Aquaculture Nutrition

Aquaculture Nutrition 2012 **18**; 620–627 doi: 10.1111/j.1365-2095.2011.00930.x

Dietary choline requirement in slight methionine-deficient diet for juvenile gibel carp (*Carassius auratus gibelio*)

Y. DUAN[1,2], X. ZHU[1], D. HAN[1], Y. YANG[1] & S. XIE[1,3]

[1] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Science, Wuhan, Hubei, China;* [2] *Graduate School of the Chinese Academy of Sciences, Beijing, China;* [3] *Aquaculture Divisions, E-Institute of Shanghai Universities, Shanghai, China*

Abstract

A 10-week feeding trial was conducted in a flow-through system to determine dietary choline requirement for juvenile gibel carp (*Carassius auratus gibelio*) (5.5 ± 0.1 g). Purified basal diet was formulated using vitamin-free casein as protein source. Choline chloride was supplemented to the basal diet to formulate seven diets containing 76.1, 163, 356, 969, 1457, 2024 and 4400 mg kg^{-1} choline. Dietary methionine was 0.58%, less than the requirement (0.69%). The results indicated that specific growth rate (SGR) was higher in the fish fed 2024 mg kg^{-1} diet than the control group. Feeding rate and feed efficiency were not significantly affected. Protein productive value increased as dietary choline increased from 76.1 to 2024 mg kg^{-1} diet and was lower in the fish fed the diet containing 4400 mg choline kg^{-1} diet. Serum high-density lipoprotein cholesterol (HDL-C) and total cholesterol significantly increased with increasing dietary choline up to 1457 mg kg^{-1}, and no differences were found with further increase. Fish carcass fat contents decreased significantly with increased dietary choline. Hepatic lipid contents increased with dietary choline up to 1457 mg kg^{-1} and then decreased. Quadric regression of SGR and plasma HDL-C indicted dietary choline requirement was 2500 and 2667 mg kg^{-1} diet, respectively.

KEY WORDS: choline chloride, gibel carp, lipid metabolism

Received 6 April 2011, accepted 18 November 2011

Correspondence: Shouqi Xie, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Science, Wuhan, Hubei 430072, China. E-mail: sqxie@ihb.ac.cn

Introduction

Choline, a vitamin-like nutrient, performs three major metabolic functions. It is required (i) for the synthesis of the neurotransmitter acetylcholine; (ii) for the synthesis of phosphatidyl choline (lecithin) and other complex choline-containing phospholipids; (iii) as a source of methyl groups, via betaine, for the synthesis of various methylated metabolites (Halver 2002). Choline has been classified as a B-complex vitamin, but it does not satisfy the standard definition of vitamin. Because there is no evidence that choline is an enzyme co-factor, it could be synthesized at adequate methyl donors when methionine, folic acid and vitamin B_{12} are present in the diet for some animals, such as pig and rat (Kroening & Pond 1967; Anderson *et al*. 1979). However, young rapidly growing fishes cannot sufficiently synthesize choline to satisfy their metabolic requirement (Wilson & Poe 1988; Craig & Gatlin 1996). Thus, choline is an essential nutrient for fish, which should be taken from the food.

The quantitative requirement of choline has been studied in many fish species. The dietary requirement has been reported to be 1000, 1500, 1700–3200, 598–634, 1000, 714–813 and 696–950 mg kg^{-1} for hybrid tilapia, *Oreochromis niloticus* × *O. aureus* (Shiau & Lo 2000), common carp, *Cyprinus carpio* L. (Ogino *et al*. 1970), white sturgeon, *Acipenser transmontanus* (Hung 1989), yellow perch, *Perca flavescens* (Twibell & Brown 2000), lake trout, *Salvelinus namaycush* (Ketola 1976), rainbow trout, *Oncorhynchus mykiss* (Rumsey 1991) and cobia, *Rachycentron canadum* (Mai *et al*. 2009), respectively. In the study of juvenile hybrid tilapia, choline deficiency has been showed to decrease fish blood triglyceride, cholesterol, phospholipids and fat concentration in liver (Shiau & Lo 2000). Deficient dietary choline could also decrease fish plasma total lipid, triacylglycerol, total cholesterol and phospholipid in sturgeon when choline was deficient in the diet (Hung 1989). Other

deficiency symptom has been reported to be poor growth and feed efficiency, fatty liver, high mortality, anorexia in common carp (Ogino *et al*. 1970), lake trout (Ketola 1976) and rainbow trout (Rumsey 1991).

Gibel carp, a warm-water omnivorous fish, is an emerging aquaculture species in China. It has almost replaced crucian carp for its higher growth rate in recent years and annual production was around 3 million tonnes (Fishery Bureau of the Ministry of Agriculture of the People's Republic of China 2010). A number of studies have been reported on feeding regime, feeding stimulants, compensatory growth and macronutrient requirement (Xie *et al*. 2001; Yang *et al*. 2006; Tan *et al*. 2009; Pei *et al*. 2004; Pan *et al*. 2009). However, many of the quantitative requirements of microelement for gibel carp have not been determined. For example, there is limited information concerning their dietary vitamin requirements. Only vitamin B_6 requirement for juvenile gibel carp was reported (Wang *et al*. 2010). As an essential nutrient, choline requirement of gibel carp is unclear.

