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Effect of Dietary Lipid Sources on Body Fatty Acid Composition of Chinese Longsnout Catfish *Leiocassis longirostris*

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Abstract

We investigated the effects of dietary lipid sources on growth and fatty acid composition of juvenile Chinese longsnout catfish. Triplicate groups of fish (initial average weight, 3.8 g) were fed four diets containing either fish oil (FO), soybean oil (SO), linseed oil (LO) and lauric acid (LA) for 10 weeks. There were no differences among the groups in body weight, feed intake, feed efficiency, protein efficiency ratio, and body proximate composition of fish fed the diets containing different lipid sources ($P > 0.05$) during the study. However, fatty acids compositions of the whole body were influenced by dietary lipid source. Fish fed the SO diet had high concentration of linoleic acid, whereas those of fish fed the LO diet were rich in linolenic acid and arachidonic acid. Fish fed the FO diet had significantly ($P < 0.05$) higher levels of monounsaturated fatty acids such as 18:1n-9 and 20:1n-9 than those of fish fed the SO and LO diets. Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) composition of body were not influenced by dietary lipid source. The results suggest that each of FO, SO, LO or LA can be used as a lipid source in the diets of Chinese longsnout catfish without any negative effects on growth and feed utilization and these data demonstrate the potential impact which dietary fat composition can change the body fatty acid profile.

Key words: Dietary lipid, Growth, Fatty acids, *Leiocassis longirostris*

Introduction

Dietary lipids play an important role in fish nutrition for provision of both essential fatty acids (EFA) and energy source (Sargent et al., 1999). Dietary lipids are also carrier of fat-soluble vitamins and provide other compounds such as polar lipid and sterols, which are important structural components of cell membrane. Dietary lipid is more important energy source than carbohydrate in feeding of carnivorous fish and has sparing function on dietary protein (Lee et al., 2002). Lipid content and source in the diet affect growth and body composition of fish. The fatty acids composition of dietary lipid has a decisive influence on the fatty acids composition in fish. Even though changes due to intermediary metabolism may take place to a certain extent, the fatty acids composition of fish reflects dietary fatty acids composition.

Major fatty acids composition varied in the different dietary lipid sources. Fish oil has been used as an important ingredient of manufactured commercial fish diets, especially for marine fish, due to its high digestibility and n-3 highly unsaturated fatty acids (HUFA) content (Kim et al., 2002; Izquierdo et al., 2003). Global fish oil production has reached a plateau and is not expected to raise much beyond the current level of production. Also, it is predicted that within a decade or so, fish oil production may not be sufficient to meet the demand of aquaculture. Therefore, it is necessary to introduce alternative lipid sources to guarantee the sustainable development of aquaculture (Jordal et al., 2007; Bouraoui et al., 2011). The most

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sustainable alternative to fish oil is vegetable oil which is rich in C_{18} polyunsaturated fatty acids (Montero et al., 2003; Lee, 2001). Research on Atlantic salmon *Salmo salar* and rockfish *Sebastes schlegeli* has shown that plant oil can be an effective substitute for fish oil without affecting the growth. However, body composition of fatty acid was changed by dietary alterations in different fish (Bell et al., 2003; Mourente and Bell, 2006; Peiedecausa et al., 2007; Pratoomyot et al., 2008; Fountoulaki et al., 2009; Bouraoui et al*.,* 2011). Dietary fatty acid profiles and n-3 to n-6 fatty acids ratios caused by the different dietary oils could change fish metabolism and health status. Therefore, the replacement of fish oil by vegetable oils would be most appropriate when sufficient quantities of essential fatty acids are present in the diets (Bell et al., 2002; Regost et al., 2003; Izquierdo et al., 2005).

