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Effect of dietary n-3 HUFA on growth performance and tissue fatty acid composition of gibel carp *Carassius auratus gibelio*

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Abstract

A 12-week growth trial was conducted with gibel carp *Carassius auratus gibelio* (initial weight: 2.69 g) to evaluate the effects of dietary n-3 highly unsaturated fatty acids (n-3 HUFA) on growth performance and tissue fatty acid composition. Five diets of different n-3 HUFA levels from 0 to 17 g kg^{-1} diet were supplemented at 80 g kg^{-1} dietary lipid by including fish oil (FO) at 0, 25, 50, 75 and 100% of supplemental lipid. The remainder was coconut oil. The results showed that fish fed FO_{25} and FO_{50} obtained highest specific growth rate and lowest with FO_0. Feed efficiency was highest at FO_{100} and lowest at FO_0. Apparent digestibility coefficient of lipid increased with increasing dietary n-3 HUFA. The fish fed FO_0 diet had the lowest thiobarbituric acid reactive substance in serum and muscle and highest moisture and lowest lipid content in viscera. Fatty acid compositions of muscle and liver were correlated with dietary fatty acids. Fish muscle concentration of 20:5n-3 increased with increasing dietary n-3 HUFA while the concentration of 22:6n-3 was distinctly reduced in FO_0 group. It suggested that 4 g kg^{-1} n-3 HUFA in diet could permit gibel carp normal growth performance and provide considerable n-3 HUFA in fish muscle. Excessive n-3 HUFA showed impact on growth performance of gibel carp.

KEY WORDS: gibel carp, growth, n-3 HUFA, tissue fatty acid composition

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Introduction

Fish, like all other vertebrates, require highly unsaturated fatty acids (HUFA) for normal growth and development to maintain the normal structure and function of cell membranes and as precursors of eicosanoids (Sargent *et al.* 1999). Generally, fresh warmwater fish have the ability to bioconvert C18 polyunsaturated fatty acids (PUFA) to the essential HUFA, and thus the requirement can be met by dietary 18:3n-3 and 18:2n-6 (Sargent *et al.* 2002). However, studies in common carp *Cyprinus carpio* L. and channel catfish *Ictalurus punctatus* showed that HUFA had higher efficiency than C18 PUFA in satisfying the dietary needs of fish for essential fatty acid (EFA) (Takeuchi & Watanabe 1977; Satoh *et al.* 1989). Otherwise, it would be desirable to increase the HUFA content in fish fillets by feeding diet containing HUFA. n-3 HUFA, especially 22:6n-3, is known to provide positive health benefits to human consumer such as against cardiovascular diseases (Sargent *et al.* 2002).

Fish oil (FO) is widely used in aquafeeds because of its high content of n-3 HUFA (Izquierdo *et al.* 2003). However, the stagnation in global FO production coupled with increased demand for its use in aquaculture feeds has greatly inflated FO prices (Barlow 2000). In addition, the high unsaturation of n-3 HUFA makes FO sensitive to lipid peroxidation (Huang *et al.* 1998; Lin & Huang 2007), and excessive HUFA could induce adverse effects on fish growth (Stickney *et al.* 1983; Ng *et al.* 2001, 2003).

Gibel carp, *Carassius auratus gibelio*, is an omnivorous fish popularly cultured in China, and the annual production was around 200 million tonnes in China (Fishery Bureau of the Ministry of Agriculture of the People's Republic of China 2007). Previous studies found that gibel carp fed the diet containing 80 g kg^{-1} FO did not show better growth compared to plant oil, which suggested gibel carp might not rely

on FO or HUFA (Chen 2008). However, the effect of n-3 HUFA on gibel carp is not clear. This study is designed to evaluate the nutrition value of n-3 HUFA by the substitution of 100, 75, 50, 25 and 0% FO with coconut oil that contains 80% saturated fatty acids (SFA) and few HUFA. Growth and feed utilization were measured along with lipid peroxidation and fatty acid composition of muscle and liver.

Material and methods

Experimental diets

Five isonitrogenous (400 g kg^{-1} crude protein) and isolipidic (80 g kg^{-1} crude lipid) diets were formulated to contain different n-3 HUFA levels ranging from 0 to 17 g kg^{-1}. For varying n-3 HUFA level, 100, 75, 50, 25 and 0% FO was substituted with coconut oil that containing 80% SFA and few HUFA. Diet formulation and chemical composition was shown in Table 1. Casein and soybean meal were used as protein sources to reduce HUFA content in the basal diet. Fatty acid composition of the experimental diets was shown in Table 2. For digestibility measurement, 5 g kg^{-1} Chromium oxide (Cr_2O_3) was used as inert marker. A lipid-free basal diet that was used as a conditioning diet was also prepared by using corn starch to maintain constant dietary energy concentration. About 0.1 g kg^{-1} BHT was mixed with the lipids as antioxidant before thoroughly mixed with other powder ingredients. All diets were made into 2 mm pellet by extrusion, air-dried and stored at −20 °C.

