Aquaculture 432 (2014) 181–191

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Conjugated linoleic acid differentially modulates growth, tissue lipid deposition, and gene expression involved in the lipid metabolism of grass carp

Gui-Fang Dong [a,c], Qi Zou [a], Huan Wang [a], Feng Huang [a,c], Xiao-Chun Liu [a], Lu Chen [b], Chao-Yu Yang [a], Yan-ou Yang [b,*]

[a] Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan, Hubei 430023, China
[b] College of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui 230000, China
[c] Freshwater Aquaculture Collaborative Innovation Center in Hubei Province, Wuhan, Hubei 430070, China

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 26 April 2014
Accepted 1 May 2014
Available online 20 May 2014

Keywords:
Conjugated linoleic acid
Fatty liver
Lipid deposition
Gene expression
Ctenopharyngodon idella

ABSTRACT

Conjugated linoleic acid (CLA) has been shown to decrease body fat and increase lean tissue in mammals. However, limited data is available about the effect of CLA on the lipid content in fish tissue, and the mechanisms underlying the beneficial effects of CLA in fish are unknown. We hypothesized that dietary CLA may induce lipid-lowering effects in grass carp (*Ctenopharyngodon idella*) tissue, and the fat reduction effect was modulated by the expression of genes involved in the lipid metabolism. A 65-day growth trial was conducted to investigate the effect of CLA on the growth, tissue lipid deposition, and gene expression involved in the lipid metabolism of grass carp. Seven isonitrogenous and isolipidic diets were formulated: 0% CLA (control); 0.5% CLA (CLA0.5); 1% CLA (CLA1); 1.5% CLA (CLA1.5); 2% CLA (CLA2); 2.5% CLA (CLA2.5); and 3% CLA (CLA3).

Results showed that only fish fed the CLA3 diet exhibited a significant reduction in feeding rate and specific growth rate than those of fish fed the control diet ($P < 0.05$). Significant decreases in the lipid content in the liver, intraperitoneal fat, and muscle were observed in fish fed with 2.5% to 3% CLA, 1.5% to 3% CLA, and 2% to 3% CLA diets, respectively ($P < 0.05$), compared to those fed with the control diet. Dose- and tissue-dependent changes were found in the mRNA expressions of fatty acid synthetase (FAS), acetyl-CoA carboxylase (ACC), lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor γ (PPARγ), and sterol regulatory element binding protein-1c (SREBP-1c). The mRNA expressions in most of the genes examined in the liver, foregut, intraperitoneal fat and muscle were highly sensitive to dietary CLA. Our results suggested that a dose-dependent effect on the reduction of fish growth induced by CLA supplementation should be carefully considered in intensive aquaculture, although lipid reduction is nutritionally important for fatty liver control in grass carp. Furthermore, our results raise the possibility that the lipid-lowering effects of dietary CLA were modulated by the gene expressions in lipogenesis (such as FAS and ACC), lipoprotein transport (such as LPL), and lipolysis (such as HSL) primarily in the liver, foregut, intraperitoneal fat, and muscle. The activation of transcription factors (such as PPARα, PPARγ, and SREBP-1c) may also be responsible for the lipid-lowering effects of dietary CLA.

1. Introduction

Dietary formulations in aquaculture likely increase lipid levels in diets to spare proteins and increase fish weight gain (Tocher, 2003). However, high lipid intake elicits adverse effects on fish. These adverse effects include the deposition of excess lipid in fish tissues (Du et al., 2006; Gao et al., 2011), induction of fatty liver syndrome (Dos Santos et al., 1993), abnormal oxidative status (Du et al., 2006), and poor nutritional value of cultured fish (Chaiyapechara et al., 2003). As such, fish farming industries have focused on the development of methods that can be used to control body lipid deposition and increase the market value of cultured fish when these diets are used (Valente et al., 2007). However, no effective treatment has been developed yet to relieve fatty liver syndrome or increase the market value of cultured fish.

Conjugated linoleic acid (CLA) is a group of positional and geometrical isomers of conjugated dienoic derivatives of linoleic acid; the two

Abbreviations: ACC, acetyl-CoA carboxylase; FAS, fatty acid synthetase; HSL, hormone-sensitive lipase; IPF, intraperitoneal fat ratio; LPL, lipoprotein lipase; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; SREBP-1c, sterol regulatory element binding protein-1c.