The objective of this study was to investigate the dietary choline requirement of juvenile gibel carp and its effect on lipid metabolism.

Materials and methods

Preparation of experimental diets

Experimental diet formulation is given in Table 1. Vitamin-free casein (Sigma Chemical Co., St. Louis, MO, USA) was used as the protein source. Corn oil and fish oil (1 : 1) were used as lipid source. The diet was formulated to contain crude protein (380 g kg^{-1} diet) and crude lipid (100 g kg^{-1} diet) according to the requirements (Pei *et al*. 2004). Choline chloride (AR grade; concentration, 98%; Shanpu Chemical Industry Co., Ltd, Shanghai, China, http://shanpuhuagong.cn.gtobal.com/) was added to the basal diet at the expense of cellulose to formulate seven purified diets containing 0, 223, 745, 2235, 3725, 5215 and 6705 mg kg^{-1} choline in diet. The analysed dietary choline concentrations were 76.1 (the control), 163, 356, 969, 1457, 2024 and 4400 mg kg^{-1} diet. All ingredients were mixed completely and made into 2-mm pellet by laboratory feed machine (SLP-45; Fishery Mechanical Facility Research Institute, Shanghai, China) at 70 ± 5 °C, oven-dried at 70 °C and stored at −20 °C until using.

Fish husbandry

Gibel carp were obtained from the hatchery of the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei, China. Before the experiment, they were acclimated to the laboratory conditions for 2 weeks and fed the control diet twice daily (0900 and 1500), and all other conditions were similar to experimental condition. The experiment was carried out in a flow-through system consisting of 21 aquariums (diameter, 70 cm; depth, 40 cm). Water flowing rate into each tank was 350 mL min^{-1}. To reduce the residual chlorine of the water, the experimental system was equipped with a large concrete filter tank filled with zeolite and active carbon. Continuous $Na_2S_2O_2{\cdot}5H_2O$ solution (20%) was added to reduce residual chlorine. Each aquarium received continuous aeration. During the experiment, water temperature was 21 ± 3 °C, pH was 6.7, and residual chlorine was <0.05 mg L^{-1}. The dissolved oxygen content was kept above 5 mg L^{-1}, and ammonia nitrogen content was less than 0.5 mg L^{-1}.

At the beginning of the experiment, fish were deprived of feed for 1 day. Twenty-five fish with an average weight of 5.5 ± 0.1 g ind^{-1} were bulk-weighed and randomly transferred into each tank. Each experimental diet was fed to triplicate tanks to apparent satiation twice a day (0900 and 1500). The uneaten feed and faeces were removed by siphoning before each feeding. The duration of the study

Table 1 Formulation and chemical composition of the basal diet (g kg^{-1} in dry matter)

Ingredient	Contents
Vitamin-free casein (89% crude protein)[1]	428.0
Corn starch[2]	239.2
Fish oil[3]	50.0
Corn oil[4]	50.0
Mineral premix[5]	50.0
Vitamin premix[6]	4.0
Cellulose	173.8
Chromic oxide	5.0
Chemical composition (g kg^{-1})	
Crude protein	380.2
Crude lipid	92.7
Gross energy (kJg^{-1})	20.5

[1] Sigma Chemical Co., St. Louis, MO, USA.
[2] Fuchen Chemical Reagent Plant, Tianjin, China.
[3] Anchovy oil from Peru, purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China.
[4] Yihai KerryY Co. Ltd., purchased from Wuhan, Hubei, China.
[5] 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, 15 500; $Ca(H_2PO_4)_2{\cdot}H_2O$, 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] Vitamin premix (mg kg^{-1} diet): thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 2; folic acid, 5; calcium pantothenate, 50; ascorbic acid, 100; inositol, 100; niacin, 100; biotin, 5; vitamin A, 110; vitamin D, 20; vitamin E, 100; vitamin K, 10.

was 10 weeks. During the experiment, fish were batch-weighed at 4w, 8w and 10w (the end of the experiment) after 1 day of food deprivation.

Sample collection and analysis

At the end of the experiment, four fish in each tank were randomly sampled and frozen at −20 °C for body composition analysis. Other ten fish per tank were randomly selected and blood samples were rapidly taken from the caudal vein using a syringe without anticoagulant. After centrifugation (3000 ***g***, 15 min, 4 °C), serum was separated and stored at −20 °C for triglyceride, cholesterol and high-density lipoprotein cholesterol concentration estimation. Then, the livers and carcass of the rest of the fish were sampled and stored at −20 °C for fat deposition analysis.