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

Diet	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
Casein	390.0	390.0	390.0	390.0	390.0
Soybean meal (oil-extracted)	120.0	120.0	120.0	120.0	120.0
Corn starch	200.0	200.0	200.0	200.0	200.0
α-starch	60.0	60.0	60.0	60.0	60.0
Vitamin premix[1]	4.0	4.0	4.0	4.0	4.0
Mineral premix[2]	50.0	50.0	50.0	50.0	50.0
Choline chloride	1.1	1.1	1.1	1.1	1.1
Cellulose	89.7	89.7	89.7	89.7	89.7
Chromic oxide	5.0	5.0	5.0	5.0	5.0
Attractant[3]	0.1	0.1	0.1	0.1	0.1
BHT[4]	0.1	0.1	0.1	0.1	0.1
FO[5]	–	20.0	40.0	60.0	80.0
Coconut oil[6]	80.0	60.0	40.0	20.0	–
Chemical composition (g kg^{-1})					
Moisture	45.9	48.7	61.6	54.4	71.2
Crude protein	401.8	402.8	421.4	401.2	402.1
Crude lipid	65.4	76.1	70.2	74.8	75.6
Ash	55.4	56.2	55.5	56.4	56.3

FO, fish oil.
[1] Vitamin premix (mg kg^{-1} diets): vitamin A, 1.83; vitamin D, 0.5; vitamin E, 10; vitamin K, 10; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamin, 20; D-calcium pantothenate, 50; biotin, 1.0; folic acid, 5; vitamin B_{12}, 2; ascorbic acid, 100; inositol, 100.
[2] Mineral premix (mg kg^{-1} diets): NaCl, 0.80; $MgSO_4 \cdot 7H_2O$, 12; $NaH_2PO_4 \cdot 2H_2O$, 20; KH_2PO_4, 25.6; $Ca(H_2PO_4)_2 \cdot H_2O$, 16; $FeSO_4 \cdot 5H_2O$, 2; $(CH_2CHCOO)_2Ca \cdot 5H_2O$, 2.8; $ZnSO_4 \cdot 7H_2O$, 0.028; $MnSO_4 \cdot 4H_2O$, 0.013; $CuSO_4 \cdot 5H_2O$, 0.0025; $CoCl_2 \cdot 6H_2O$, 0.0008; KIO_3, 0.0024; cellulose, 0.36.
[3] Attractant: 0.0026 g Asp + 0.0063 g Thr+0.001 g DMPT per kg diets (Zhao 2007).
[4] BHT: 2,6-Di-tert-butyl-4-methylphenol, Sigma.
[5] Anchovy oil from Peru, purchased from Coland Feed Co. Ltd. Wuhan, P. R. China.
[6] Jin-yang Oil Company, Suzhou, China.

Experimental condition, fish and feeding

The growth trial was carried out in a recirculation system consisting of 15 fibreglass tanks (60 × 45 × 50cm, water volume: 100 L). During the experiment, continuous aeration was supplied to each tank. Water temperature was recorded daily and ranged between 24 and 30 °C. The photoperiod was 12 L/12 D with the light period from 08:00 to 20:00 h. Dissolved oxygen, ammonia nitrogen and residual chlorinate (>5, <0.5 and <0.05 mg L^{-1}, respectively) in water were measured weekly, pH was around 6.8.

Gibel carp were obtained from Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, P. R. China and stocked in 10 reservoir tanks for acclimatization 4 weeks prior to experiment. During the first 2 weeks of acclimatization, all fish were fed a practical diet to satiation twice a day (09:00 and 15:00 h). Then, the fish were fed a lipid-free diet to deplete their lipid reserves for 2 weeks. At the beginning of the experiment, apparent healthy fish (initial body weight about 2.69 g) were batch weighed after 24 h feed deprivation and randomly distributed into each tank. Triplicate tanks were randomly allotted to each of five experimental diets.

The growth trial lasted for 12 weeks. During the trial, fish were hand-fed to satiation twice a day (09:00 and 15:00 h), and uneaten feed was collected by siphoning 1 h after feeding. To calibrate the amount of feed intake, leaching rate was measured by placing weighted diet in tanks without fish for 1 h and then collecting, drying and weighing. From the third week of the experiment, intact fresh faeces in each tank was collected 1 h after feeding twice daily by siphoning and dried at 60 °C for digestibility determination.

Sampling procedure

At the end of the experiment, the fish of each tank were batch-weighted after 24 h feed deprivation. Six fish per tank were randomly selected, killed by a blow to the head, and blood

Table 2 Fatty acid composition of the experimental diets (% in total fatty acids)