* Corresponding author at: College of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui 230000, China. Tel./fax: +86 551 5786397.
E-mail addresses: cdyyo@126.com (Y. Yang), dongguifang2002@126.com (G.-F. Dong).

http://dx.doi.org/10.1016/j.aquaculture.2014.05.008
0044-8486/

main natural isomers are *cis*-9 and *trans*-11 as well as *trans*-10 and *cis*-12 (Pariza et al., 2001). Dietary CLA also decreases body fat in mice (Takahashi et al., 2002), rats (Sisk et al., 2001), pigs (Dugan et al., 1997), and chickens (Badinga et al., 2003). Furthermore, in mammals, CLA is responsible for these antiobesity properties via multiple mechanisms, including increasing energy expenditure (Choi et al., 2004), regulating adipocytes metabolism (Park et al., 2004), modulating adipokines and cytokines (Inoue et al., 2004), and promoting fatty acid oxidation (Wahle et al., 2004). In addition, CLA can alter the expression of numerous important genes to maintain lipid and fatty acid homeostasis (Ide, 2005), such as acetyl-CoA carboxylase (ACC, EC 6.4.1.2), peroxisome proliferator-activated receptors (PPARs), and sterol regulatory element binding protein-1 (SREBP-1). Therefore, CLA-induced effects on mammal lipid deposition may be of considerable interest in aquaculture.

However, considerable discrepancies among results in the literature exist, especially in the lipid-lowering effects of CLA in fish. For example, a significant reduction in the lipid contents of the liver is obtained in hybrid striped bass and yellow perch when these organisms are fed with 1% CLA (Twibell et al., 2000, 2001), whereas no significant effects were observed in the liver, muscle or whole body in channel catfish, gilthead sea bream, and European sea bass when these organisms are fed with the same CLA concentration (1% CLA) (Makol et al., 2009, 2012; Twibell and Wilson, 2003). These findings imply that the lipid-lowering effects of CLA in fish depend on species, fish size, administration duration, CLA dose, and chemical form of CLA included in the diets (Makol et al., 2012). Therefore, much more study should be taken to investigate dietary CLA intake on the lipid deposition. On the other hand, a considerable number of studies on the effects of dietary CLA on fish growth performance have been conducted (Bandarra et al., 2006; Makol et al., 2009, 2012; Tan et al., 2010), but the underlying molecular process and metabolic mechanism involved in such lipid-lowering effects of CLA in fish has received less attention. To date, the possible mechanisms for this fat reduction by dietary CLA in fish remain unclear.

Grass carp (*Ctenopharyngodon idella*) is an important economic farmed fish in China, where more than 3 million tons of the fish are produced per year (Cheng et al., 2009). Compared with carnivorous fish, grass carp exhibits low lipid demands and poor lipid utilization (Du et al., 2008; Gao et al., 2011). Therefore, excess lipid intake in grass carp not only causes loss of appetite, growth delay, and fat accumulation but also affects lipoprotein synthesis and induces lipid peroxidation (Du et al., 2008; Ji et al., 2011). In addition, some diseases related to fish lipid metabolism, such as fatty liver syndrome, increase yearly in cultured grass carp (Cheng et al., 2009). If studies could show that dietary CLA may induce lipid-lowering effects in grass carp without eliciting any negative effects on fish growth; as such, the effective prevention and treatment of fatty liver problems in grass carp and other species in aquaculture may be elucidated. Therefore, the present study aimed to investigate the effects of dietary CLA on the growth and tissue lipid deposition in grass carp. The underlying molecular process (such as gene expression) involved in lipid metabolism was also evaluated to elucidate the mechanisms of lipolysis, lipid deposition, and lipid homeostasis.

2. Material and methods

2.1. Fish and experimental diets

Grass carp were obtained from the Sanhu hatchery farm in Jiayu, Hubei, China and acclimated in two quadrate concrete tanks (diameter = 200 cm, height = 160 cm, water volume = 3000 L) for 20 d before the experiment was conducted. During acclimation period, the fish were fed to satiation with the control diet twice daily (09:00 and 15:00 h).