Dietary choline concentration was determined using a spectrophotometric method (Venugopal 1985). The testing principle is that choline is extracted by alkali treatment. The extracts were loaded on the glass column (30 cm × 0.8 cm ID) equipped with florisil (Shimadzu Co., Kyoto, Japan) for purification and then reacted with reineckate to form pink chromophore with maximum absorption at 526 nm. Serum triglyceride and cholesterol concentrations were determined by the method of Carson & Goldfard (1979). Remaining serum sample was send to Zhongnan Hospital of Wuhan University, Hubei, China, to determine the levels of serum high-density lipoprotein cholesterol (HDL-C) by the automatic biochemical analyser (Abbott-AEROSET, Chicago, IL, USA) using direct method. The measurement principle is that chylomicron, VLDL-C and LDL-C can be eliminated by cholesterol esterase, cholesterol oxidase and subsequently catalase, and HDL receives no influence so that HDL-C content can be determined directly.

Proximate composition analysis was conducted for the experimental diets and fish body. The liver and carcass samples were analysed for lipid content. Dry matter was determined by drying at 105 °C to constant weight (AOAC 1984). Crude protein content was measured using 2300 Kjeltec Analyzer Unit (Foss Tecator AB, Hoganas, Sweden), lipid by ethyl ether extraction using a Soxtec system (Soxtec System HT6; Tecator), ash by combustion at 550 °C and gross energy by combustion in a microbomb calorimeter (Phillipson micro-bomb calorimeter; Gentry Instruments Inc., Aiken, SC, USA). All analyses were performed in duplicate.

Statistical analysis

The homogeneity and significance of means were analysed by one-way ANOVA using Statistica 6.0 (StatSoft, Tulsa, OK, USA). When ANOVA identified significant difference, Duncan's multiple range tests were used to test the difference between groups. Dietary choline requirement for juvenile gibel carp was estimated by the broken-line regression.

Results

Growth performance was significantly affected by dietary choline levels ($P < 0.05$) (Table 2). No symptom of nutritional deficiency was observed during the experiment. Specific growth rate (SGR) increased with increased dietary

Table 2 Growth performance of juvenile gibel carp fed diets with different choline levels (mean ± SE)*

Dietary choline (mg kg^{-1} diet)	IBW[1]	FBW[2]	HSI[3]	SGR[4]	FR[5]	FE[6]	PPV[7]
76.1	5.52 ± 0.02	14.70 ± 0.07^{a}	7.84 ± 1.69	1.38 ± 0.01^{a}	1.79 ± 0.18	67.2 ± 4.15	24.9 ± 1.02^{a}
163	5.48 ± 0.02	15.88 ± 0.88^{ab}	8.59 ± 1.00	1.49 ± 0.08^{ab}	1.87 ± 0.03	71.6 ± 4.11	26.9 ± 1.07^{b}
356	5.48 ± 0.05	15.54 ± 0.81^{ab}	7.60 ± 0.40	1.46 ± 0.06^{ab}	2.01 ± 0.03	65.7 ± 2.44	25.5 ± 1.29^{ab}
969	5.49 ± 0.03	14.73 ± 0.49^{a}	8.17 ± 1.15	1.39 ± 0.04^{ab}	1.87 ± 0.03	70.3 ± 1.82	25.8 ± 1.03^{ab}
1457	5.45 ± 0.05	16.33 ± 0.72^{ab}	8.27 ± 0.99	1.54 ± 0.07^{ab}	1.99 ± 0.03	70.1 ± 3.71	26.7 ± 1.24^{b}
2024	5.52 ± 0.02	17.10 ± 0.95^{b}	5.86 ± 0.24	1.59 ± 0.09^{b}	1.92 ± 0.04	74.4 ± 2.74	28.6 ± 1.95^{b}
4400	5.52 ± 0.04	15.43 ± 0.29^{ab}	6.35 ± 0.17	1.45 ± 0.03^{ab}	1.99 ± 0.05	66.6 ± 0.87	24.8 ± 0.35^{a}

* Means in the same column with different superscripts are significantly different ($P < 0.05$).
[1] IBW: initial body weight (g).
[2] IBW: initial body weight (g).
[3] HSI: hepatosomatic index (%) = 100 × liver wet weight/body weight.
[4] SGR: specific growth rate (% day^{-1}) = 100 × [ln (final body weight) − ln (initial body weight)]/days.
[5] FR: feeding rate (% body weight day^{-1}) = 100 × total feed intake/[days × (initial body weight + final body weight)/2].
[6] FE: feed efficiency (%) = 100 × wet weight gain/dry feed intake.
[7] PPV: protein productive value (%) = 100 × protein retained in fish body/protein intake.