Fatty acid	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
8:0	4.6	3.8	1.6	0.8	0.3
10:0	4.1	3.4	1.7	0.9	0.4
12:0	36.4	27.5	16.3	7.9	1.3
14:0	17.8	14.4	11.4	8.0	5.2
16:0	14.5	15.1	16.8	17.5	17.3
18:0	6.0	5.9	6.3	6.1	6.0
SFA[1]	84.3	71.0	54.8	41.9	31.1
16:1n-9	0.3	1.2	2.4	3.2	4.2
18:1n-9	10.5	11.9	14.0	15.5	16.2
20:1n-9	0.0	0.3	0.6	0.8	1.1
MUFA[2]	10.8	13.6	17.2	19.9	21.9
18:2n-6	4.6	9.0	14.1	18.5	21.8
20:4n-6	0.0	0.2	0.4	0.6	0.8
n-6[3]	4.6	9.2	14.5	19.2	22.8
18:3n-3	0.4	1.1	2.0	2.8	3.4
20:5n-3	0.0	2.5	5.6	8.0	10.4
22:6n-3	0.0	2.7	5.9	8.3	10.5
n-3[4]	0.4	6.3	13.5	19.0	24.2
PUFA[5]	5.0	15.5	28.0	38.2	47.0
n-3 HUFA[6]	0.0	5.2	11.5	16.2	20.8
n-3/n-6	0.1	0.7	0.9	1.0	1.1

FO, fish oil.
[1] SFA, saturated fatty acids: 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0.
[2] MUFA, monounsaturated fatty acids: 14:1n-9, 16:1n-9, 18:1n-9, 20:1n-9.
[3] n-6: 18:2n-6, 18:3n-6, 20:4n-6.
[4] n-3: 18:3n-3, 20:5n-3, 22:6n-3.
[5] PUFA, polyunsaturated fatty acids: 18:2n-6, 18:3n-3, 18:3n-6, 20:4n-6, 20:5n-3, 22:6n-3.
[6] n-3 HUFA, highly unsaturated fatty acids: 20:5n-3, 22:6n-3.

samples were rapidly taken from the caudal vein using a syringe. After centrifugation (3000 ***g***, 10 min, 4 °C), serum was separated for thiobarbituric acid reactive substance (TBARS) values determination. Then viscera and muscle sample from the region underneath the dorsal fin were dissected and stored at −20 °C for fat deposition and TBARS analysis.

Liver and muscle samples were removed from eight other fish of each tank and were immediately placed in liquid nitrogen for fatty acid analysis. Similar samples were also taken before 2-week lipid-free diet conditioned and at the beginning of the experiment.

Triplicates of fish (10 fish each sample) at the beginning and five fish from each tank at the end of the experiment were sacrificed and stored at −20 °C for fish body composition analysis.

Chemical analyses

Muscle was homogenized, and protein concentration was determined by the method of Bradford (1976). Serum and muscle TBARS were measured according to Uchiyama & Mihara (1978) and presented as nmol malonaldehyde (MDA) ml^{-1} or mg $prot^{-1}$, respectively.

Proximate composition analysis was conducted for the experimental diets, fish body and faeces. Tissue samples were analysed for moisture and 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, Hoganas, Sweden), lipid by ethyl ether extraction using a Soxtec system (Soxtec System HT6; Tecator, Hoganas, Sweden), and ash by combustion at 550 °C. The content of Cr_2O_3 in the diets and faeces were determined as described by Bolin *et al.* (1952). All analyses were performed in duplicate.

The samples of liver and muscle were freeze-dried and powdered for fatty acid analysis. Lipids were extracted from tissue using Folch method (Folch *et al.* 1957) and then methylated to fatty acid methyl esters using BF_3 in methanol. The esters were separated and quantified by a 14-C gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector (ID) and a capillary column (60 m × 0.25 mm ID). Nitrogen was used as carrier gas, and column temperature was programmed to increase (130 °C : 1 min; 130–170 °C : 6.5 °C min^{-1};170–215 : 2.75 °C min^{-1}; 215 °C : 12 min; 215–230 °C : 40 °C min^{-1}; 230 °C : 3 min). Injection and detector temperatures were maintained at 270 °C and 280 °C, respectively. Fatty acids were identified by comparison with known standards.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. Differences were regarded as significant when $P < 0.05$. Statistica 6.0 was used to perform statistical calculations.

Results

Growth performance and feed utilization

Table 3 shows that feeding rate (FR) decreased with increasing dietary n-3 HUFA ($P < 0.05$). Final body weight and specific growth rate (SGR) of fish fed the FO_{25} and FO_{50} diets were the highest, followed by fish fed the FO_{75} and FO_{100} diets, and the lowest in fish fed the FO_0 diet ($P < 0.05$). Feed efficiency (FE) was the highest in the fish fed the FO_{100} diet, followed by fish fed the FO_{50} diet, then fish fed the FO_{25} and FO_{75} diets, and the lowest in fish fed the FO_0 diet ($P < 0.05$). Fish fed the FO_0 diet had the lowest

Table 3 Growth and feed utilization of gibel carp fed different diets (Mean ± SE)*