Chinese longsnout catfish *Leiocassis longirostris* Günthe is a commercially important aquaculture fish species in Asia. In recent years, cultures of this fish species have been expanding due to an increasing demand from consumers. Some studies on protein, lipid and carbohydrate requirements of this species have been conducted (Lim et al., 2013; Tan et al., 2006, 2007; Pei et al., 2004, 2005). In considering studies on optimum levels of lipid, 7-14% seems to be enough for energy need of this species, but limited information is available on using different lipid source in diet. Therefore, we studied the effects of feeding diets containing fish oil, soybean oil, linseed oil and lauric acid on the growth and fatty acid profile of juvenile Chinese longsnout catfish.

Materials and Methods

Experimental diets

Ingredient and nutrient contents of the experimental diets are presented in Table 1. Casein and pollack fish meal as the primary protein source and wheat flour as carbohydrate source were used. The four isonitrogenous and isolipidic diets containing fish oil (FO), soybean oil (SO), linseed oil (LO) and lauric acid (LA) at level of 10% were formulated. Crude protein and lipid levels in experimental diets were maintained 42% and 10%, respectively. The experimental diets were pelletized by a laboratory pellet machine after 30 g water was mixed with 100 g of ingredients and dried overnight at room temperature. All diets were stored at -30°C until used. Fatty acid compositions of the experimental diets are summarized in Table 2.

Experimental fish and feeding conditions

Juvenile Chinese longsnout catfishs were obtained from Inland Fisheries Research Institute (Jinhae, Korea). These fishes were acclimated to laboratory conditions by feeding commercial pellets for 2 weeks before starting the feeding trial. After this conditioning period, juvenile Chinese longsnout catfish averaging body weight (3.9 g) was randomly distributed in 12 tanks (400 L plastic tanks) at a density of 20 fish per tank. Each experimental diet was fed to three groups of fish to visual satiation two times per day (9:00 and 17:00 h) for 10 weeks.

Table 1. Ingredient and proximate composition of experimental diets

Ingredients (%)	Diets			
	FO	SO	LO	LA
Casein	24.0	24.0	24.0	24.0
Pollack fish meal	24.0	24.0	24.0	24.0
Wheat flour	20.0	20.0	20.0	20.0
α-potato starch	19.0	19.0	19.0	19.0
Squid liver oil	10.0	-	-	-
Soybean oil	-	10.0	-	-
Linseed oil	-	-	10.0	-
Lauric acid	-	-		10.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	1.0	1.0	1.0	1.0
Vitamin C (50%)	0.5	0.5	0.5	0.5
Chorine salt (50%)	0.3	0.3	0.3	0.3
Taurine	0.2	0.2	0.2	0.2
Nutrients composition (%, dry matter basis)				
Crude protein	42.7	42.5	43.0	43.0
Crude lipid	10.1	10.3	9.6	9.6

[1]Vitamin premix contained the following vitamins diluted in cellulose (g/kg premix): α-tocopheryl acetate, 14.5; thiamin, 2.1; riboflavin, 7.0; pyridoxine, 1.4; niacin, 27.8; Ca-D-pantothenate, 9.7; myo-inositol, 139.1; D-biotin, 4.2; folic acid, 0.5; p-amino benzoic acid, 13.9; K_3, 1.4; A, 0.6; D_3, 0.002; cyanocobalamin, 0.003.

[2]Mineral premix contained the following minerals (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80; $NaH_2PO_4 \cdot 2H_2O$, 370; KCl, 130; Ferric citrate, 40; $ZnSO_4 \cdot 7H_2O$, 20; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

Filtrated water was supplied at a flow rate of 5 L/min in each tank and the mean water temperature was 23.0 ± 0.8°C. The photoperiod was left under natural conditions during the feeding trail. Records were kept of daily feed consumption, mortalities, and feeding behavior.

Fish sampling

At the end of the feeding trial, all of the fish in each tank were collectively weighed after anesthetizing with tricaine methanesulfonate (MS222, Sigma, St. Louis, MO, USA) at a concentration of 100 ppm after starvation for 24 h. Blood was drawn from the caudal vessel with 1 mL heparinized syringes from three fish in each tank and transferred to microcentrifuge tubes. The collected blood was centrifuged at 4,500 *g* for 10 min and the plasma was separated and stored in a -75°C freezer. Before freezing, plasma was divided into separate aliquots for analyses of glucose, total protein, GTP, phospholipid, triglyceride and cholesterol in the plasma. Proximate composition was analyzed according to standard methods (AOAC, 1995).

