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Interactions between dietary protein levels, growth performance, feed utilization, gene expression and metabolic products in juvenile grass carp (*Ctenopharyngodon idella*)

Yan Jin [a,b], Li-xia Tian [a], Shi-wei Xie [a], Ding-qian Guo [a], Hui-jun Yang [a], Gui-ying Liang [a], Yong-jian Liu [a,*]

[a] Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou, China
[b] School of Medicine, University of St Andrews, St Andrews, United Kingdom

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ABSTRACT

The present study aims to evaluate the possible interactions between dietary protein levels, growth performance, gene expression and volatile compounds in the plasma and liver of juvenile grass carp. Juvenile grass carp (4.27 ± 0.01 g) were fed with six extruded purified diets containing different protein levels (200, 250, 300, 350, 400, 450 g kg^{-1} dry diet) for 8 weeks. The best growth performance appeared in the 400 g kg^{-1} dietary protein group, which had highest percent weight gain, protein retention, condition factor and lowest lipid retention, feed conversion ratio and whole body crude lipid among all the treatments. In addition, we found that fish fed with higher protein content diet was apt to have lower lipid content in muscle and liver. It may relate to the expression patterns of neuropeptide Y in the hypothalamus and acetyl-coA carboxylase 1 in both liver and hypothalamus. An analysis of volatile compounds in the plasma and liver showed that the concentration of several types of fatty acid and amino acid changed between the 400 g kg^{-1} dietary protein group and 200 g kg^{-1} dietary protein group. This may also contribute to the final growth performance.

1. Introduction

Dietary protein level is an essential factor in increasing the growth performance and production of reared fish. Dietary protein usually makes up the largest single cost factor in feeds (Hatlen et al., 2005; Islam and Tanaka, 2004). It is well known that fish preferentially utilize dietary protein as an energy source rather than lipid or carbohydrate, so it is important to optimize protein utilization for tissue synthesis rather than for energy purposes (Sadasivam and Aires de, 1985). Therefore, understanding how fish use dietary protein and what happens in vivo during the growth period is fundamental in fish culture management, which leads to maximized feed conversion efficiency and cost savings.

The grass carp (*Ctenopharyngodon idella*) is a typical herbivorous agastric finfish that is one of the most important species cultured in China (Jin et al., 2013). Although studies on the nutrition of grass carp were initiated as far back as the late 1970s (Dabrowski, 1977; Law, 1986), there is no adequate knowledge on the interactions between dietary protein levels, gene expression and metabolic products in juvenile grass carp. Only the dietary requirement of protein (Dabrowski, 1977) has been studied. It is necessary to explore the mechanism behind the phenomenon, so that we can achieve optimum growth rates in aquaculture.

Using the combined technologies of molecular biology and metabolomics it will be possible to construct complete metabolic profiles for each feed trial. Metabolomics allows the investigation of changes in metabolic profile of a broad range of small molecular weight metabolites, including lipids, sugars and amino acids in biological samples (Fiehn, 2002), whereas gene expression may offer us some idea of what enzymes and transporters are responsible for these changes.

The aim of present study was to evaluate the possible interactions between dietary protein levels and growth performance, gene expression and the appearance of volatile metabolites in liver and plasma in the juvenile grass carp. In the present study, we chose to measure the gene expression of hypothalamic neuropeptide Y (*npy*) and acetyl-CoA carboxylase 1(*acc1*), together with hepatic *acc1*, uncoupling protein 1 (*ucp1*) and peroxisome proliferator-activated receptor γ (*ppar*γ). NPY has been established as an important regulator of food intake (Volkoff,

Abbreviations: NPY, neuropeptide Y; ACC1, acetyl-CoA carboxylase 1; UCP1, uncoupling protein 1; PPARγ, peroxisome proliferator-activated receptor γ; PWG, percent weight gain; FCR, feed conversion ratio; AST, aspartate aminotransferase; ALT, alanine transaminase; TG, triglycerides; CHO, cholesterol; BSTFA, bis(trimethylsilyl)trifluoroacetamide; TMCS, trimethylchlorosilane; ECF, ethyl chloroformate; TIC, total ion current; PLS-DA, partial least squares discriminant analysis; VSI, viscerasomatic index; HIS, hepatosomatic index; IPF, intraperitoneal fat ratio; CF, condition factor; PCA, principal component analysis; VIP, variable importance in the projection.

