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Growth performance, digestive enzyme, transaminase and GH-IGF-I axis gene responsiveness to different dietary protein levels in broodstock allogenogynetic gibel carp (*Carassius auratus gibelio*) CAS III

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ABSTRACT

A 60-day growth trial was conducted to investigate the effect of dietary protein levels on growth performance, digestive enzyme activities, transaminase activities and GH-IGF-I axis gene expression of broodstock gibel carp (initial body weight, 180.3 ± 0.4 g) using diets containing white fishmeal and casein as the main protein sources. Six isolipidic (100 g kg^{-1}) and isoenergetic (gross energy 17.5 kJ g^{-1}) diets were prepared, with protein levels ranging from 200 to 400 g kg^{-1} at 40 g kg^{-1} increments. Each diet was fed by hand to triplicate groups of 22 fish each to apparent satiation four times a day. Results indicated that specific growth rate (SGR), feed efficiency (FE) and protein retention efficiency (PRE) significantly increased with the increase of dietary protein levels from 200 to 360 g kg^{-1}, after that, SGR and FE plateaued while PRE notably decreased. Whole-body and muscle crude lipid increased and moisture decreased with increasing dietary protein levels; whereas crude protein and ash in whole-body and muscle, condition factor, vicerosomatic index and hepatosomatic index showed no obvious differences between treatments. Plasma ammonia concentration, hepatic alanine transaminase and aspartate transaminase activities markedly enhanced with the increase of dietary protein level up to 320–360 g kg^{-1}. Intestinal trypsin activity peaked in fish fed protein level of 320 g kg^{-1}, and then remarkably descended; while no significant differences were observed in intestinal lipase and α-amylase. The relative mRNA abundance of insulin like growth factor-I (IGF-I) in liver notably up-regulated with dietary protein level increasing to 360 g kg^{-1}, and down-regulated at higher protein level. Pituitary growth hormone (GH) mRNA showed an almost opposite trend with IGF-I. In conclusion, based on a broken-line analysis of SGR, the recommended dietary protein level for broodstock gibel carp was 369 g kg^{-1}, which was higher than that for sub-adult fish.

Statement of relevance

This study provides basic data of nutritional requirement for broodstock gibel carp.

1. Introduction

Protein is a major organic material in fish tissue, taking a proportion of about 65–75% of the total on a dry-weight basis, and the deposition of protein appears to be the main determinant of live weight gain in fish (Wilson, 2002). Protein, as the most expensive ingredient in fish feeds, plays a key role not only in maintenance and repairing of broken tissues, but also in production of enzymes, hormones and antibodies required for many vital processes. Protein deficiency in the diet leads to a reduction of growth and loss of weight. But on the other hand, excessive protein in diet usually converts to energy and increases nitrogenous excretions into water, which affects the voluntary feed intake and growth of fish (McGoogan and Gatlin, 1999). Therefore, it is necessary to estimate the optimum dietary protein level for fish to achieve best growth at the lowest cost.

Up to now, the protein requirements for fish are determined in various species. However, these researches are mainly focus on juveniles (NRC, 2011). As reported, the protein requirement of fish is influenced by fish growth stage and body size (Abdel-Tawwab et al., 2010; Arnason et al., 2010; Page and Andrews, 1973). Generally, the protein requirement of fish decreased with the increase of age and weight (Wilson, 2002). Whereas, it was reported that protein had significant effects on fish reproduction such as gonadal maturation, fecundity, fertilization, embryo development, and larvae quality (Gunasekera and Lam, 1997; Gunasekera et al., 1995). Therefore, dietary protein requirement

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for broodstock could be different from those of rapidly growing juveniles and sub-adults. However, broodstock nutrition including protein requirement is limited to a few species and remains one of the most poorly understood areas of fish nutrition due to the limitation of culture facilities for adult fish and the cost of conducting extended broodstock feeding trials (Izquierdo et al., 2001).