Seven isonitrogenous (crude protein: 29%) and isolipidic (crude lipid: 4.5%) diets were prepared: 0% CLA (control: CLA0); 0.5% CLA (CLA0.5); 1% CLA (CLA1); 1.5% CLA (CLA1.5); 2% CLA (CLA2); 2.5% CLA (CLA2.5); and 3% CLA (CLA3). CLA was supplied by AUHAI (Qingdao Auhai Biotech. Company, Qingdao, China) as a supplement and was added to the diets as a substitute of fish oil to maintain a constant lipid level in dietary treatments. The fatty acid compositions of the CLA oil (g/100 g of total fatty acids) were C16:0, 0.6; C18:0, 0.4; C18: 1 C9, 2.7; C18: 2 C9, C12, 1.5; *cis*-9, *trans*-11 CLA isomer, 43.8; *trans*-10, and *cis*-12 CLA isomer, 47.3. The diet formulations and chemical compositions are shown in Table 1. Experimental diets were prepared to form pellets (1 mm to 3 mm in diameter) by using a laboratory presser, oven-dried at 60 °C, and stored at −10 °C before use.

2.2. Growth trial

The experiment was conducted in a flow-through system containing 21 concrete tanks (diameter = 200 cm, water volume = 3000 L). At the beginning of the trial, the fish weighing 7.51 ± 0.05 g (mean ± SE) were subjected to fasting for 24 h. The fish of equal size were randomly selected, weighed, and stocked in each tank (50 fish per tank). Three tanks were randomly assigned to each of the seven diet groups. Three groups of ten fish each were sampled to determine the initial body composition. The feeding trial lasted 65 d.

All of the fish were held under a natural photoperiod during the experiment. Oxygen was supplied by aeration with a minimum level at 7.0 mg L^{-1}. Water temperature was recorded daily and ranged from 24.5 °C to 28.4 °C. pH was approximately 7.0. Ammonia-N was measured weekly and retained at less than 0.1 mg L^{-1}. Fish were then hand-fed to apparent satiation twice daily (09:00 and 15:00 h). The supplied daily feed was recorded, and the uneaten feed was siphoned 1 h after they were fed. These uneaten feed were dried and then reweighed. The leaching rate of uneaten feed was estimated by placing the weighed feed in tanks without fish for 1 h. Afterward, the uneaten feed was collected, dried, and reweighed. The average leaching rate was used to calibrate the amount of uneaten feed.

Table 1
Formulation and chemical composition of the experimental diets (g/100 g in dry matter).

Ingredients	Diets						
	CLA0	CLA0.5	CLA1	CLA1.5	CLA2	CLA2.5	CLA3
White fish meal[a]	10	10	10	10	10	10	10
Soybean meal (oil-extracted)[b]	47	47	47	47	47	47	47
Corn starch	18	18	18	18	18	18	18
α-Starch	6	6	6	6	6	6	6
Mineral premix[c]	4	4	4	4	4	4	4
Vitamin premix[d]	1	1	1	1	1	1	1
Fish oil	3	2.5	2	1.5	1	0.5	0
CLA[e]	0	0.5	1	1.5	2	2.5	3
Choline chloride	1	1	1	1	1	1	1
Cellulose	10	10	10	10	10	10	10
Chemical composition (in dry matter)							
Crude protein (%)	29.64	29.87	30.12	29.31	30.04	29.71	29.97
Crude lipid (%)	4.66	4.53	4.71	4.42	4.65	4.5	4.42
Ash (%)	8.52	8.64	8.46	8.67	8.75	8.7	8.54
Gross energy (kJ/g)	15.97	15.78	15.86	15.44	15.39	15.51	15.67

[a] White fish meal: American Seafood Company, Seattle, Washington, USA.
[b] Soybean meal: oil-extracted soybean, Wuhan Coland Feed Co., Ltd., Wuhan, China.
[c] Mineral premix (mg kg^{-1} diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 7500; $NaH_2PO_4 \cdot 2H_2O$, 12,500; KH_2PO_4, 16,000; $Ca(H_2PO_4) \cdot 2H_2O$, 10,000; $FeSO_4$, 1250; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750; $ZnSO_4 \cdot 7H_2O$, 176.5; $MnSO_4 \cdot 4H_2O$, 81; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.5; KI, 1.5; Starch, 225. 6 BHT: 2,6-Di-tert-butyl-4-methylphenol.
[d] Vitamin premix (mg kg^{-1} diet): Vitamin A, 110; Vitamin D_3, 20; Vitamin E, 100; Vitamin K_3, 10; ascorbic acid, 111; Thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 2; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 5; rice bran, 3226.
[e] Qingdao Auhai Biotech. Co. Ltd., Qingdao, China.