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# Effects of dietary soybean oil inclusion to replace fish oil on growth, muscle fatty acid composition, and immune responses of juvenile darkbarbel catfish, *Pelteobagrus vachelli*

**Xueqin Jiang[1], Liqiao Chen[1]*, Jianguang Qin[2], Chuanjie Qin[1],Haibo Jiang[1] and Erchao Li[1]**

[1]School of Life Science, East China Normal University, Shanghai, 200062 China.
[2]School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia.

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**An 80 day feeding trial was conducted to evaluate the effects of dietary inclusion of soybean oil to replace fish oil in the diet on growth, muscle fatty acids and immune responses of *Pelteobagrus vachelli.* Isonitrogenous and isocaloric diets were formulated with four fish oil to soybean oil ratios at 8:0 (FO, control), 6:2 (MO1), 2:6 (MO2) and 0:8 (SO) in triplicate. Each diet was fed to juvenile darkbarbel catfish (1.0 ± 0.02 g) twice daily. No significant differences were found in weight gain, special growth rate, hepatosomatic index and intraperitoneal fat ratio among all the treatments. Incorporation of soybean oil significantly modified the fatty acid composition and n-3 and n-6 PUFAs ratio in muscle of fish. Fish fed MO1 showed significantly higher serum lysozyme activity, complement C3 and C4 contents and total IgM content than those fed other diets. These results indicate that partial replacement of fish oil with soybean oil does not compromise fish growth, but improve fish immunity. This study suggests that 25% fish oil replacement with soybean oil can be used in the practical diet of this fish.**

**Key words:** Darkbarbel catfish, *Pelteobagrus vachelli*, soybean oil, growth, fatty acid composition, immune responses.

## INTRODUCTION

Fish oils are considered the main source of lipid in aquaculture feeds to promote growth and development of farmed species by providing essential polyunsaturated fatty acids (PUFAs), especially high unsaturated fatty acids (HUFAs) (Sargent et al., 2002). However, in recent years, the increasing demand with limited supply of fish oil (Barlow, 2000; Petropoulos et al., 2009) necessitates the search for alternative lipids to replace fish oil in aquaculture feeds (Mourente and Bell, 2006). Thus, vegetable oils are potential and sustainable candidates to partially replace fish oils in aquaculture feeds (Montero et al., 2003; Lin and Shiau, 2007). Among available vegetable oils, soybean oil has been sought after due to its availability, affordable price and rich content of essential fatty acids (Bell et al., 2001; Caballero et al., 2003).

Like in mammals, nutritional status can affect immune system and disease resistance in fish (Blazer, 1992;

*Corresponding author. E-mail: lqchen@bio.ecnu.edu.cn. Tel/fax: +86 21 62233637.

Calder, 2001). Studies on dietary lipid and fish immune system have been emphasized on because incorporation of vegetable oils in diets may result in the potential imbalance of n-3 to n-6 fatty acids and thereafter directly or indirectly affect immune system and disease resistance in fish (Pablo et al., 2002; Montero et al., 2003). Recent research has shown that fish immunity is compromised when fish oil is replaced with vegetable oils in *Epinephelus malabaricus* (Lin and Shiau, 2007), *Ictalurus punctatus* (Fracalossi and Lovell, 1994), *Psetta maxima* (Regost et al., 2003), *Salmo salar* L. (Brandsen et al., 2003) and *Sparus aurata* (Montero et al., 2008).

Darkbarbel catfish, *Pelteobagrus vachelli* is an important freshwater species in aquaculture in Asia because of its high nutritional and market values (Xu et al., 2012). Currently, nutrition research on this species has been limited to define the environmental and nutritional requirements for optimal growth (Lu et al., 2008; Huang et al., 2009; Tan et al., 2009; Ye et al., 2009; Zheng et al., 2010). However, information on nutritional modulation of fish immunity by dietary lipid is scarce, especially on the immune response of fish when fish oil is replaced with vegetable oils. The purpose of this study was to investigate the effect of inclusion of soybean oil at various levels in feed on growth, muscle fatty acid composition and immune responses of this species.

## MATERIALS AND METHODS

### Experimental diets

Four isonitrogenous and isocaloric diets (Table 1) were formulated with four levels of fish oil and soybean oil at 8:0 (FO, control), 6:2 (MO1), 2:6 (MO2) and 0:8 (SO). Fish meal and soybean meal were used as a dietary protein source, and wheat starch was used as a dietary carbohydrate source. Diet samples were analyzed for crude protein, lipid, dry matter and ash content (Table 1) according to the standard methods of AOAC (1995), followed by the analysis of fatty acid composition (Table 2). The diet containing 51% crude protein and 11% lipid is sufficient to support the optimal growth of darkbarbel catfish (Ye et al., 2009). Diet ingredients were ground into fine powder and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried in a ventilated oven at 40°C to moisture less than 10%. After being dried, the diets were processed into pellets (1 mm dia×2 mm length), and stored at -20°C until use.

