Aquaculture 457 (2016) 43–49

Contents lists available at ScienceDirect

Aquaculture

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

Adaptations of lipid metabolism and food intake in response to low and high fat diets in juvenile grass carp (*Ctenopharyngodon idellus*)

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ARTICLE INFO

Article history:
Received 13 September 2015
Received in revised form 18 January 2016
Accepted 19 January 2016
Available online 20 January 2016

Keyword:
Dietary fat level
Growth
Lipid homeostasis
Appetite
Leptin

ABSTRACT

This study was conducted to examine the systemic metabolic strategies of grass carp to maintain lipid homeostasis when fed with low- or high-fat diets. The isonitrogenous diets with different fat levels (9.3, 48.7 and 107.9 g kg^{-1}) were fed to grass carp for 8 weeks. After the feeding trial, the growth rate and feed intake of grass carp fed with low-lipid (LL) diet or high-lipid (HL) diet were lower than fish fed with medium-lipid (ML) diet. Serum triglyceride (TG) and total body fat contents were significantly increased in grass carp with increasing lipid intake. Gene expression data indicated that fish increased hepatic *gk* and *pk* expressions to elevate glycolysis, and enhanced *acc* and *fas* expressions to accelerate biosynthesis of fatty acid (FA) to adapt to low lipid intake. Meanwhile, fish fed with LL diet decreased hepatic *cpt1* expression to depress lipolysis, leading to low contents of serum TG and body fat. In contrast, excess lipid intake increased *g6pase*, *pepck* and *pparα* expressions to stimulate gluconeogenesis and β-oxidative, while decreased *acc* and *fas* expressions to reduce lipid synthesis in fish liver. Moreover, increased β-oxidative-induced FA or gluconeogenesis-induced serum glucose might induce the appetite suppression by high dietary fat through modulation of *leptin* expression. This study could be a reference in the systemic adaptation of lipid metabolism responding to dietary fat in fish.

Statement of relevance

Systemic adaptation of lipid metabolism responding to dietary lipid.

1. Introduction

Dietary fat provides essential fatty acids, phospholipids for maintaining cell normal structure and biological function (Sargent et al., 1999). It is well known that appropriate levels of non-protein energy sources determine the efficiency of protein utilization (Wilson and Halver, 1986). Dietary fat as an energy source have been widely used in economic fish aquaculture to save dietary protein and increase feed efficiency (Hillestad et al., 1998; Boujard et al., 2004). However, several studies have revealed that excessive fat in diets has a negative effect for fish growth, such as unwanted hepatic fat deposition (Lee et al., 2002; Du et al., 2005).

Animals have developed an accurate and complicated metabolic system to adapt to different nutritional states (Soengas, 2014). In mammals, the mechanism of lipid metabolism responding to different dietary fat has been extremely discussed, especially high-fat diet (Lin et al., 2000; Buettner et al., 2007; Kohsaka et al., 2007). Mammals mostly store excess energy as neutral lipid (TG) in white adipose tissue. However, lipid also can be stored in liver and muscle in fish (Ando et al., 1993; Kaneko et al., 2013). A number of lipometabolic genes in some fishes have been cloned, and preliminary functions have also been illustrated (Morash et al., 2009; Cheng et al., 2011; Leng et al., 2012; He et al., 2014, 2015). Although a lot of existing literature dealing with lipid metabolism in many teleost species (Wang et al., 2005; Du

Abbreviations: *acc*, acetyl-CoA carboxylase; *agrp*, agouti-related protein; *b2m*, beta-2-microglobulin; *β-actin*, actin isoform B; *cart*, cocaine and amphetamine regulated transcript; *cck*, cholecystokinin; CHO, cholesterol; *cpt1*, carnitine palmitoyltransferase 1; *ef1α*, elongation factor 1-alpha; *fas*, fatty acid synthase; FI, feed intake; *g6pase*, glucose-6-phosphatase*gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *gk*, glucokinase; *gp*, glycogen phosphorylase; *gs*, glycogen synthase; HDL, high density lipoprotein; LDL, low density lipoprotein; *npy*, neuropeptide Y; *pepck*, phosphoenol pyruvate carboxykinase; PER, protein efficiency ratio; *pk*, pyruvate kinase; *pomc*, proopiomelanocortin; *pparα*, peroxisome proliferator-activated receptor type *α*; *rpl13a*, ribosomal protein L13a; SGR, specific growth ratio; SR, survival ratio; TG, triglyceride; *tubα*, tubulin alpha; WG, weight gain.