	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
IBW[1]	2.68 ± 0.01	2.69 ± 0.01	2.68 ± 0.01	2.69 ± 0.00	2.69 ± 0.01
FBW[2]	11.50 ± 0.11[a]	13.87 ± 0.14[c]	13.94 ± 0.16[c]	13.02 ± 0.09[b]	13.12 ± 0.11[b]
FR[3]	2.27 ± 0.02[c]	2.23 ± 0.01[c]	2.13 ± 0.00[b]	2.12 ± 0.01[b]	2.02 ± 0.01[a]
SGR[4]	1.73 ± 0.02[a]	1.95 ± 0.02[c]	1.96 ± 0.02[c]	1.87 ± 0.02[b]	1.89 ± 0.02[b]
FE[5]	65.3 ± 1.74[a]	72.1 ± 0.87[b]	75.8 ± 0.55[c]	73.7 ± 0.95[b]	77.8 ± 0.60[d]
PRE[6]	25.8 ± 0.67[a]	28.9 ± 0.37[b]	29.0 ± 0.59[b]	28.2 ± 0.71[b]	29.7 ± 0.45[b]
PER[7]	1.75 ± 0.02[a]	1.90 ± 0.01[b]	1.91 ± 0.00[b]	1.92 ± 0.02[b]	2.03 ± 0.01[c]

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

Table 4 Apparent digestibility coefficient (ADC) of experimental diets in gibel carp (Mean ± SE)*

	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
ADC_d[1]	81.8 ± 0.46	82.8 ± 0.21	81.6 ± 0.63	83.1 ± 0.17	82.7 ± 0.60
ADC_p[2]	95.3 ± 0.13[a]	96.2 ± 0.10[b]	96.6 ± 0.19[c]	96.1 ± 0.07[b]	96.4 ± 0.07[bc]
ADC_l[3]	86.9 ± 0.51[a]	88.9 ± 0.34[b]	87.3 ± 0.50[a]	90.7 ± 0.31[c]	91.1 ± 0.44[c]

FO, fish oil.
* Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1] ADC_d: ADC of dry matter (%) = 100 × [1 − (Cr_2O_3 in the diet/ Cr_2O_3 in the faeces)].
[2] ADC_p: ADC of protein (%) = 100 × [1 − (Cr_2O_3 in the diet/Cr_2O_3 in the faeces)] × (crude protein in the faeces/crude protein in the diet)].
[3] ADC_l: ADC of lipid (%) = 100 × [1 − (Cr_2O_3 in the diet/Cr_2O_3 in the faeces)] × (crude lipid in the faeces/crude lipid in the diet)].

protein retention efficiency and protein efficiency ratio ($P < 0.05$).

Apparent digestibility coefficient (ADC)

There was no significant difference in the ADC of dry matter ($P > 0.05$) (Table 4). ADC of protein was highest in the FO_{50} group and the lowest in the FO_0 group ($P < 0.05$). ADC of lipid increased with increasing dietary n-3 HUFA ($P < 0.05$).

Serum and muscle TBARS

Table 5 shows that serum and muscle TBARS were significantly lower in fish fed the FO_0 diet compared with all other treatments ($P < 0.05$). The relationship between serum TBARS or muscle TBARS and dietary n-3 HUFA can be described as (Fig. 2):

$$TBARS_{serum} = 0.39\ DH + 12.19$$
$$TBARS_{muscle} = 0.21\ DH + 4.68$$

where DH is dietary n-3 HUFA (% of total fatty acids).

Table 5 Serum and muscle TBARS of gibel carp fed different diets for 12 weeks (Mean ± SE)*

Diet	Serum TBARS (nmol MDA mL^{-1})	Muscle TBARS (nmol MDA mg $prot^{-1}$)
FO_0	10.20 ± 0.53[a]	3.65 ± 0.43[a]
FO_{25}	15.82 ± 0.33[b]	6.73 ± 0.86[b]
FO_{50}	17.79 ± 1.70[b]	8.07 ± 0.97[b]
FO_{75}	19.47 ± 0.53[b]	7.68 ± 0.65[b]
FO_{100}	18.37 ± 0.82[b]	8.73 ± 0.54[b]

FO, fish oil; MDA, malonaldehyde; TBARS, thiobarbituric acid reactive substance.
* Means in the same column with different superscripts are significantly different ($P < 0.05$).

Body composition

Table 6 shows that dry matter of the final fish body was the highest in fish fed the FO_{50} diet, followed by fish fed the FO_{100} diet and the lowest in fish fed the FO_0 diet ($P < 0.05$). Ash content decreased with increasing dietary n-3 HUFA ($P < 0.05$), while there was no significant difference in the crude protein and lipid of the final fish body ($P > 0.05$).

Table 6 Body and tissue composition of gibel carp fed different diets for 12 weeks (g kg^{-1} in wet weight) (Mean ± SE)*

Diet	Initial fish	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
Body						
Dry matter	250.9 ± 6.8	272.3 ± 1.1^{a}	276.4 ± 1.4^{ab}	285.3 ± 1.4^{c}	273.5 ± 1.8^{ab}	279.0 ± 2.9^{b}
Crude protein	154.7 ± 4.3	149.0 ± 1.6	152.5 ± 2.0	152.2 ± 2.4	148.6 ± 2.8	148.2 ± 1.4
Crude lipid	52.8 ± 0.4	49.1 ± 2.4	54.5 ± 2.0	64.6 ± 3.4	50.6 ± 4.1	57.4 ± 4.9
Ash	33.4 ± 1.4	28.7 ± 0.3^{c}	28.2 ± 0.2^{bc}	27.5 ± 0.3^{ab}	27.7 ± 0.0^{ab}	27.2 ± 0.2^{a}
Muscle						
Moisture		768.0 ± 1.3	767.0 ± 1.1	764.1 ± 3.2	765.8 ± 0.9	766.9 ± 0.5
Crude lipid		14.4 ± 1.0	14.7 ± 0.1	15.9 ± 1.5	14.1 ± 0.7	13.4 ± 0.4
Viscera						
Moisture		702.3 ± 5.8^{b}	666.5 ± 5.2^{a}	667.7 ± 4.0^{a}	671.0 ± 3.7^{a}	672.1 ± 5.1^{a}
Crude lipid		99.1 ± 15.8^{a}	136.9 ± 3.7^{b}	131.3 ± 0.6^{b}	141.6 ± 5.3^{b}	139.8 ± 1.5^{b}