* Corresponding author at: Nurtrition Laboratory, Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, Guangzhou 510275,China. Tel.: +86 02 84110789.
E-mail address: edls@mail.sysu.edu.cn (Y. Liu).

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2006; Yokobori et al., 2012). PPARγ is a member of the ligand-activated nuclear receptor superfamily. PPAR-γ-activating ligands improve adipose tissue function by up-regulating genes encoding molecules that promote a combination of lipid storage and lipogenesis (Evans et al., 2004). ACC1 is situated at the branch point of the metabolic crossroad for acetyl-CoA, linking the Kreb's cycle and lipid metabolism. UCP1 are important enzymes correlating to energy metabolism, there was a dose-related reduction in *ucp* mRNA produced by the NPY treatment in rat (Billington et al., 1994). Metabolic profile of liver and plasma were observed, because liver is the metabolic center of grass carp and plasma is the major transporter.

2. Materials and Methods

2.1. Experimental design and diets

Six purified experimental diets with different protein levels (200, 250, 300, 350, 400, 450 g kg^{-1} dry diet) were used. The formulation and approximate composition analysis of the feeds are shown in Table 1. Casein (Hulunbeier Sanyuan Milk Co., Ltd, Inner Mongolia, China) and gelatin (Rousselot Gelatin Co., Ltd, Guangdong, China) were used as the protein source in the ratio, 9:1. All the diet ingredients were ground through a 60 mm mesh before use. All the powdered ingredients were weighed and mixed for 15 min, and then fish oil and corn oil were added and mixed for 15 min. Distilled water was added to the premixed dry ingredients and thoroughly mixed until homogenous in a Hobart-type mixer. The pellets were obtained (1.5 mm in diameter) using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) and air-dried to give a water content of approximately 100 g kg^{-1}. The pellets were ground, sieved and stored in plastic bags at −20 °C until used.

Table 1
Formulation and composition of experimental diets.

	Dietary protein levels(g kg^{-1}diet)					
	200	250	300	350	400	450
Ingredients(g kg^{-1} diet)						
Casein	216	265	317	369	423	477
Gelatin	24	29	35	41	47	53
Cellulose	200	200	200	200	200	200
Corn starch	418	364	306	248	188	128
Corn oil	20	20	20	20	20	20
Fish oil	20	20	20	20	20	20
Vitamin mix[1]	10	10	10	10	10	10
Mineral mix[2]	80	80	80	80	80	80
Choline chloride (50%)	5	5	5	5	5	5
vitamin C	2	2	2	2	2	2
Taurine	5	5	5	5	5	5
Chemical analysis(g kg^{-1}diet)						
Moisture	91.1	91.4	88.4	86.6	90.6	103.2
Crude protein	228.9	269.8	319.6	368.5	427.6	473.9
Crude lipid	34.6	32.4	34.5	33.3	34.2	35.1
Digestible energy (Mcal/kg)[3]	2974.68	2975.05	2974.61	2974.17	2973.99	2973.81

[1] Vitamin mix (mg kg^{-1} diet): thiamine (98%), 50; riboflavin (80%), 50; vitamin A, 25 000 IU; vitamin E (50%), 400; vitamin D3, 24 000 IU (Roche Taishan Vitamin Products Ltd., Shanghai, P.R. China); menadione (43%), 40 (Zhejiang Brother chemical Company Ltd., Zhejiang Province,P.R. China); pyridoxine HCl (98%), 40 (Hubei Xian ning Second Pharmaceutical Factory, Xian ning,Hubei Province, P.R. China); cyanocobalamin (1%), 0.1 (Junchi Biological Technology Co., Ltd.,Tianjing, P.R. China); biotin (2%), 6 (Sumitomo chemical Co., Ltd., Osaka Japan); calcium pantothenate (98%), 100 (Dahchi Pharmaceutical Co., Ltd., Tokyo, Japan); folic acid (97%), 15 (Jinan Xinfa Pharmaceutical Co., Ltd., Jinan, Shandong Province, P.R. China); niacin (99%), 200 (Lonza Guangzhou Ltd., Co., Guangzhou, Guangdong Province, P.R. China); inositol (98%), 2000 (Shanghai Yiran Industrial Limited Company, Shanghai, P.R. China); and cellulose was used as a carrier.