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http://dx.doi.org/10.1016/j.aquaculture.2016.01.014
0044-8486/

et al., 2006; Morash et al., 2009; Chatzifotis et al., 2010), the adaptive strategies to different fat intake have not been well understood.

Appetite of mammals could be regulated by energy status (Gélineau and Boujard, 2001; Gélineau et al., 2001). Hypothalamic detection of nutrients, directly or indirectly afferent information, activates neurocircuits involved in the regulation of food intake, glucose homeostasis, lipid metabolism, and energy expenditure (Morton et al., 2006; Blouet and Schwartz, 2010). In addition to responding to changes in circulating metabolite levels, those neurons contain receptors for several hormones (such as leptin, ghrelin and cholecystokinin) allowing them to integrate multiple endocrine signals with information of nutritional status and energy reserves (Levin et al., 2004; Blouet and Schwartz, 2010). In teleost fish, evidences obtained in recent years pointed to the presence of sensor systems for glucose and fatty acids at central and peripheral locations, which are related to the control of food intake through changes in the expression of orexigenic (*npy/agrp*) and anorexigenic (*cart/pomc*) neuropeptides (Conde-Sieira et al., 2010; Librán-Pérez et al., 2012). However, it is still unclear that how metabolic information is integrated with the decision of changing food intake accordingly in fish brain (Soengas, 2014).

Previous studies have reported that food intake of some carnivorous fish could be regulated by the nutrients level such as glucose and fatty acids through changes in the expression of anorexigenic and orexigenic neuropeptides (Librán-Pérez et al., 2012, 2015). However, the adaptation strategy of energy metabolism and metabolic feedback in herbivorous fish needs to be assessed (Soengas, 2014). In the present study, we investigated the effects of dietary fat on growth performance, food intake, and expressions of anorexigenic and orexigenic neuropeptide genes and hepatic glucose and lipid metabolic genes in juvenile grass carp (*Ctenopharyngodon idellus*), a typical herbivorous fish, in an attempt to clarify the systemic metabolic strategies to maintain lipid homeostasis in herbivorous fish with low or high lipid intake.

2. Materials and methods

2.1. Experimental diets

Using casein as protein source, corn starch as the carbohydrate source, fish oil and soybean oil as the lipid source, three diets were formulated to contain three crude lipid levels (9.3, 48.7 and 107.9 g kg^{-1} named as LL, ML and HL). The composition and chemical analysis of the three experimental diets are shown in Table 1. All the ingredients were from mainland of China and purchased from Shentianyu and Fulong Dietary Company (Wuhan, China). The diets were pelleted (2 mm diameter) by a laboratory pellet machine within 30 min after the ingredients were thoroughly mixed. Then the pellets were air-dried and stored in a freezer at −20 °C until used.

2.2. Fish and experimental conditions

Experimental grass carp were obtained from the Fish Center of Xiantao, Hubei, China. Prior to the experiment, the fish were distributed into 4 tanks (1000-L) provided with flow-through water for 15 days. Then they were selected and randomly distributed into 12 tanks (300-L) where the fish were acclimated to the experimental conditions for 2 weeks. After the 2-week acclimation, fish were then starved for 24 h to measure the body length and weight at the beginning of the experiment. The stocking density was 25 fish (mean weight was about 12 g) per tank (300-L) and each diet was fed to triplicate randomly assigned tanks. During the experimental period, the temperature ranged from 22 to 26 °C, the ammonia content was about 0.27 ± 0.02 mg L^{-1} the pH ranged from 7.11 to 7.59. The aerated and filtered flow-through water was kept at a flow-rate of 3 L min^{-1}. The dissolved oxygen value was 7.26–7.86 mg L^{-1}. During whole feeding trial, the fish were fed to apparent satiation twice daily at 08:00 and 16:00 for 8 weeks. Uneaten feed was collected after feeding by siphoning, then dried for about 12 h in a ventilated oven at 60 °C to determine feed intake.