Table 3 Whole-body and tissue composition of gibel carp fed diets with different choline levels (g kg^{-1} wet weight) (mean ± SE)*

Dietary choline (mg kg^{-1} diet)	Carcass lipid	Liver lipid	Whole-body composition (g kg^{-1} fresh weight)			
			Moisture	Crude protein	Crude lipid	Ash
76.1	73.7 ± 1.2^{bc}	46.1 ± 2.4^{ab}	709.3 ± 2.8	146.8 ± 2.1	73.4 ± 1.9	33.3 ± 0.3^{a}
163	69.8 ± 0.9^{ab}	43.0 ± 6.4^{ab}	715.7 ± 4.4	149.0 ± 1.5	74.4 ± 3.9	33.8 ± 0.6^{ab}
356	71.9 ± 2.1^{abc}	48.4 ± 6.7^{ab}	711.7 ± 1.2	152.5 ± 1.3	72.0 ± 5.3	35.4 ± 1.2^{b}
969	68.0 ± 1.6^{bc}	30.8 ± 1.7^{a}	717.4 ± 2.9	147.3 ± 2.3	68.1 ± 3.1	34.0 ± 0.3^{ab}
1457	74.6 ± 0.4^{c}	49.3 ± 8.1^{b}	714.2 ± 2.4	150.3 ± 1.7	72.3 ± 3.6	32.7 ± 0.4^{a}
2024	70.4 ± 0.5^{bc}	54.2 ± 0.9^{b}	710.7 ± 3.4	151.0 ± 2.3	74.9 ± 1.0	34.3 ± 0.5^{ab}
4400	68.8 ± 1.4^{a}	41.6 ± 2.8^{ab}	716.6 ± 3.0	150.4 ± 1.2	73.0 ± 1.6	34.5 ± 0.4^{ab}

* Means in the same column with different superscripts are significantly different ($P < 0.05$).

choline ($P < 0.05$) whereas showed no significant difference between groups when dietary choline was higher than 163 mg kg^{-1} ($P > 0.05$). Feeding rate and feed efficiency were not significantly affected by choline levels ($P > 0.05$). Protein productive value (PPV) increased when dietary choline increased from 163 to 2024 mg kg^{-1} diet ($P < 0.05$) whereas decreased in the fish fed the diet containing 4400 mg kg^{-1} dietary choline. Fish hepatosomatic index was not affected by dietary choline ($P > 0.05$).

Carcass lipid content was highest when dietary choline was 1457 mg kg^{-1} diet and lowest at highest dietary choline (4400 mg kg^{-1}) ($P < 0.05$). Liver lipid contents were significantly higher at 1457 and 2024 mg kg^{-1} dietary choline ($P < 0.05$). Fish body moisture, crude protein and crude lipid were not affected by dietary choline ($P > 0.05$) while ash content was higher at 356 mg kg^{-1} dietary choline ($P < 0.05$; Table 3).

Serum triglycerides showed increase with increased dietary choline chloride, but no significant difference was observed ($P > 0.05$). Serum high-density lipoprotein cholesterol and total cholesterol significantly increased with increasing dietary choline chloride levels up to 1457 mg choline kg^{-1} diet ($P < 0.05$), and no significant differences were found at higher dietary choline ($P > 0.05$; Table 4).

The relationship between fish SGR, PPV, serum high-density lipoprotein cholesterol (HDL-C) and dietary choline level is shown in Figs 1, 2 & 3. Quadric regression showed that dietary choline requirement of juvenile gibel

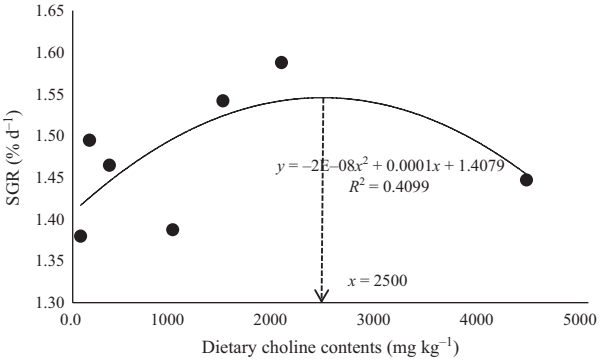

Figure 1 Relationship between specific growth rate (SGR) of gibel carp and dietary choline contents. Each point represents the mean of triplicate groups of fish. Requirements derived with the quadric regression method for SGR is 2500 mg kg^{-1} diet.

Table 4 Serum triglycerides (TG), cholesterol (CHOL) and high-density lipoprotein cholesterol (HDL-C) of gibel carp fed diets containing graded levels of choline (mean ± SE)*

Dietary choline (mg kg^{-1} diet)	TG (mg dL^{-1})	HDL-C (mg dL^{-1})	CHOL (mg dL^{-1})
76.1	186.3 ± 30.29	9.80 ± 0.32^{a}	347.2 ± 14.46^{a}
163	184.4 ± 14.77	10.54 ± 0.54^{ab}	347.0 ± 16.54^{a}
356	164.1 ± 13.96	10.73 ± 0.37^{ab}	362.0 ± 12.28^{ab}
969	170.2 ± 15.40	10.98 ± 0.66^{abc}	376.2 ± 17.26^{ab}
1457	209.7 ± 13.09	12.30 ± 0.09^{c}	420.3 ± 8.53^{b}
2024	200.6 ± 21.47	11.81 ± 0.49^{bc}	375.1 ± 16.81^{ab}
4400	194.6 ± 12.15	11.93 ± 0.33^{bc}	414.3 ± 29.68^{b}

* Means in the same column with different superscripts are significantly different ($P < 0.05$).