Chemical analysis

Ten fish per tank at the end of the feeding trials were sampled and stored at -25°C for proximate composition analysis. Crude protein was determined by Kjeldahl method using Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was analyzed with ether extraction in a soxhlet extractor (SER 148, VELP Scientifica, Milano, Italy). Moisture was determined using a dry oven at 105°C for 6 h and also Ash content was determined after combustion at 550°C for 4 h in a muffle furnace. Fatty acid methyl esters were analyzed by using a gas chromatography (PerkinElmer, Clarus 600, GC, USA) with a flame ionization detector, equipped with SP™-2560 capillary column (100 m × 0.25 mm i.d., film thickness 0.20 μm; Supelco, Bellefonte, PA, USA). Injector and detector temperature were 260°C. The column temperature was programmed from 140 to 240°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (PUFA 37 component FAME Mix; Supelco).

Table 2. Major fatty acid composition (% of the total fatty acids) of the experimental diets

Fatty acids	Diets			
	FO	SO	LO	LA
C12:0	0.7	0.7	0.4	82.0
C14:0	4.6	0.5	0.4	0.5
C16:0	17.1	14.2	9.7	4.0
C16:1n	6.6	0.6	0.5	0.4
C18:0	2.9	3.2	4.7	0.7
C18:1n-9	19.6	21.7	15.3	3.4
C18:2n-6	21.0	46.3	17.6	4.0
C18:3n-3	2.8	5.3	40.5	
C20:0	0.4	0.6	4.4	0.1
C20:1	2.4	0.2	0.1	0.7
C20:1n-9	3.7	0.4	0.5	0.4
C20:4n-6	0.4			
C20:5n-3	9.5	2.9	2.6	1.7
C22:5n-3	0.8			
C22:6n-3	7.0	3.7	3.5	2.1
SFA[1]	25.7	19.2	19.6	87.3
MFA[2]	32.3	22.9	16.4	4.9
n-3 HUFA[3]	16.5	6.6	6.1	3.8

[1]Saturated fatty acids.
[2]Monounsaturated fatty acids.
[3]Highlyunsaturated fatty acids (C ≥ 20).

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant ($P < 0.05$) differences were found, Duncan's multiple range test (Duncan 1955) was used to rank the groups. The data are presented as mean ± SE of triplicate groups. All statistical analyses were carried out using SPSS Version 19 (SPSS, Michigan Avenue, Chicago, Illinois, USA).

Results

There were no significant differences in weight gain (42-75%), feed efficiency (59-68%), protein efficiency ratio (1.38-1.57), and daily feed intake (1.60-1.78%) of among juvenile Chinese longsnout catfish fed the experimental diets ($P > 0.05$). Also, the moisture (74.2-75.1%), crude protein (14.1-14.3%), crude lipid (6.1-7.2%) and ash (2.8-3.3%) contents of whole were not affected by dietary lipid source. However, fatty acid compositions of the whole body of juvenile Chinese longsnout catfish were affected the dietary lipid sources (Table 3). Significant differences among the four feeding treatments were noted in the content of saturated and unsaturated fatty acids ($P < 0.05$). Percentages of saturated fatty acids such as C12:0 or C14:0 of fish were higher in fish fed LA diet than fish fed other diets. Percentages of monounsaturated fatty acids such as C18:1n or 20:1n of fish fed FO diet were significantly higher than those in fish fed other diets. Percentages of n-6 polyunsaturated fatty acid (PUFA) such as linoleic acid were significantly highest in whole body of fish fed SO diet. While, the values of n-3 PUFA such as linolenic acid or arachidonic acid in whole body of fish fed LO diet were the highest among the treatments.

