Comparative Biochemistry and Physiology, Part B 226 (2018) 18–25

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Influence of dietary lipid levels on growth, nutrient utilization, tissue fatty acid composition and desaturase gene expression in silver barb (*Puntius gonionotous*) fingerlings

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ARTICLE INFO

Keywords:
Silver barb
Fish oil
Lipid level
Growth
Fatty acid
Δ6 fad

ABSTRACT

Silver barb (*Puntius gonionotus*) is considered as a promising medium-sized carp species for freshwater aquaculture in Asia. This study in silver barb was carried out to evaluate the effects of increasing dietary levels of lipid on growth, nutrient utilization, whole-body composition, tissue fatty acid composition and Δ6 fatty acyl desaturase (*Δ6 fad*) gene expression. Fish (11.3 ± 0.23 g of initial body weight) was fed for 60 days with five experimental diets: FO-0 (control feed); FO-30; FO-60; FO-90 and FO-120 containing 0, 30, 60, 90 and 120 g fish oil kg^{-1} diet, respectively. Among the diets, the highest specific growth rate (SGR), protein efficiency ratio (PER) and whole-body lipid content, and the lowest feed conversion ratio (FCR) were recorded with FO-120 diet. The saturated fatty acids (SFA) level in the muscle was significantly ($P < .05$) increased with the enhanced FO supplementation, whereas monounsaturated fatty acids (MUFA) level decreased. Increased level of fish oil in the diet also enhanced the n-3 PUFA and n-3 LC-PUFA (long-chain polyunsaturated fatty acid) in the muscle and liver. The expression of *Δ6 fad* gene was downregulated, whereas the serum biochemical constituents were either remain unchanged or enhanced with increased FO supplementation in the diets of silver barb.

1. Introduction

Fish oil has received much attention in human health studies due to presence of health promoting n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). LC-PUFAs are the essential components of cell membranes and tissues, and play crucial roles in growth, ontogenesis, reproduction, stress, immune responses as well as metabolic disorders, cardiovascular and neurological diseases (Sargent et al., 2002; Van der Merwe et al., 2013; Awada et al., 2013; Muhlhausler and Ailhaud, 2013). Fish oil (FO) is used as the main source of lipids in commercial aqua feeds. The quantitative and qualitative lipid compositions of feeds for farmed fish should be calculated to meet their essential fatty acid requirements, make an optimal contribution to their gross energy needs and contribute to their nutritional value for consumers. Fish require specific fatty acids for optimum growth (Chatzifotis et al., 2010; Peres and Oliva-Teles, 1999; Wang et al., 2014; El-Kasheif et al., 2011; López et al., 2009; Al-Souti et al., 2012). The tissue fatty acids composition of fish are influenced by the dietary fatty acids (Grigorakis et al., 2002; Chen et al., 1995). Supplementation of FO in the diets significantly increased the tissue n-3LC-PUFA concentrations in various fish species (Manning and Li, 2002; Lim et al., 2010; Al-Souti et al., 2012). Over the years, intensive research has been carried to modulate the LC-PUFA biosynthesis in species of aquaculture interest through dietary supplementations (Vagner and Santigosa, 2011). Biosynthesis of LC-PUFA in fish involves the sequential desaturation and elongation of precursor C18 PUFAs (Alpha linolenic acid, ALA and linoleic acid, LA) and Δ6 desaturase and Elovl5 elongase are the critical enzymes in the biosynthetic pathway of LC-PUFA (Tocher et al., 2004; Zheng et al., 2004). Several earlier studies have shown that Δ6 fatty acyl desaturase (*Δ6 fad*) mRNA levels were higher in freshwater fish fed on vegetable oil (VO) supplemented diets in comparison to that of fish fed with FO containing feeds (Seiliez et al., 2001; Zheng et al., 2004; Ren et al., 2012). Some studies have also revealed that the LC-PUFA inhibit the *Δ6 fad* in freshwater fish as well as in mammals (Christiansen et al., 1991; Ulmann et al., 1992).

Puntius gonionotus, commonly known as 'silver barb' is a promising medium-sized carp species across the Asian countries, such as

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https://doi.org/10.1016/j.cbpb.2018.08.005
Received 31 March 2018; Received in revised form 8 August 2018; Accepted 8 August 2018
Available online 15 August 2018

Indonesia, Bangladesh, Thailand, Malaysia and Myanmar (Sarker et al., 2002) because of its high consumer's preference, fast growth, easy and year round reproduction and wide adaptability to culture conditions (Hussain et al., 1989). In India, silver barb has recently been included as a new species in carp aquaculture practices and a study have revealed that it can bioconvert C18 PUFA to LC-PUFA when fish oil in the diet is substituted with vegetable oil sources (Nayak et al., 2017). However, there is no information available on the requirement of optimum dietary lipid level in the practical diets for its growth and fillet n3 LC-PUFA deposition. Therefore, the present study was conducted to investigate the effect of feeding diets supplemented with graded levels of lipid on growth, feed utilization, whole body composition and fatty acids profile in the muscle and liver of silver barb fingerlings. In addition, the effect of FO supplementation on differential expression of *Δ6 fad* gene in various organs/tissues in silver barb was also investigated.

2. Materials and methods

2.1. Ethical procedures

All animal handling procedures were approved by the Ethics and Animal Care Committee of the ICAR-CIFA established norms.