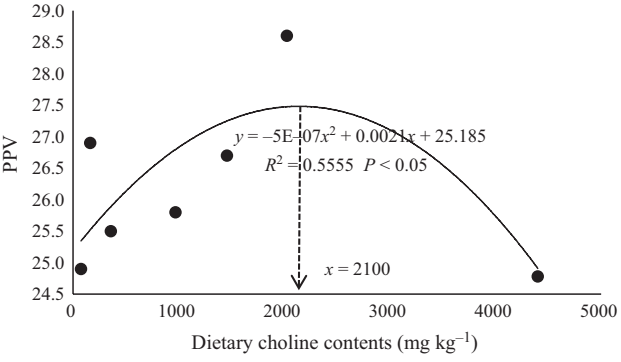

Figure 2 Relationship between protein productive value (PPV) and dietary choline contents.

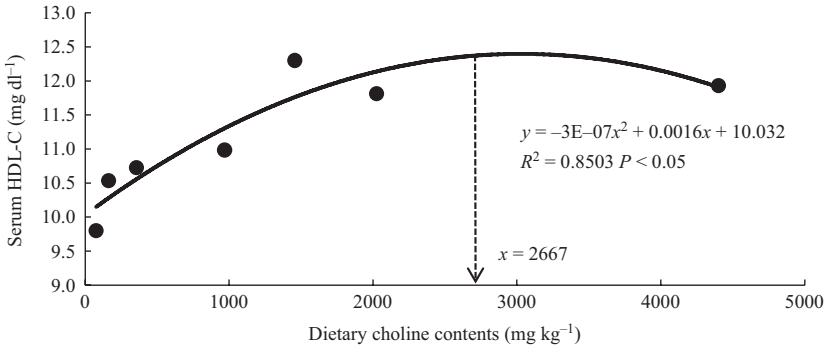

Figure 3 Relationship between serum high-density lipoprotein cholesterol (HDL-C) in gibel carp and dietary choline contents. Each point represents the mean of three groups of fish. Requirements derived with the quadric regression method for HDL-C is 2667 mg kg^{-1} diet.

carp was 2500 mg kg^{-1} based on SGR, 2100 mg kg^{-1} based on PPV and 2667 mg kg^{-1} based on HDL-C. Fish liver lipid increased with increasing carcass lipid and then kept constant (Fig. 4).

Discussion

Fish in the present study showed normal growth as other reports in gibel carp (Pan *et al.* 2008, 2009; Chen *et al.* 2010). The essentiality of dietary choline was confirmed in the present study. The supplementation of choline significantly promoted the growth, protein utilization and lipid utilization of juvenile gibel carp. Fish fed insufficient dietary choline showed poor growth and poor protein utilization. It is in agreement with the results in common carp (Ogino *et al.* 1970) and lake trout (Ketola 1976). Based on the SGR, the optimal choline requirement for juvenile gibel carp was 2500 mg kg^{-1} diet, which was lower than those reported for yellowtail *Seriola lalandi* (2920 mg kg^{-1}, Shimeno 1991) and grass carp *Ctenopharyngodon idella* (3000 mg kg^{-1}, Wang *et al.* 1995) and higher than those for juvenile hybrid tilapia (1000 mg kg^{-1}, Shiau & Lo 2000), lake trout (1000 mg kg^{-1}, Ketola 1976), blue tilapia, *Tilapia aurea* (500 mg kg^{-1}, Roema *et al.* 1990), hybrid striped bass, *Monrone saxatilis* × *M. chrysops* (500 mg kg^{-1}, Griffin *et al.* 1994) and yellow perch (568–634 mg kg^{-1}, Twibell & Brown 2000). The high choline requirement in this study can be attributed to many factors, such as the low initial body weight (5.5 ± 0.1 g). It has been suggested that dietary choline requirement in aquatic animals was inversely related to fish body size (Griffin *et al.* 1994). Rumsey (1991) reported that choline requirement varied from 813 to 774 g kg^{-1} when trout initial body weight varied from 1.4 to 3.2 g. The rearing system could also affect the requirement results, and the flow-through rearing system could reduce the environmental choline concentration. Roema *et al.* (1990) reported that blue tilapias did not require additional pantothenic acid or choline in the diet when held in the recirculating systems. It is unlikely that this species has no requirement for these vitamins, one possible reason of which is the dissolved vitamin in the recirculating system.

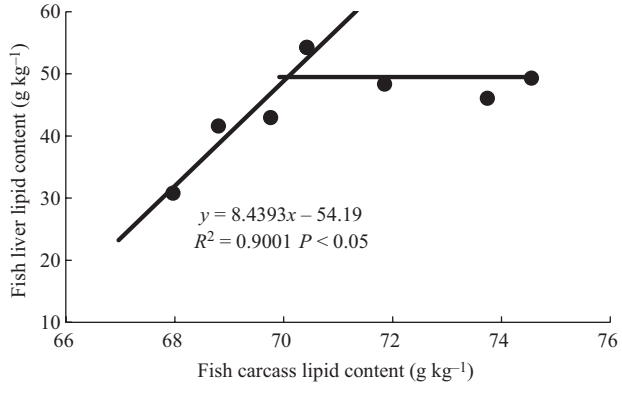

Figure 4 Relationship between fish liver lipid content and fish carcass lipid content.