Hematological change of the plasma in juvenile Chinese longsnout catfish is presented in Table 4. The plasma contents of protein, glucose, GPT, phospholipid and triglyceride did not show significant differences among the treatments ($P > 0.05$). Plasma cholesterol content of fish fed LA diet was higher than that of fish fed vegetable oils (SO and LO) diets.

Discussion

Because of the limited supply and price increases of FO, recent studies have attempted to investigate the use of vegetable oils as an alternative to FO in fish feed (Francis et al., 2006; Mourente and Bell, 2006; Peng et al., 2008). The present study showed that juvenile Chinese longsnout catfish fed the different dietary oils did not have any adverse effect on growth performance and feed utilization. These results suggest that the use of SO or LO as an alternative lipid source to FO is appropriate for the normal growth of Chinese longsnout catfish. Similarly, the results obtained in Atlantic salmon *Salmo salar* (Bell et al., 2003), Atlantic cod *Gadus morhua* L (Bell et al., 2006), Murray cod *Maccullochella peelii peelii* (Francis et al., 2006), red sea bream *Pagrus auratus* (Glencross et al., 2003) and sharp snout sea bream *Diplodus puntazzo* (Piedecausa et al., 2007) showed that total replacement of dietary FO by vegetable oils had no significant effect in growth rates.

Table 3. Major fatty acid composition (% of the total fatty acids) of the whole body of juvenile Chinese longsnout catfish fed the diets containing various lipid sources for 10 weeks

Fatty acids	Diets				
	Initial	FO	SO	LO	LA
C12:0	0.1	0.1 ± 0.01^{a}	0.3 ± 0.19^{a}	0.1 ± 0.03^{a}	11.1 ± 1.10^{b}
C14:0	2.3	3.1 ± 0.13^{a}	2.4 ± 0.88^{a}	1.6 ± 0.03^{a}	6.3 ± 0.40^{b}
C16:0	17.7	17.0 ± 0.15^{ns}	17.9 ± 0.24	14.1 ± 0.26	15.4 ± 0.23
C16:1	5.5	6.0 ± 0.15^{b}	3.7 ± 0.66^{a}	2.8 ± 0.03^{a}	5.9 ± 0.10^{b}
C18:0	5.0	4.8 ± 0.07^{ab}	5.4 ± 0.39^{bc}	5.5 ± 0.09^{c}	4.3 ± 0.06^{a}
C18:1n-9	33.7	36.1 ± 0.62^{b}	32.8 ± 1.55^{a}	32.2 ± 0.20^{a}	33.5 ± 0.44^{ab}
C18:2n-6	19.5	11.1 ± 0.24^{ab}	17.9 ± 5.17^{b}	10.1 ± 0.20^{ab}	4.7 ± 0.47^{a}
C18:3n-3	4.6	1.6 ± 0.15^{a}	2.8 ± 0.15^{b}	16.3 ± 0.17^{c}	1.1 ± 0.29^{a}
C20:0	0.7	0.3 ± 0.01^{a}	0.6 ± 0.03^{b}	2.1 ± 0.01^{c}	0.2 ± 0.06^{a}
C20:1n-9	1.0	4.9 ± 0.12^{c}	2.0 ± 0.73^{a}	2.0 ± 0.06^{a}	3.3 ± 0.01^{b}
C22:1n-9	0.4	0.7 ± 0.03^{ab}	1.0 ± 0.27^{b}	0.5 ± 0.03^{a}	0.3 ± 0.01^{a}
C20:3n-3	1.7	0.2 ± 0.03^{ns}	0.9 ± 0.060	1.0 ± 0.12	0.4 ± 0.3
C20:4n-6	0.5	0.7 ± 0.06^{b}	0.6 ± 0.10^{ab}	1.4 ± 0.03^{c}	0.5 ± 0.03^{a}
C20:5n-3	1.8	3.9 ± 0.15^{ns}	2.7 ± 0.95	2.3 ± 0.15	2.8 ± 0.30
C22:3n-3	0.4	0.3 ± 0.01^{ns}	0.2 ± 0.07	0.2 ± 0.06	0.1 ± 0.07
C22:4n-6	0.1	0.1 ± 0.01^{ns}	0.2 ± 0.06	0.2 ± 0.01	0.1 ± 0.01
C22:5n-3	0.9	1.9 ± 0.03^{ns}	1.4 ± 0.35	1.4 ± 0.06	1.5 ± 0.09
C22:6n-3	3.1	7.6 ± 0.32^{ns}	6.7 ± 1.18	5.9 ± 0.34	8.0 ± 0.52
SFA[1]	25.9	25.2 ± 0.21^{a}	26.7 ± 3.67^{a}	23.5 ± 0.35^{a}	37.4 ± 1.62^{b}
MFA[2]	40.9	47.7 ± 0.75^{d}	39.7 ± 1.86^{ab}	37.7 ± 0.32^{a}	43.1 ± 0.52^{b}
n-3 PUFA[3]	12.5	15.5 ± 0.67^{a}	14.8 ± 3.30^{a}	27.0 ± 0.63^{b}	14.0 ± 0.86^{a}
n-6 PUFA[3]	20.8	12.3 ± 0.20^{ab}	19.8 ± 5.47^{b}	12.2 ± 0.17^{ab}	5.9 ± 0.56^{a}
n-3 HUFA[4]	7.9	13.9 ± 0.52^{ns}	11.9 ± 3.18	10.8 ± 0.68	12.8 ± 0.62