FO, fish oil.

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

Fish fed the FO_0 diet showed the highest moisture and the lowest lipid content in viscera ($P < 0.05$), whereas the moisture and lipid content of muscle were not significantly affected by dietary n-3 HUFA ($P > 0.05$).

Tissue fatty acid composition

Fatty acid composition of muscle and liver in fish was shown in Tables 7 & 8. After 2 week conditioning with lipid-free diet, liver SFA (mainly 16 : 0) decreased while monounsaturated fatty acids (MUFA) (mainly 18:1n-9) increased. Liver PUFA concentration also reduced, especially 22:6n-3 and 20:5n-3. Conversely, muscle fatty acids showed negligible variation after conditioning.

The fatty acid compositions of muscle and liver reflected the variation in dietary lipid. Plots of fatty acid concentration in both tissue lipid (% in total fatty acids) (Tables 7 & 8) against their concentration in dietary lipid (Table 2) yielded linear relationships (Fig. 1). However, different fatty acids showed different pattern.

Table 7 Fatty acid composition of muscle lipid in gibel carp fed different diets for 12 weeks (% in total fatty acids)

Fatty acid	Before conditioning	After conditioning	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
12:0	–	–	5.9	5.3	2.5	1.9	0.4
14:0	2.7	2.6	6.7	5.8	4.0	3.6	2.5
16:0	28.0	27.4	22.6	21.8	23.7	23.9	26.3
18:0	10.0	8.9	6.3	5.9	6.3	6.8	7.3
20:0	0.3	0.3	0.3	0.2	0.2	0.2	–
SFA[1]	40.9	39.1	41.7	39.1	36.7	36.4	36.4
16:1n-9	3.5	3.3	7.8	6.0	5.7	5.3	5.9
18:1n-9	26.9	28.9	33.5	29.5	29.0	27.6	28.0
20:1n-9	2.0	2.1	1.9	2.1	1.9	2.2	2.2
MUFA[2]	32.4	34.8	43.7	37.9	36.8	35.3	36.1
18:2n-6	14.9	14.6	5.2	6.2	9.7	9.6	10.5
20:4n-6	1.3	1.5	2.8	3.3	2.4	2.2	1.8
n-6[3]	16.2	16.1	8.4	9.5	12.2	11.7	12.3
18:3n-3	0.9	0.9	0.3	0.4	0.8	0.7	0.8
20:5n-3	1.9	1.9	0.6	1.6	1.9	2.5	2.5
22:6n-3	7.8	7.1	5.2	11.6	11.7	13.5	11.9
n-3[4]	10.6	10.0	6.1	13.6	14.3	16.7	15.2
HUFA[5]	11.0	10.5	8.6	16.5	16.0	18.2	16.2
PUFA[6]	26.8	26.1	14.5	23.1	26.5	28.4	27.5
n-3/n-6	0.7	0.6	0.7	1.4	1.2	1.4	1.2

FO, fish oil.

[1] SFA, saturated fatty acids: 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0.

[2] MUFA, monounsaturated fatty acids: 14:1n-9, 16:1n-9, 18:1n-9, 20:1n-9.

[3] n-6: 18:2n-6, 18:3n-6, 20:4n-6.

[4] n-3: 18:3n-3, 20:5n-3, 22:6n-3.

[5] HUFA, highly unsaturated fatty acids: 20:4n-6, 20:5n-3, 22:6n-3.

[6] PUFA, polyunsaturated fatty acids: 18:2n-6, 18:3n-3, 18:3n-6, 20:4n-6, 20:5n-3, 22:6n-3.