[2] Mineral mix (g kg^{-1} diet): calcium biphosphate, 9.8; calcium lactate, 37.9; sodium chloride, 2.6; potassium sulphate, 13.1; potassium chloride, 5.3; ferrous sulphate, 0.9; ferric citrate, 3.1; magnesium sulphate, 3.5; zinc sulphate, 0.04; manganese sulphate, 0.03; cupric sulphate, 0.02; cobalt chloride, 0.03; potassium iodide, 0.002; and cellulose 42. All minerals were supplied by Guangzhou Chemical Reagent Factory, except that calcium lactate was supplied by Yinchuan Jintaiyang Calcium Lactate Co. Ltd., Yinchuan, Ningxia Province, P.R. China.

3 The available energy was calculated using 4.13 Mcal/kg for casine, 2.8 Mcal/kg for gelatin, 4 Mcal/kg for corn starch, 8.44Mcal/kg for fish oil, 8.75Mcal/kg for corn oil respectively (China feed database).

2.2. Experimental fish and experimental conditions

A group of 700 juvenile grass carp were obtained from a local hatchery. Before starting the experiment, the fish were acclimated to the experimental diet containing 300 g kg^{-1} dietary protein (Table 1) for two weeks. After acclimatization, healthy fish were sorted by weight into uniform groups. The fish were randomly distributed to a recirculating system comprising 18 experimental glass-fiber tanks (98 L × 48 W × 42 H cm, water volume of 200 L) with an equal stocking density of 30 fish per tank. The initial body weight of fish averaged 4.27 ± 0.01 g. Each diet was assigned to triplicate tanks. The feeding trial lasted for 8 weeks. The fish were fed twice a day with a ration of 40–50 g kg^{-1} of body weight. The fish were weighed every two weeks for daily ratio adjustment. Natural light-dark cycle was used during the trial. A third of the rearing water was replaced weekly and water quality parameters were determined before and after refreshing the water. Water quality parameters monitored weekly were as follows (mean ± SD): temperature, 26.9 ± 0.5 °C; dissolved oxygen, 7.05 ± 0.09 mg L^{-1}; total ammonia–nitrogen, 0.082 ± 0.006 mg L^{-1} and pH, 7.3 ± 0.2, respectively.

2.3. Sample collection and analytical methods

At the beginning of the feeding trial, 10 juvenile fish were randomly selected and sampled for analysis of whole body composition prior to dietary administration. At the termination of the feeding trial, all fish were fasted for 24 h, all the fish of each tank were counted and weighed to determine percent weight gain (PWG), feed conversion ratio (FCR) and nutrients retention. Fourteen fish from each tank were randomly selected and anaesthetized (MS222; Sigma, St Louis, MO, USA, 50 mg L^{-1}). Two for analysis of whole body composition and six measured for body length and weight, before blood collection into heparinized syringes by cardiac puncture. The blood samples were centrifuged (4000 g, 10 min) at 4 °C (centrifuge MR23i, Jouan, France). The plasma was separated and stored at −80 °C until analyzed. The fish were then dissected to obtain viscera, liver, mesenteric fat and white muscle. White muscle was from both sides of the fillets without skin. The last six fish was exsanguinated by snipping gilled before collection of the liver and hypothalamus, which were immediately frozen in liquid nitrogen and stored at -80 °C until used.

Diets and fish samples (including whole body, white muscle and liver) were analyzed in duplicate for approximate composition of moisture, crude protein and crude lipid. Moisture, crude protein and crude lipid were determined using standard methods (AOAC, 1995). Moisture was determined by drying in an oven at 105 °C for 24 h until constant weight; crude protein (N × 6.25) was analyzed by the Kjeldahl method after acid digestion (1030-Auto-analyzer; Tecator, Höganäs, Sweden); crude fat was determined by the ether-extraction method by Soxtec System HT (Soxtec System HT6; Tecator). Digestible energy content was calculated using 4.13 Mcal/kg for casine, 2.8 Mcal/kg for gelatin, 4 Mcal/kg for corn starch, 8.44Mcal/kg for fish oil, 8.75Mcal/kg for corn oil (China feed database; Du et al., 2009). Activities of plasma aspartate aminotransferase (AST) and alanine transaminase (ALT) along with the concentrations of plasma triglycerides (TG) and cholesterol (CHO) were assayed within 3 days by enzymatic procedures using automatic biochemical analyzer (Hitachi 7170; DAICHI, Tokyo, Japan) in a clinical laboratory of the First Affiliated Hospital of Sun Yat-sen University.