kg diet) of experimental diets.

Ingredient	Experimental diets[a]	
	FO	BVO
Casein	309.2	309.2
Gelatin	60.0	60.0
Palm oil[b]	–	40.0
Linseed oil	–	30.0
Olive oil	–	15.0
Sunflower oil	–	15.0
Peruvian fish oil	100.0	–
Dextrin	287.4	287.4
Vitamin mix[c]	30.0	30.0
Mineral mix[d]	40.0	40.0
Dicalcium phosphate	10.0	10.0
Chromic oxide	5.0	5.0
Carboxymethyl cellulose	15.0	15.0
alpha-cellulose	143.4	143.4
Proximate composition		
Dry matter	909.8	904.7
Protein	344.2	343.9
Lipid	81.8	83.2
Ash	44.7	44.7
Nitrogen-free extract[e]	529.3	528.2

[a] Experimental diets nomenclature: Fish Oil (FO), and Blended Vegetable Oil (BVO).
[b] Refined, bleached, deodorized (RBD) palm olein.
[c] Vitamin mix (g/kg premix) = Ascorbic acid, 45.00; inositol, 5.00; choline bitartrate, 136.06; niacin, 4.50; riboflavin, 1.00; pyridoxine·HCl, 1.00; thiamin·HCl 0.92; D-calcium panthothenate, 3.00; retinyl acetate, 0.60; cholecalciferol, 0.083; menadione 1.67; DL-α-tocopheryl acetate (250 IU/g), 8.00; D-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; and cellulose, 783.167.
[d] Mineral mix (g/kg premix) = Calcium phosphate monobasic, 135.49; calcium L-lactate hydrate, 327.00; ferric citrate, 29.70; magnesium sulphate·$7H_2O$, 132.00; potassium phosphate dibasic, 239.80; sodium phosphate monobasic·H_2O, 87.20; sodium chloride, 43.50; potassium iodide, 0.15; cuprous chloride, 0.20; manganous sulphate·H_2O, 0.80; cobalt chloride·$6H_2O$, 1.00; zinc sulphate·$7H_2O$, 3.00; and sodium selenite, 0.011.
[e] Nitrogen-free extract = 100 − (% ash + % protein + % lipid + % fiber).

2.2. Fish and experimental conditions

Red hybrid tilapia (*Oreochromis sp.*) fingerlings were purchased from a local fish farm (Sungai Petani, Kedah, Malaysia) whilst GIFT tilapia (*O. niloticus*) fingerlings were obtained from the Aquaculture Extension Centre, Jitra, Malaysia. All fish were acclimated to the experimental system for two weeks before the start of the feeding trial. Fish were fed with a conditioning diet with similar protein and energy content to the experimental diets but containing no added lipid during the acclimatization period.

A completely randomized factorial design 2×2 (diet×tilapia genotype) in quadruplicate was used. At the start of the feeding trial, groups of 20 fish of about 9 g body weight were randomly selected, weighed (to the nearest 0.1 g) and stocked into 16 aquaria (eight aquaria for each tilapia genotype). Each experimental diet was fed to four replicate groups of each tilapia strain ($n=4$). Filtered water flowed into each 95-L aquarium continuously. All aquaria were