2.2. Experimental diets

Five isoproteic diets: FO-0 (control feed); FO-30; FO-60; FO-90 and FO-120 were formulated to contain 0, 30, 60, 90 and 120 g fish oil kg^{-1} diet, respectively (Table 1). Ground nut oil cake, soybean meal and fish meal were used for protein sources. Carboxymethyl cellulose was used as binder. The powdered major feed ingredients were mixed thoroughly for each diet, pressure-cooked at 2.7 kg pressure cm^{-2} for 15 min, cooled, fortified with a vitamin–mineral mixture and oil and blended. The dough was extruded through a 1.0-mm diameter die in a feed pelletizer. The resultant pellets were dried overnight at 45 °C crumbled and stored frozen at −20 °C in airtight plastic jars.

Table 1
Ingredient (g kg^{-1}) and chemical composition (g kg^{-1}dry matter) of the experimental diets.[1]

	FO-0	FO-30	FO-60	FO-90	FO-120
Ingredients					
Fish meal	100	100	100	100	100
Soybean meal	260	260	280	380	360
Groundnut oil cake	350	350	350	260	300
De-oiled rice bran	250	220	170	130	80
Fish oil	0	30	60	90	120
Carboxymethyl cellulose	10	10	10	10	10
Vitamin[a] and Mineral mixture[b]	30	30	30	30	30
Chemical composition					
Crude protein	319	316	313	321	316
Crude lipid	27	54	85	112	143
Ash	56	60	60	57	62
NFE	563	535	500	463	427
Gross energy(MJ kg^{-1})	18.3	18.8	19.3	20.0	20.5

NFE: nitrogen free extract.
Values are mean of triplicate analysis.
[1] Experimental diet nomenclature: FO-0, FO-30, FO-60, FO-90 and FO-120 are diets with 0 (control), 30, 60, 90 and 120 g fish oil (FO) supplementation kg^{-1}.
[a] Vitamin (IU or g kg^{-1} premix): retinol palmitate, 50,000 I·U; thiamine, 5; Riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalomin, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotine, 0.25
[b] Minerals (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4.7\ H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4.\ H_2O$, 3.12; $ZnSO4.7H_2O$, 4.67; $CuSO_4.5H_2O$, 0.62; KI, 0.16; $CoCl_2.6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02

2.3. Fish, culture system and feeding

Puntius gonionotous fingerlings were procured from the Institute's farm at ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India and were acclimatized to laboratory conditions in 500 l fibre-reinforced plastic (FRP) tanks. During acclimatization, fishes were fed on a standard carp diet for two weeks, developed at the institute. During the experiment, fish were handled as per the normal ethical practice of the institute. One hundred and fifty fingerlings of mean body weight (11.30 ± 0.23 g) were randomly allocated to 15 FRP tanks, each of 150 l capacity and triplicate tanks were allocated in each dietary treatment. Each tank was stocked with 10 fish. All the tanks were individually plumbed with flow-through system, and the flow rate of water was maintained at 1 l min^{-1} throughout the experiment. To ensure oxygen saturation, the water in each tank was continuously aerated by air stones. During the experiment, the daily water quality parameters including temperature, pH, dissolved oxygen, NH_4^+ ranged from 29 to 30 °C, 7.5–7.6, 7.0–7.3 ppm and 0.020–0.022 ppm, respectively, and there were no significant influence of dietary treatments on these parameters. The water quality parameters were within the acceptable range, reported for other carp rearing (Jena et al., 2002). The fish were hand fed to apparent satiation twice daily at 9.00 and 16.00 h for 60 days. Feed consumption was recorded during the entire study period.

Growth performance and feed utilization efficiency were assessed by recording final body weight, determining net body weight gain, percent weight gain, specific growth rate (SGR), feed intake, feed conversion ratio (FCR) and protein efficiency ratio (PER) as follows:

$$\text{Net weight gain (g)} = W_f - W_i$$
$$\text{Weight gain (\%)} = 100\ (W_f - W_i)\ W_i^{-1}$$
$$\text{Specific growth rate (\%)} = 100\ (\text{Ln}\ (W_f)) - \text{Ln}\ (W_i))\ T^{-1}$$
$$\text{Feed conversion ratio} = \text{WTFC}\ (W_f - W_i)^{-1}$$
$$\text{Protein efficiency ratio} = (W_f - W_i)\ W_{prot,f}^{-1}$$
$$\text{Feed Intake} = 100\ (W_i)\ T^{-1}$$

where W_i and W_f are the initial and final body weight (g), WTFC is the weight of feed consumed (g), T is duration of the experiment (days) and $W_{prot,f}$ is the weight of intake of dietary crude protein.

2.4. Sampling and analytical methods

At the end of the 60 days feeding trial, the fish were starved for 24 h, anesthetized and weighed. The procedure followed as described by Nayak et al., 2017 for collecting sample for *Δ6 fad* gene expression. Five fish per replicate were frozen at −20 °C for whole body proximate composition determination ($n = 15$ for each treatment). Liver, muscle, intestine and brain samples of two fish from each replicate were used for RNA extraction. For RNA extractions tissue samples were quickly removed and frozen immediately in liquid nitrogen and stored at −80 °C for further use. The remaining muscle and liver tissues were frozen at −20 °C for fatty acid analysis.

2.5. Chemical analysis

The chemical composition of experimental diets and whole-body samples were analyzed as per standard procedures (AOAC, 1990). Briefly, moisture content was determined by drying samples in an oven at 100 °C for 24 h, protein was determined by Kjeldahl method (nitrogen x 6.25) using an automated Kjeldahl distillation systems (Vapodest; Gerhardt Analytical System, Germany). Lipids were extracted by chloroform: methanol mixture (2:1, *v*/v) containing 0.01% butylated hydroxytolune (BHT) to reduce lipid oxidation during processing (Folch et al., 1957). Total ash was determined by incinerating the samples in a muffle furnace at 550 °C for 3 h. Nitrogen free extract (NFE) was calculated by difference. Gross energy was determined using an adiabatic