Choline could be synthesized in animal body by phosphotidylethanolamine *N*-methyltransferase from phosphatidyl ethanolamine with the methyl from *S*-adenosyl-L-methionine. Choline is an important intermediate in the catabolic pathway that begins with methionine (Vemury *et al.* 1980). Therefore, dietary methionine could affect the choline requirement. Warm-water fishes have high ability to synthesize choline (Zhang & Wilson 1999). In the study of tilapia, methionine concentrations were 11.3 g kg^{-1} diet, amounts that are far exceeding the requirements of 0.75 g kg^{-1} for nile tilapia. The excessive methionine concentration may have flooded the sulphur amino acid

catabolic pathway with sufficient metabolic precursors for synthesis of choline, thereby masking any effect from choline (Santiago & Lovell 1988). Craig *et al.* (2000) also reported that it was unclear whether dietary choline was essential for tilapia when the methionine is sufficient, but choline was clearly required when the dietary sulphur amino acid concentrations was minimal. In the present study, dietary methionine was reduced to be less than the requirement of gibel carp (6.9 g kg^{-1} of dry matter) to limit the endogenous synthesis of choline from methionine. Thus, the choline requirement obtained for juvenile gibel carp in the present study might represent the maximal dietary choline requirement but not the minimal requirement.

Choline is a major component of phosphatidylcholine, which plays an important role in lipoprotein synthesis. Lipoproteins of all animals act as major carriers of lipid. Serum lipoproteins consisted of low-density lipoprotein (LDL) and high-density lipoprotein (HDL). HDL is the main lipoprotein for fish, and the primary function is to transport cholesterol from peripheral tissues to liver. Then liver metabolizes the extra cholesterol to bile acid, which is secreted by intestine for lipid absorption (Hayashi & Kumagai 2008). In the study in carp and mammals, HDL exerted growth-promoting effect, although the mechanism is uncertain (Tauber *et al.* 1980; Kondo & Watabe 2006). In the present study, serum HDL-C showed the same trend with SGR and significantly increased with dietary choline, suggesting that HDL-C showed growth-promoting effect. The sensitivity of serum HDL-C to dietary choline level suggests that it could be used to evaluate choline nutrition status of the fish. The choline requirement based on SGR was higher than that on PPV, but lower than that on serum HDL-C. It suggested that choline is more important in lipid metabolism and growth.

In all kinds of fish fed choline-deficient diets, blood lipid classes showed the same trend and were low, probably due to the substantial contribution of choline to the synthesis of phospholipids (Lombardi 1971). There are reversed results in liver lipid content for different species. Choline deficiency caused accumulation of liver lipid in channel catfish, *Ictalurus punctatus* (Wilson & Poe 1988), lake trout (Ketola 1976) and hybrid striped bass (Griffin *et al.* 1994), which was attributed to impaired hepatic lipoprotein secretion and subsequent accumulation of triacylglycerol (Chan 1991). However, in the present study, dietary choline deficiency did not cause excess accumulation of liver lipid in gibel carp. It is similar to the report in red drum, *Sciaenops ocellatus* (Craig & Gatlin 1996). In the present study, when dietary choline concentration was up to 1457 mg kg^{-1}, the liver lipid started to increase significantly. The same response was also reported in hybrid tilapia (Shiau & Lo 2000). The mechanism is uncertain. It could be due to the increased lipid metabolism in liver with increased dietary choline (Shiau & Lo 2000).

In the study of mammals, choline could alter body fat distribution (Bryant *et al.* 1999) to reduce carcass fat (Fernández *et al.* 1998). The major lipid storage sites in fish are variable with species, but include liver, perivisceral adipose tissue and carcass (Ackman 1980). In the study of aquatic animals, such as hybrid tilapia, red drum and cobia, choline could also affect lipid accumulation in fish liver and muscle (Craig & Gatlin 1996; Shiau & Lo 2000; Mai *et al.* 2009). In the present study, both liver lipid and carcass lipid contents were affected by the dietary choline level. Figure 4 showed that fish liver lipid increased with carcass lipid and then kept constant with the further increase in carcass lipid. It indicated that most of the lipid in fish was not stored in liver and it could be stored in carcass or viscera.

The present results showed that a certain range of dietary choline could increase protein utilization (PPV). Increased protein efficiency ratio was reported with increasing choline in Nile tilapia (El-Husseiny *et al.* 2008), kuruma shrimp (Michael *et al.* 2006; Michael & Koshio 2008) and shrimp (Michael *et al.* 2007). The increased protein utilization could be due to the improved utilization of the non-protein energy (lipid and/or carbohydrate). However, high dietary choline up to 4430 mg kg^{-1} decreased PPV in the present study. PPV has been reported to be stable when dietary choline was higher than optimal level (Michael *et al.* 2006; El-Husseiny *et al.* 2008). Further investigations are required to investigate the negative impact of high dietary choline on protein utilization.