Values (means ± SE of triplicate groups) with each row with the different superscripts are significantly different ($P < 0.05$).
[1]Saturated fatty acids.
[2]Monounsaturated fatty acids.
[3]Polyunsaturated fatty acids (C ≥ 18).
[4]Highly unsaturated fatty acids (C ≥ 20).

Table 4. Hematological change of the plasma in juvenile Chinese longsnout catfish fed the diets containing various lipid sources for 10 weeks

	Diets			
	FO	SO	LO	LA
Total protein (g/dL)	3.3 ± 0.14^{ns}	3.0 ± 0.16	3.3 ± 0.16	3.2 ± 0.13
Glucose (mg/dL)	15.7 ± 2.19^{ns}	15.3 ± 1.67	18.7 ± 1.76	19.0 ± 3.21
Cholesterol (mg/dL)	128 ± 9.8^{ab}	109 ± 12.7^{a}	115 ± 4.4^{a}	161 ± 16.6^{b}
GPT (IU/L)	7.3 ± 1.45^{ns}	8.3 ± 1.86	12.0 ± 2.65	8.3 ± 1.33
Phospholipid (mg/dL)	433 ± 25.7^{ns}	360 ± 32.2	377 ± 8.3	405 ± 34.3
Triglyceride (mg/dL)	367 ± 54.3^{ns}	348 ± 87.1	309 ± 27.7	525 ± 33.0

Values (mean ± SE of triplicate groups) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

However, total FO substitution by vegetable oils diets in sea bass *Dicentrarchus labrax* (Izquierdo et al., 2003), black sea bream *Acanthopagrus schlegeli* (Peng et al., 2008) and gilthead sea bream *Sparus aurata* (Montero et al., 2008) reduced fish growth. These variations may be related to the particular essential fatty acid requirements of the studied species, the dietary inclusion of fish meal or other fatty acid sources and the lipid content of diets assayed for each species and the ability to accept vegetable oils of the target fish (Bell et al., 2010).