It is generally accepted that the growth hormone (GH)-insulin-like growth factor-I (IGF-I) axis plays a pivotal role in the neuroendocrine regulation of vertebrate growth (Wood et al., 2005). Moreover, this progress is directly or indirectly affected by nutritional status. For instance, prolonged starvation generally leads to suppressed body growth, with concomitant reductions in the levels of circulating IGF-I. It was further shown that dietary protein content had a close correlation with GH and IGF-I or their mRNAs in Asian seabass *Lates calcarifer* (Dyer et al., 2004), gilthead sea bream *Sparus aurata* (Pérez-Sánchez et al., 1995), and Nile tilapia *Oreochromis niloticus* (Qiang et al., 2012). The mechanism is not very clear and may be partially ascribed by the modulating transcriptional activities of the GH-IGF-I genes and serum binding proteins (Wood et al., 2005). However, study of simultaneous and nutritionally regulated changes in mRNA transcripts of GH-IGF-I axis is limited (Gómez-Requeni et al., 2004). Also, relevant effect in gibel carp (*Carassius auratus gibelio*) remains unknown.

Gibel carp CAS III, a selected omnivorous warm water fish, is widely cultured in China with its higher growth, flesh yield and stronger resistibility (Gui and Zhou, 2010). Gibel carp reaches first maturation around one year old and keeps on growing after spawning. In our laboratory, dietary protein requirements for 3.7 g and 85.2 g gibel carp var. CAS III were estimated to be 360 g kg^{-1} and 330 g kg^{-1} dry matter respectively, when fishmeal and casein were used as protein sources (Ye, 2013). But, no information is available about the protein requirement of broodstock gibel carp and few data is found for the effects of dietary protein levels on digestive capacity, protein metabolism and the endocrine regulation related to fish growth in gibel carp. The purpose of the present study was to estimate the dietary protein requirement and the effects of protein levels on digestive enzyme activities, transaminase activities and GH-IGF-I axis gene expression of broodstock gibel carp.

2. Materials and methods

2.1. Experimental diets

Formulation and chemical composition of experimental diets were shown in Table 1 and amino acid composition of diets was presented in Table 2. Six isolipidic (100 g kg^{-1} dry matter) and isoenergetic (gross energy 17.5 kJ g^{-1}) diets were formulated to contain crude protein levels of 200, 240, 280, 320, 360 and 400 g kg^{-1}. White fishmeal (200 g kg^{-1} dry matter) and casein were used as protein sources, fish oil and corn oil (1/1, w/w) as lipid sources. Corn starch and rice bran were added to diets to modulate gross energy. All ingredients passed through a 375 μm sieve before completely mixed. Diets were prepared in a laboratory extruder (SLP-45, Fishery Mechanical Facility Research Institute, Shanghai, China) and made into a 3.6 mm pellet. The pellets were oven-dried at 70 °C and stored at −20 °C until used.

2.2. Experimental fish and feeding trial

Two-yearling broodstock gibel carp CAS III (all of them were postpartum females and have naturally spawned in April) was obtained from an experimental station of the Institute of Hydrobiology, the Chinese Academy of Sciences, Hubei, China. Prior to the experiment, all fish were cultured in floating net cages (2.0 m × 2.0 m × 2.0 m, water depth: 1.7 m) in the center of pond (70 m × 30 m × 3.5 m, water depth: 3 m, located in Shishou Original Seed Stock Farm of Four Major Carps, Hubei, China) and fed with equal mixture of the experimental diets 4 times a day for 2 weeks to make fish adapted to the experimental feeds and rearing system. At the beginning of the feeding trial, fish fasted for 24 h. Fish with similar size (mean initial weight 180.3 ± 0.4 g) were selected, bulk weighted and randomly distributed into 18 net cages at a density of 22 fish per cage. Each experimental diet was randomly assigned to triplicate cages. Fish were hand-fed to apparent satiation 4 times daily at 08:30, 11:30, 15:30 and 18:30. Feed

Table 1
Formulation and chemical composition of experimental diets.