### Experimental fish, feeding and sampling

Juvenile darkbarbel catfish were obtained from a fish hatchery in Shanghai, China. Fish were fed with the control diet for two week prior to the growth trial. Fish (1.0 ± 0.02 g) were randomly distributed into 12 tanks (0.8 × 0.6 × 0.6 m) with 30 fish in each tank. Fish were fed each experimental diet in triplicate at 5% fish weight twice a day at 0800 and 1800 h, and were bulk weighed every two weeks to adjust the daily ration. All tanks were configured in a recirculation system with flow-through dechlorinated water. Dissolved oxygen in water was maintained at about 8.0 mg/L by continuous aeration; ammonia-N was less than 0.1 mg/L; pH varied from 7.0 to 7.3; water temperature ranged from 25 to 28°C. A light/dark period was set as 12 h:12 h during the feeding trial. Fish were fasted for 24 h before sampling. The trial lasted 80 days. At the end of the feeding trial, all fish were weighed, and five fish per tank were taken for blood sampling from the caudal vein with a 1.0 ml heparinized syringe, subsequently allowed to clot for 1 h in microtubes at room temperature and centrifuged at 1500×*g* for 5 min at 4°C to recover serum which was frozen at -20°C until use (Montero et al., 2003). While livers and intraperitoneal fats were obtained from these fish and weighed, dorsal muscle was taken for fatty acid analysis (Piedecausa et al., 2007). Growth variables were calculated as follows:

Weight gain (WG) = $(Wt-W_0)/W_0 \times 100$
Specific growth rate (SGR) = $(LnWt-LnW_0) \times 100/t$
Hepatosomatic index (HSI) = (liver wet weight/body wet weight)×100
Intraperitoneal fat ratio (IPF) = (intraperitoneal wet weight/body wet weight)×100

where $W_0$ and $Wt$ were initial and final fish weight (g), respectively; $t$ is duration of experiment (day).

### Biochemical composition analysis

All diets were analyzed for proximate composition following the standard methods (AOAC, 1995). Moisture was determined by drying in an oven at 105°C to a constant weight. Then dry matter was digested with nitric acid and incinerated in a muffle furnace at 600°C overnight for ash content. Crude protein was measured with the Kjeldahl method and crude lipid was determined by the ether extraction method using the Soxhlet System (2055 Soxhlet Avanti; Foss Tecator, Hoganas, Sweden).

Diets and fish muscles were dried by lyophilization for fatty acid analysis. The analysis was performed using gas chromatography (GS, HP6890, USA) with minor modifications as described by Mourente et al. (1999) and Satoh et al. (1989). Briefly, total lipid was extracted using chloroform: methanol (2:1, v/v) according to the method of Folch et al. (1957). The capillary gas chromatography (GC) method was employed to determine the fatty acid profile. The HP6890 (FID detector) and SPTM-2380 column (30 m×0.25 mm×0.20 ìm) were used on a GC machine. Separation was carried out with nitrogen as carrier gas. The column temperature was programmed from 140 to 240°C at 4°C/min, held for 5 min at 140°C and 10 min at 240°C, with a detector at 260°C. A split injector (50:1) at 260°C was used. Fatty acids were identified by comparing their retention time to the chromatographic standard (Sigma). Peak areas were determined using Varian software.

### Immune parameters assay

#### *Serum lysozyme activity*

The serum lysozyme activity was determined as described by Ellis (1990) and Alcorn et al. (2002), using a detection kit (Nanjing Jiancheng Bioengineering Institute, China). One unit of enzyme activity was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per min per ml serum.