In conclusion, SGR of gibel carp improved at an optimal dietary choline level, and dietary choline requirement for gibel carp is estimated to be 2500 mg kg^{-1} diet for maximum growth.

Acknowledgements

The authors would like to thank Mr Guanghan Nie for his technical support with the aquarium. The project was funded by National Key Basic Research Program (NKBRP) (2009CB118702) and partly supported by the earmarked fund for China Agriculture Research System (CARS-46-19) and Special Fund for Agro-scientific Research in the Public Interest (201003020).

References

Ackman, R.G. (1980) Fish lipids, Part 1. In: Advances in Fish Science and Technology (Connell, J.J. ed.), pp. 86–103. Fishing News Books Ltd, Farnham.

Anderson, P.A., Baker, D.H., Sherry, P.A. & Corbin, J.E. (1979) Choline methionine interrelationships in feline nutrition. *J. Anim. Sci*., **49**, 522–527.

AOAC (1984) Animal feed. In: Official Methods of Analysis, 14th edn (Williams, S. ed.), pp. 152. Association of Official Analytical Chemists, Washington, DC.

Bryant, T.C., Rivera, J.D., Galyean, M.L., Duff, G.C., Hallford, D.M. & Montgomery, T.H. (1999) Effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on growth and serum metabolites in lambs. *J. Anim. Sci*., **77**, 2893–2903.

Carson, S.E. & Goldfard, S. (1979) A sensitive enzymatic method for determination of free and esterified tissue cholesterol. *Clin. Chim. Acta*, **79**, 575–585.

Chan, M.M. (1991) Choline. In: Handbook of Vitamins, 2nd edn (Machlin, L.J. ed.), pp. 537–556. Marcel Dekker, New York, NY.

Chen, J., Zhu, X., Han, D., Yang, Y., Lei, W. & Xie, S. (2010) Effect of dietary n-3 HUFA on growth performance and tissue fatty acid composition of gibel carp, *Carassius auratus gibelio*. *Aquacult. Nutr*., **17**, e476–e485.

Craig, S.R. & Gatlin, D.M. (1996) Dietary choline requirement of juvenile Red drum, *Sciaenops ocellatus*. *J. Nutr*., **126**, 1696–1700.

Craig, S.R., Kasper, C.S., White, M.R. & Brown, P.B. (2000) Choline is required by tilapia when methionine is not in excess. *J. Nutr*., **130**, 238–242.

El-Husseiny, O.M., Din, G.E., Abdul-Aziz, M. & Mabroke, R.S. (2008) Effect of mixed protein schedules combined with choline and betaine on the growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquacult. Res*., **39**, 291–300.

Fernández, C., Gallego, L. & Lopez-Bote, C.J. (1998) Effect of betaine on fat content in growing lambs. *Anim. Feed Sci. Technol*., **73**, 329–338.

Fishery Bureau of the Ministry of Agriculture of the People's Republic of China (2010) *China Fishery Bulletin*, pp. 70. Chinese Agricultural Press, Beijing, P.R. China.

Griffin, M.E., Wilson, K.A., White, M.R. & Brown, P.B. (1994) Dietary choline requirement of juvenile hybrid striped bass. *J. Nutr*., **124**, 1685–1689.

Halver, J.E. (2002) The vitamins. In: Fish Nutrition, 3rd edn (Halver, J.E. ed.), pp. 111. Academic Press, New York, NY.

Hayashi, S. & Kumagai, A. (2008) Studies on eel liver functions using perfused liver and primary cultured hepatocytes. *AquaBiosci. Monogr*., **1**, 1–57.

Hung, S.S.O. (1989) Choline requirement of hatchery-produced juvenile white sturgeon, *Acipenser transmontanus*. *Aquaculture*, **78**, 183–194.

Ketola, H.G. (1976) Choline metabolism and nutritional requirement of Lake trout, *Salvelinus namaycush*. *J. Anim. Sci*., **43**, 474–477.

Kondo, H. & Watabe, S. (2006) Growth promoting effects of carp serum components on goldfish culture cells. *Fish. Sci*., **72**, 884–888.

Kroening, G.H. & Pond, W.G. (1967) Methionine, choline and threonine interrelationships for growth and lipotropic action in the baby pig and rat. *J. Anim. Sci*., **26**, 352–360.

Lombardi, B. (1971) Effects of choline deficiency on rat hepatocytes. *Fed. Proc*., **30**, 139–142.

Mai, K., Xiao, L., Ai, Q., Wang, X., Xu, W., Zhang, W., Liufu, Z. & Ren, M. (2009) Dietary choline requirement for juvenile cobia, *Rachycentron canadum*. *Aquaculture*, **289**, 124–128.

Michael, F.R. & Koshio, S. (2008) Biochemical studies on the interactive effects of dietary choline and inositol in juvenile Kuruma shrimp, *Marsupenaeus japonicus* Bate. *Aquaculture*, **285**, 179–183.