Ingredients	Dietary protein level (g kg^{-1} dry matter)					
	200	240	280	320	360	400
White fishmeal[a]	200	200	200	200	200	200
Casein[b]	36.6	86.2	135.8	186.5	234.9	284.4
Fish oil[c]	28.55	28.85	29.15	29.45	29.8	30.1
Corn oil	28.55	28.85	29.15	29.45	29.8	30.1
Corn starch	390.6	329.1	267.6	204.6	144.6	83.1
Rice bran	150	140	130	120	110	100
Mineral premix[d]	13.8	13.8	13.8	13.8	13.8	13.8
Vitamin premix[e]	3.9	3.9	3.9	3.9	3.9	3.9
Choline chloride	1.1	1.1	1.1	1.1	1.1	1.1
Carboxymethyl cellulose	30	30	30	30	30	30
Cellulose	79.7	101	122.3	144	164.9	186.3
Y_2O_3	1	1	1	1	1	1
Chemical composition (g kg^{-1} dry matter)						
Crude protein	198.8	245.6	275.2	314.9	362.0	397.2
Crude lipid	91.4	90.5	91.1	90.8	91.0	90.5
Ash	95.3	91.3	96.0	95.7	93.8	93.5
Moisture (g kg^{-1} diet)	68.3	63.7	76.2	58.8	67.3	78.3
Gross energy[f] (kJ g^{-1})	17.44	17.59	17.33	17.25	17.25	17.36
P/E ratio (mg kJ^{-1})	11.40	13.97	15.88	18.26	20.99	22.88

[a] Pollock fishmeal from American Seafood Company, Seattle, Washington, USA.
[b] Purchased from Lanzhou Longruan Casein Co., Ltd., Lanzhou, Gansu, China.
[c] Anchovy oil from Peru purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China.
[d] Mineral premix (mg kg^{-1} diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 8155.6; $NaH_2PO4 \cdot 2H_2O$, 12500.0; KH_2PO_4, 16,000.0; $CaHPO_4 \cdot H_2O$, 7650.6; $FeSO_4 \cdot 7H_2O$, 2286.2; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750.0; $ZnSO_4 \cdot 7H_2O$, 178.0; $MnSO_4 \cdot H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 7H_2O$, 34.5; KI, 114.8; and corn starch, 753.7.
[e] Vitamin premix (mg kg^{-1} diet): thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 0.020; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 0.1; starch, 645.2; ascorbic acid, 100; vitamin A, 110; vitamin D, 20; vitamin E, 50; and vitamin K, 10.
[f] Obtained through calorimetry, discounting the energy originated from cellulose.

Table 2
Amino acid composition of the experimental diets.

Amino acid	Dietary protein level (g kg^{-1} dry matter)					
	200	240	280	320	360	400
Arg	10.6	11.8	13.0	14.2	15.3	16.5
His	4.1	5.0	6.0	6.9	7.8	8.8
Ile	8.8	10.5	12.3	14.0	15.7	17.4
Leu	14.0	17.5	21.0	24.6	28.1	31.6
Lys	14.3	17.2	20.1	23.0	25.9	28.7
Met	4.4	5.4	6.4	7.4	8.3	9.3
Phe	7.7	9.5	11.3	13.1	14.8	16.6
Thr	7.8	9.4	10.9	12.4	13.9	15.4
Val	10.1	12.5	15.0	17.5	19.9	22.3
Cys	6.5	8.4	10.3	12.2	14.1	16.0
Tyr	1.8	2.1	2.2	2.3	2.5	2.6
Ala	16.4	19.0	21.7	24.3	26.9	29.5
Asp	8.9	9.6	10.2	10.9	11.5	12.2
Gly	28.2	36.3	44.4	52.7	60.6	68.7
Glu	9.8	10.9	12.0	13.2	14.3	15.4
Pro	8.4	10.2	12.1	14.0	15.8	17.7
Ser	9.3	12.2	15.0	18.0	20.8	23.7
EAAI[a]	99.7	99.3	99.0	98.7	98.5	98.3
AAI[b]	98.6	97.6	95.9	94.7	93.8	93.0

[a] EAAI: essential amino acid index = $100 \times ((a/A) \times (b/B) \ldots\ldots \times (k/K))^{1/n}$; a, b, …… k: essential amino acid content (g 16 g N^{-1}) in diet; A, B, …… N: essential amino acid content (g 16 g N^{-1}) in white fishmeal; n: number of essential amino acids.
[b] AAI: amino acid index = $100 \times ((a/A) \times (b/B) \ldots\ldots \times (k/K))^{1/n}$; a, b, …… k: amino acid content (g 16 g N^{-1}) in diet; A, B, …… N: amino acid content (g 16 g N^{-1}) in white fishmeal; n: number of amino acids.