Table 1
Compositions of experimental diets.

Item	Experimental diets		
	LL	ML	HL
Casein	190.0	190.0	190.0
Gelatin	70.2	70.2	70.2
Fish oil	0	20	50
Soybean oil	0	20	50
DL-Met (99%)	1.6	1.6	1.6
α-Starch	100	100	100
Corn starch	326.5	326.5	326.5
Cellulose	100	60	0
$Ca(H_2PO_4)_2$	20	20	20
Mineral mix[b]	20	20	20
Vitamin mix[a]	10	10	10
Choline chloride	6	6	6
Ethoxyquin	0.5	0.5	0.5
Total	1000	1000	1000
Compositions (g kg^{-1} diet)			
Crude protein	316.5	315.8	314.2
Crude lipid	9.3	48.7	107.9
Ash	68.5	65.6	63.2
Moisture	95.3	92.2	86.8
Gross energy (kJ g^{-1})	11.67	12.41	13.52

[a] Vitamin premix (per kg of diet): vitamin A, 2000 IU; vitamin B1 (thiamin), 5 mg; vitamin B2 (riboflavin), 5 mg; vitamin B6, 5 mg; vitamin B12, 0.025 mg; vitamin D3, 1200 IU; vitamin E 21 mg; vitamin K3 2.5 mg; folic acid, 1.3 mg; biotin, 0.05 mg; pantothenic acid calcium, 20 mg; inositol, 60 mg; ascorbic acid (35%), 110 mg; niacinamide, 25 mg.
[b] Mineral premix (per kg of diet): $MnSO_4$, 10 mg; $MgSO_4$, 10 mg; KCl, 95 mg; NaCl, 165 mg; $ZnSO_4$, 20 mg; KI, 1 mg; $CuSO_4$, 12.5 mg; $FeSO_4$, 105 mg; Na_2SeO_3, 0.1 mg; Co, 1.5 mg.

2.3. Sample collection and chemical analyses

At the end of the 8-week feeding trial, approximately 2 h after the last feeding, all the fish were anesthetized with MS-222 (Argent Chemical Laboratories, Redmond, WA, USA) and then weighed and counted. In each cage, three fish were randomly captured for body chemical analysis, livers from another three fish were dissected and separated for tissue lipid contents detection; other six fish for molecular experiments were randomly captured and killed by immediate spinal destroying for measure and dissection. The small pieces of fish liver and brain samples for gene expression assay were immediately collected and frozen in liquid nitrogen and stored at −80 °C for RNA isolation and subsequent analysis. Blood was collected from the caudal vein of other three fish from each cage and centrifuged at 3500 g for 10 min, and then serum was separated and stored at −80 °C until used.

Crude protein, crude lipid, moisture and ash of diets were determined by standard methods (A.O.A.C., 1995). Crude protein (N × 6.25) was determined following the Kjeldahl method after an acid digestion using a Kjeltec system (Kjeltec 2300 Analyzer, Foss Tecator, Sweden). Crude lipid was evaluated by the ether-extraction method using Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Ash was measured using a muffle furnace at 550 °C for 12 h. Moisture was determined by oven drying at 105 °C for 6 h. Energy content of the diets was measured by bomb calorimetry using a Parr 6200 calorimeter equipped with a Parr 1108 Oxygen Bomb and a Parr 6510 water handling system (Parr Instrument Company, Moline, IL, USA).

CHO, TG, HDL, LDL and glucose contents were determined using an automatic biochemical analyzer [Abbott Aeroset Analyzer (Abbott Laboratories, Abbott Park, IL, USA)] in the Zhongnan Hospital of Wuhan University (Wuhan, Hubei, China).

2.4. RNA isolation, reverse transcription and gene expression analysis

The liver total RNA of grass carp consuming diets with varying lipid levels was extracted by SV Total RNA Isolation System kit (Promega, USA) following the manual, then its purity and quantity were measured