Table 8 Fatty acid composition of liver lipid in gibel carp fed different diets for 12 weeks (% in total fatty acids)

Fatty acid	Before conditioning	After conditioning	FO_0	FO_{25}	FO_{50}	FO_{75}	FO_{100}
12:0	–	0.1	7.0	7.4	4.0	3.4	0.9
14:0	3.9	2.8	8.5	8.6	6.0	5.8	4.1
16:0	37.4	26.2	24.2	26.2	29.8	29.2	31.9
18:0	12.4	10.2	7.4	7.1	8.3	8.2	8.1
20:0	0.6	0.2	–	–	0.5	–	–
SFA[1]	54.4	39.6	51.4	49.3	48.5	46.7	44.9
16:1n-9	4.1	4.6	9.0	6.6	6.7	7.0	7.0
18:1n-9	27.7	44.9	28.6	31.9	31.9	35.1	33.3
20:1n-9	2.3	2.4	2.2	2.1	2.4	2.7	2.9
MUFA[2]	34.1	52.0	40.4	40.6	41.0	44.8	43.2
18:2n-6	7.1	6.7	3.9	6.6	5.4	5.6	8.0
20:4n-6	0.5	0.5	2.0	1.2	1.3	0.6	0.9
n-6[3]	7.6	7.1	6.5	7.8	6.7	6.2	8.8
18:3n-3	0.3	0.4	0.3	0.7	–	–	–
20:5n-3	0.8	0.2	–	–	0.6	–	–
22:6n-3	2.9	0.8	1.4	1.7	3.2	2.3	3.0
n-3[4]	4.0	1.3	1.7	2.3	3.8	2.3	3.0
HUFA[5]	4.1	1.4	3.4	2.9	5.1	2.9	3.9
PUFA[6]	11.6	8.4	8.2	10.1	10.5	8.5	11.9
n-3/n-6	0.5	0.2	0.3	0.3	0.6	0.4	0.3

FO, fish oil.
[1] SFA, saturated fatty acids: 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0.
[2] MUFA, monounsaturated fatty acids: 14:1n-9, 16:1n-9, 18:1n-9, 20:1n-9.
[3] n-6: 18:2n-6, 18:3n-6, 20:4n-6.
[4] n-3: 18:3n-3, 20:5n-3, 22:6n-3.
[5] HUFA, highly unsaturated fatty acids: 20:4n-6, 20:5n-3, 22:6n-3.
[6] PUFA, polyunsaturated fatty acids: 18:2n-6, 18:3n-3, 18:3n-6, 20:4n-6, 20:5n-3, 22:6n-3.

SFA (12:0, 14:0 and 16:0) in muscle and liver were positively correlated with their concentration in dietary lipid, respectively. Liver MUFA was positively correlated with dietary MUFA while muscle MUFA negatively correlated. PUFA (mainly 18:2n-6) in muscle increased with increasing dietary PUFA, whereas liver PUFA showed negligible variation.

20:4n-6 in both muscle and liver were negatively correlated with their dietary concentration. Conversely, muscle 20:5n-3 gradually increased with dietary 20:5n-3. The same pattern of 22:6n-3 showed in liver also. However, 22:6n-3 and HUFA in muscle were distinctly reduced in fish fed the FO_0 diet.

Discussion

In this study, gibel carp fed the FO_{25} and FO_{50} diets showed better growth performance. The n-3 HUFA concentration in the FO_0, FO_{25}, FO_{50}, FO_{75} and FO_{100} diets accounted for 0, 5.17, 11.48, 16.22 and 20.84% in total fatty acids, which equivalents to 0, 4, 9, 13 and 17 g kg^{-1} of the diet. This might suggest that 4–9 g kg^{-1} n-3 HUFA was appropriate for maximal growth for gibel carp. It is similar with other freshwater fishes including rainbow trout *Oncorhynchus mykiss* (Takeuchi & Watanabe 1976), channel catfish (Satoh *et al*. 1989) and hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus* (Chou *et al*. 2001), which required 4-5, 5–7.5 and 5 g kg^{-1} n-3 HUFA for maximal growth, respectively.

This study observed that gibel carp fed the diet without n-3 HUFA resulted in the lowest SGR and FE. This could be because of the deficiency of EFA or imbalanced fatty acids. There was only small amount of 18:2n-6 (4.57% in total fatty acids, equal to 4 g kg^{-1} in the diet), and none HUFA included in the FO_0 diet, whereas 10 g kg^{-1} 18:2n-6 should be provided to meet the requirement of common carp (Takeuchi & Watanabe 1977) and grass carp *Ctenopharyngodon idella* (Takeuchi *et al*. 1991), respectively. Simultaneous, higher moisture and lower lipid content in body and tissue was also found in this study, which has been suggested to be an indicator of EFA deficiency in rainbow trout (Watanabe *et al*. 1974a,b) and common carp (Watanabe *et al*. 1975a,b). However, many reports showed that FO in freshwater fish could be totally replaced by other lipid sources, even by oils containing few PUFA, such as coconut oil or animal oils (Stickney & Andrews 1972; Legendre *et al*. 1995; Ballestrazzi *et al*. 2006). Our previous study in gibel carp (Chen 2008) also elicited that the fish fed with coconut oil showed well growth performance. These present results