The importance of essential fatty acids for fish has been well studied in several commercial fish species (Castell et al., 1972; Sargent et al., 1999; Lee et al., 2001). Essential fatty acids and the requirements of fish differ among species. Freshwater fish require 18:2n-6 and/or 18:3n-3 (0.5-2%), while marine fish require n-3HUFA (0.5-2%) due to absence of ability to elongate and desaturate fatty acids with low level of carbon fatty acids (18:2n-6 or 18:3n-3) into HUFA de novo, particularly, EPA and DHA (NRC 1993). In this study, growth and feed efficiency of juvenile Chinese longsnout catfish were not affected by dietary fatty acids kind and level such as 18:2n-6 (0.5-5.2%), 18:3n-3 (0-4.5%), EPA (0.2-1.1%) and DHA (0.2-0.8%). This indicates that 0.5% 18:2n-6 or 0.4% n-3HUFA (EPA+DHA) might be sufficient to meet the essential fatty acids requirement of juvenile Chinese longsnout catfish to maintain normal growth. The essential fatty acid was reported in several freshwater species: 0.7-1.0% 18:2n-6 for rainbow trout *Oncorhynchus mykiss* (Castell et al., 1972), 1.0% 18:2n-6 and 0.5-1.0% 18:3n-3 for common carp *Cyprinus carpio* (Takeuchi and Watanabe., 1977), 0.5% 18:2n-6 and 0.5% 18:3n-3 for Japanese eel *Anguilla japonicas* (Takeuchi et al., 1980), 1.0% 18:2n-6 for tilapia *Tilapia zilli* (Kanazawa et el., 1980), 1.0% 18:2n-6 and 0.5% 18:3n-3 for grass carp *Cyprinus carpio* (Takeuchi et al., 1991). However further study concerning essential fatty acid requirement for this fish species is necessary.

In this study, the body fatty acid compositions were directly influenced by the dietary fatty acid profile. It is well established that fatty acids pattern of fish body lipid reflects the fatty acids composition of dietary lipid (Bell et al., 1994). The major sources of saturated and monounsaturated fatty acids of fish in this study were 16:0 and 18:1n-9, respectively. The body composition of 18:1n-9 was higher than that of the feed regardless of the dietary lipid source as described for other species (Skonberg et al. 1994; Montero et al. 2005). The compositions of saturated (12:0 and 14:0), monounsaturated (16:1 and 18:1), n-6 PUFA (18:2n-6) and n-3 PUFA (18:3n-3) were the highest of fish fed the LA, FO, SO and LO diet, respectively. The similar results have also been observed in many other studies (Caballero et al., 2002; Montero et al., 2005; Piedecausa et al., 2007) showing that fish fed diet containing SO showed a significantly higher 18:2n-6 compared to those of fish fed FO or LO. Fish fed LO diet exhibited a significantly higher 18:3n-3 than the other diets. However, the concentrations of 18:2n-6 and 18:3n-3 in juvenile Chinese longsnout catfish was lower than those in diets. These data suggest that these fatty acids were readily oxidized and selectively utilized when present at high concentration in diet.

In this study, the composition of n-3HUFA, such as 20:5n-3 and 22:6n-3 was not influenced by dietary lipid source. The reason of this phenomenon is probably owing to their ability to convert C18 into HUFA (Izquierdo et al., 2005). Although marine fish species cannot synthesize EPA and DHA from C18 (Izquierdo et al., 2005; Mourente and Bell, 2006; Peng et al., 2008), freshwater fish could convert 18:3n-3 to n-3HUFA (EPA+DHA). Bell et al. (1997) reported the hepatocytes of parr fed the vegetable oil diet had an enhanced ability to further chain elongate and desaturate 18:2n-6 and especially 18:3n-3 to their respective C20 and C22 end-product PUFA, as compared to parr fed the fish oil diet, American catfish *Pseudoplatystoma fasciatum* could convert 18:2n-6 to 20:4n-6 and 18:3n-3 to 22:6n-3 (Yamada et al., 1980; Kissil et al., 1987).

The results of this study suggest that each of FO, SO, LO or LA can be used as a lipid source in the diets of Chinese longsnout catfish without any negative effects on growth and feed utilization as the dietary essential fatty acid requirement of Chinese longsnout catfish may be satisfied and these data demonstrate the potential impact which dietary fat composition can change the body fatty acid profile.

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