Michael, F.R., Koshio, S., Teshima, S.-I., Ishikawa, M. & Uyan, O. (2006) Effect of choline and methionine as methyl group donors on juvenile kuruma shrimp, *Marsupenaeus japonicus* Bate. *Aquaculture*, **258**, 521–528.

Michael, F.R., Teshima, S., Koshio, S., Ishikawa, M., Uyan, O. & Ren, T. (2007) Effect of two choline sources on the performance of postlarval, *Marsupenaeus japonicus*. *Aquacult. Nutr*., **13**, 59–64.

Ogino, C., Uki, N., Watanabe, T., Iida, Z. & Ando, K. (1970) B vitamin requirements of carp-IV. Requirements for choline. *Bull. Jpn. Soc. Sci. Fish*., **36**, 1140–1146.

Pan, L., Xie, S., Zhu, X., Lei, W., Han, D. & Yang, Y. (2008) Effects of dietary manganese on growth and tissue manganese concentrations of juvenile gibel carp, *Carassius auratus gibelio*. *Aquacult. Nutr*., **14**, 459–463.

Pan, L., Xie, S., Zhu, X., Lei, W., Han, D. & Yang, Y. (2009) The effect of different dietary iron levels on growth and hepatic iron concentration in juvenile gibel carp (*Carassius auratus gibelio*). *J. Appl. Ichthyol*., **25**, 428–431.

Pei, Z., Xie, S., Lei, W., Zhu, X. & Yang, Y. (2004) Comparative study on the effect of dietary lipid level on growth and feed utilization for gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther). *Aquacult. Nutr*., **10**, 209–216.

Roema, A.J., Stickneyb, R.R. & Kohler, C.C. (1990) Vitamin requirements of blue tilapias in a recirculating water system. *Prog. Fish-Cult*., **52**, 15–18.

Rumsey, G.L. (1991) Choline-betaine requirements of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **95**, 107–116.

Santiago, C.B. & Lovell, R.T. (1988) Amino acid requirements for growth of Nile tilapia. *J. Nutr*., **118**, 1540–1546.

Shiau, S.-Y. & Lo, P.-S. (2000) Dietary choline requirements of juvenile hybrid Tilapia, *Oreochromis niloticus* × *O. aureus*. *J. Nutr*., **130**, 100–103.

Shimeno, S. (1991) Yellowtail *Seriola quinqueradiata*. In: Handbook of Nutrient Requirments of Finfish (Wilson, R.P. ed.), pp. 181–191. CRC Press, Boca Raton, FL.

Tan, Q., Fen, W., Xie, S., Zhu, X., Lei, W. & Shen, J. (2009) Effect of high dietary starch levels on the growth performance, blood chemistry and body composition of gibel carp (*Carassius auratus gibelio*). *Aquacult. Res*., **40**, 1011–1018.

Tauber, J.P., Cheng, J. & Gospodarowicz, D. (1980) Effect of high and low density lipoproteins on proliferation of cultured bovine vascular endothelial cells. *J. Clin. Invest*., **66**, 696–708.

Twibell, R.G. & Brown, P.B. (2000) Dietary choline requirement of juvenile yellow perch (*Perca flavescens*). *J. Nutr*., **130**, 95–99.

Vemury, M.K.D., Kies, C. & Fox, H.M. (1980) L-Methionine/choline/inorganic sulfur interrelationships in soy based diets fed to human adults. *Nutr. Rep. Int*., **22**, 369–382.

Venugopal, P.B. (1985) Choline. In: Methods of Vitamin Assay (Augustin, J., Klein, B.P., Becker, D. & Venugopal, P.B. eds), pp. 555–573. John Wiley and Sons, New York, NY.

Wang, D., Zhao, L. & Tan, Y. (1995) Requirement of the fingerlings grass carp (*Ctenopharyngodon idella*) for choline. *J. Fish. China*, **19**, 132–139 (In Chinese with English abstract).

Wang, J., Xie, S., Zhu, X., Lei, W., Han, D. & Yang, Y. (2010) Dietary vitamin B_6 requirement of juvenile gibel carp, *Carassius auratus gibelio*. *Acta Hydrobiol. Sin.*, **35**, 98–104 (In Chinese with English abstract).

Wilson, R.P. & Poe, W.E. (1988) Choline nutrition of fingerling channel catfish. *Aquaculture*, **68**, 65–71.

Xie, S., Zhu, X., Cui, Y., Wootton, R.J., Lei, W. & Yang, Y. (2001) Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. *J. Fish Biol.*, **58**, 999–1009.

Yang, Y., Xie, S., Cui, Y., Lei, W., Zhu, X. & Yang, Y. (2006) Partial and total replacement of fishmeal with poultry by-product meal in diets for gibel carp, *Carassius auratus gibelio*. *Aquacult. Res.*, **37**, 40–48.

Zhang, Z. & Wilson, R.P. (1999) Reevaluation of the choline requirement of fingerling channel catfish (*Ictalurus punctatus*) and determination of the availability of choline in common feed ingredients. *Aquaculture*, **180**, 89–98.