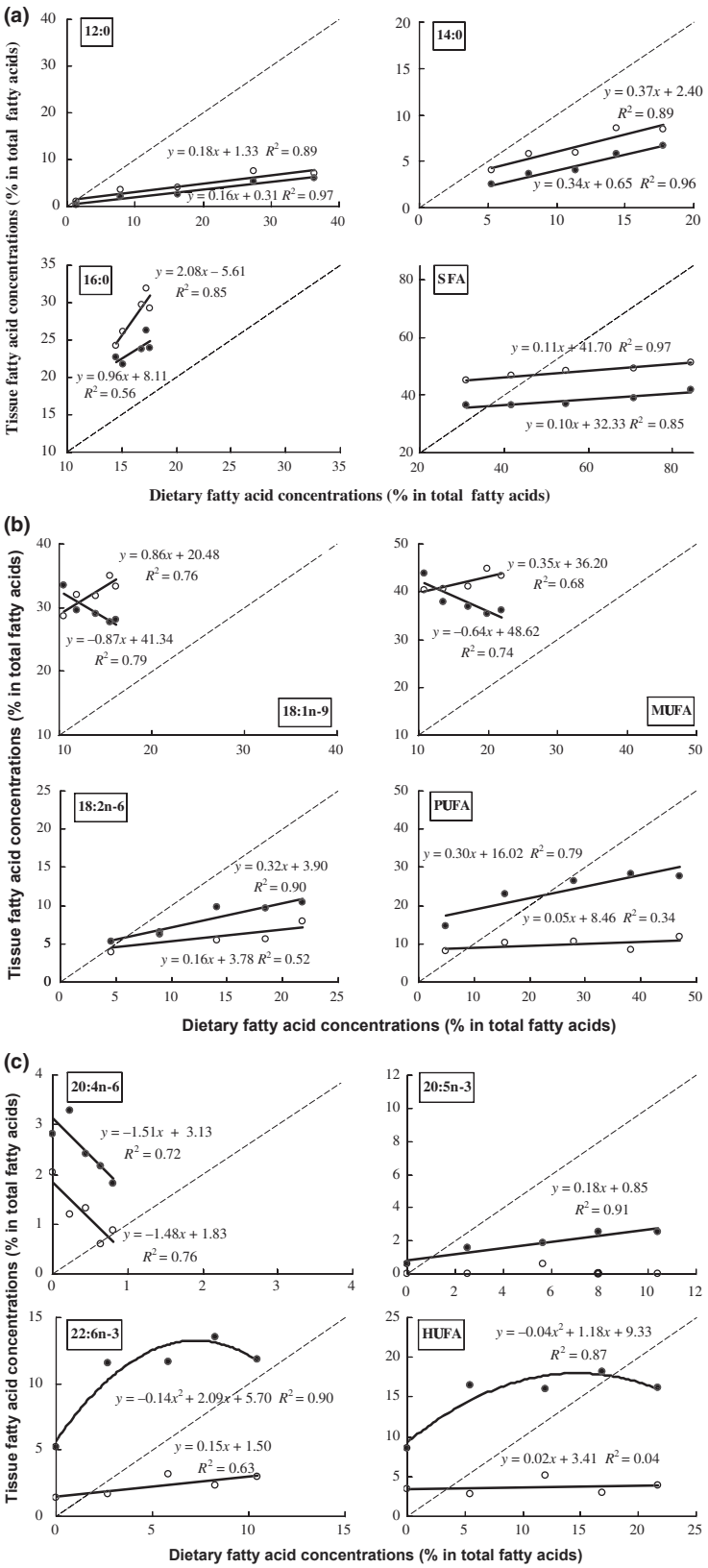

Figure 1 Relationship between muscle (–●–) or liver (–○–) fatty acid concentrations and dietary concentrations of SFA (a), MUFA and PUFA (b) and HUFA (c). HUFA, highly unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

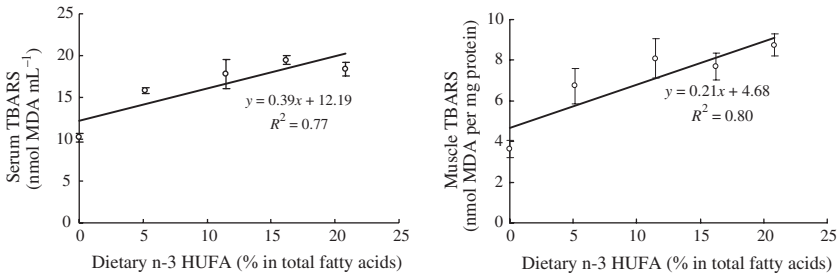

Figure 2 Relationship between serum or muscle thiobarbituric acid reactive substance and dietary n-3HUFA concentrations. HUFA, highly unsaturated fatty acids.

might be attributed to the following two reasons: (i) the residual EFA from fish meal or other ingredient could meet the minimal EFA requirement of fish. (ii) Freshwater fish could tolerate EFA deficiency during a short period growth study (Watanabe *et al*. 1975a,b; Corraze 2001). Thus, using of a basal diet without HUFA and conditioning with a lipid-free diet would be more appropriate for the evaluation of the contribution from n-3 HUFA during a relative short experimental period.

The growth of gibel carp investigated in this study was slightly reduced when dietary n-3 HUFA elevated to 13 g kg^{-1}. Similar growth depression has also been reported in some other freshwater fishes, such as tilapia (Kanazawa *et al*. 1980; Ng *et al*. 2001), African catfish *Clarias gariepinus* (Hoffman & Prinsloo 1995; Ng *et al*. 2003) and channel catfish (Stickney *et al*. 1983). There are two possible reasons accounting for this: (i) the higher n-3 HUFA in the diets was highly susceptible to oxidation and might led to poor palatability or digestibility (Chou *et al*. 2001; Du *et al*. 2008). FO_{25} and FO_{50} groups showed the best growth performance and lower FR, whereas higher FE was observed in FO_{100} group. Higher or similar ADC of dry matter, protein and lipid was also observed for the diets containing high n-3 HUFA. It is possible that the total digestible energy of feed can be partially modified, resulting in variations in feed intake (Turchini *et al*. 2009). These suggested that decreased FR might be the main reason of the slight depressed growth at high n-3 HUFA inclusion. (ii)The normal requirement of fish for HUFA was about 5–10 g kg^{-1} in dry matter, and excess HUFA possibly induced lower growth (Takeuchi & Watanabe 1979; Sargent *et al*. 2002; Du *et al*. 2008). For gibel carp, a warmwater omnivorous fish, up to 13 g kg^{-1} HUFA could not be normal situation in natural diet and might create an obligation for the animal to metabolically respond to these changes (Yu & Sinnhuber 1976; Lochmann & Gatlin 1993; Ibeas *et al*. 2000).

It has been reported that fish fed lipids with high proportions of unsaturated fatty acids tend to be more susceptible to lipid peroxidation than those fed lipids with low unsaturated fatty acids contents (Stéphan *et al*. 1995; Mourente *et al*. 2000; Menoyo *et al*. 2004; Lin & Shiau 2007). This was also observed in this study, as the lipid peroxidation indicator, TBARS values of serum and muscle showed positive linear relationship with dietary n-3HUFA concentration (Fig. 2). However, TBARS was the lowest in the fish fed the diet without n-3 HUFA, while no significant increase in TBARS with increasing dietary n-3 HUFA. This might be explained by the similar tissue HUFA concentrations in these four groups, which was considered as the major cause for inducing tissue peroxidation (Menoyo *et al*. 2002; Lin & Huang 2007). Alternatively, the level of endogenous antioxidant (e.g. Vitamin E) in tissues of fish fed higher FO might be high enough to keep the TBARS production at a similar variation (Huang *et al*. 1998). No data in this regard was obtained in this study and further investigation is suggested.

Fatty acid compositions of fish tissues were closely influenced by dietary fatty acid composition (Sargent *et al*. 2002; Francis *et al*. 2007). This study showed similar trend, as linear correlations were observed between tissue fatty acid concentrations and their concentrations in dietary lipid (Fig. 1). However, individual fatty acids were selectively retained or utilized in different tissues, because the incorporation of fatty acids in tissues were also modulated by various factors such as preferential incorporation, β-oxidation or fatty acid elongation and desaturation processes (Robin *et al*. 2003; Lin *et al*. 2007). Despite positive correlation of fatty acid between tissue and diet, the present results suggested that SFA in muscle and liver lipid (36.4–41.7% and 44.9–51.4% in total fatty acids, respectively) showed a narrower range than their relative dietary concentrations (31.1–84.3% in total fatty acids). This was consistent with previous studies in rainbow trout (Yu *et al*. 1977; Greene & Selivonchick 1990), Atlantic salmon *Salmo salar* (Bell *et al*. 2002) and red hybrid tilapia *Oreochromis* sp.(Ng *et al*. 2001), probably reflecting a homeostatic relationship that the fish tended to maintain to obtain optimal membrane function (Olsen & Henderson 1997).

Although present similar dietary concentration, 22:6n-3 had higher proportion than 20:5n-3 in fish muscle, it

suggested the important role and selective deposition of 22:6n-3. This selective deposition of 22:6n-3 in muscle, probably because of the high specificity of fatty acyl transferases for 22:6n-3 or relative resistance to β-oxidation, has also been demonstrated in other studies (Bell *et al*. 2002; Izquierdo *et al*. 2003). In addition, unlike 20:5n-3 that closely reflected dietary concentration, percentage of muscle 22:6n-3 was lowest in the fish fed the diet without n-3 HUFA, and then reached a plateau when dietary n-3 HUFA elevated to 4 g kg^{-1}. This suggested that 4 g kg^{-1} n-3 HUFA in diet could provide considerable n-3 HUFA in fish muscle. It had been reported in Atlantic salmon that 40–50% of dietary FO could be replaced by vegetable oil with only modest decrease in flesh 22:6n-3 (Bell *et al*. 2002; Bransden *et al*. 2003). Similar phenomenon was also found in Nile tilapia *O. niloticus* (Ng *et al*. 2006). However, many other studies reported that substitution of FO with other lipid sources would decrease flesh n-3 HUFA (Chou *et al*. 2001; Bell *et al*. 2003). The inconsistent results might be related to different fish species or size, the relative proportions of neutral lipids and polar lipids in the tissue and experiment period (Olsen & Henderson 1997; Turchini *et al*. 2009).

In summary, this study suggested that 4 g kg^{-1} n-3 HUFA could permit gibel carp normal growth performance and provide considerable n-3 HUFA in fish muscle. However, excessive n-3 HUFA supplement might cause negative effect on the growth of gibel carp.

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