#### *Total immunoglobulin M (IgM) in serum*

Total IgM was determined according to the method of Siwicki and Anderson (1993). As modified by Tang et al. (2008), the assay was based on the measurement of total protein contents in plasma using a micro protein determination method (C-690; Sigma) prior to and after precipitating down the IgM molecules employing a 12%

**Table 1.** Formulation and proximate composition of the experimental diets.

| Ingredients (g·kg$^{-1}$) | Diets* | | | |
|---|---|---|---|---|
| | FO | MO1 | MO2 | SO |
| Fish meal[a] | 500 | 500 | 500 | 500 |
| Soybean meal | 280 | 280 | 280 | 280 |
| Wheat starch | 64 | 64 | 64 | 64 |
| Fish oil | 80 | 20 | 60 | |
| Soybean oil | | 60 | 20 | 80 |
| Mineral mix[b] | 50 | 50 | 50 | 50 |
| Vitamin mix[c] | 5 | 5 | 5 | 5 |
| Choline chloride | 1 | 1 | 1 | 1 |
| CMC | 20 | 20 | 20 | 20 |
| **Proximate composition of diets (g·kg$^{-1}$ dry matter)** | | | | |
| Dry matter | 910.0 | 920.8 | 920.1 | 910.2 |
| Crude protein | 510.0 | 510.1 | 520.9 | 520.3 |
| Crude fat | 110.1 | 110.0 | 110.4 | 110.0 |
| Ash | 90.0 | 90.6 | 90.4 | 90.2 |

*Diet abbreviations are as follows: FO=fish oil; MO1=mixture oil 1 (fish oil:soybean oil=1:3); MO2= mixture oil 2 (fish oil:soybean oil=3:1); SO= soybean oil. [a]Made in New Zealand. [b]Mineral mixture (mg/Kg diet): MnSO4.7$H_2O$, 399; Ca ($H_2PO_4$)$_2$, 3; $AlCl_3$.6$H_2O$, 21; $ZnSO_4$ 7$H_2O$, 60; KI, 0.15; $K_2HPO_4$, 0.6; $FeSO_4$.7$H_2O$, 105; $CuSO_4$.5$H_2O$, 30; NaCl, 150 ; cellulose, 231.25. [c]Vitamin mixture (mg/kg diets, NRC 1977): retinol acetate, 5500 IU; cholecalciferol, 1000 IU; a-tocopherol acetate, 50 IU; menadione, 10 ; choline chloride, 550; nicotinic acid,100; riboflavin, 20; pyridoxine hydrochloride, 20; thiamin hydrochloride, 20; biotin,0.1; folic acid,, 5; vitamin B12, 20; L-ascorbyl-2-monophosphate Mg, 100; myo-inositol, 100. All ingredients were diluted with a-cellulose to 1 g.

**Table 2.** Fatty acid composition of experimental diets for darkbarbel catfish *Pelteobagrus vachelli* (% total fatty acids).

| Fatty acids[a] | Diets | | | |
|---|---|---|---|---|
| | FO | MO1 | MO2 | SO |
| C8:0 | 0.08 | 0.61 | 1.67 | 2.20 |
| C12:0 | 0.10 | 0.17 | 0.30 | 0.36 |
| C14:0 | 7.19 | 6.04 | 3.54 | 3.13 |
| C15:0 | 0.65 | 0.63 | 0.34 | 0.35 |
| C16:0 | 18.77 | 19.11 | 19.67 | 20.12 |
| C17:0 | 0.87 | 0.83 | 0.51 | 0.54 |
| C18:0 | 3.44 | 4.53 | 6.70 | 7.78 |
| ∑SFA[1] | 31.95 | 32.62 | 34.93 | 36.12 |
| C16:1 | 10.98 | 6.33 | 3.92 | 3.98 |
| C18:1n-9 | 15.64 | 19.45 | 18.78 | 20.72 |
| C20:1n-9 | 5.09 | 1.76 | 4.21 | 0.84 |
| ∑MUFA[2] | 33.31 | 31.43 | 28.39 | 26.08 |
| C18:2n-6 | 4.01 | 10.44 | 19.21 | 24.27 |
| 20:4n-6 | 3.59 | 3.01 | 1.38 | 0.50 |
| ∑n-6[3] | 8.11 | 13.65 | 21.58 | 25.26 |
| C18:3n-3 | 1.43 | 1.75 | 2.19 | 2.70 |
| C20:5n-3 | 12.8 | 10.72 | 6.45 | 5.01 |
| C22:6n-3 | 12.4 | 9.83 | 5.46 | 3.80 |
| ∑n-3[4] | 26.64 | 23.52 | 15.1 | 12.1 |
| ∑PUFA | 34.74 | 35.95 | 36.68 | 37.8 |
| n-3/ n-6 | 3.28 | 1.72 | 0.69 | 0.47 |

[a]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.[1]Total SFA includes *C4:0, C6:0, C8:0, C10~17:0, C18:0, C21:0, C22:0,C23:0* and *C24:0.* [2]Total MUFA includes *C14:1, C15:1, C16:1, C17:1, 18:1n-9* and *C24:1n-9*. [3]Total *n*-6 includes *C18:3n-6, C18:2n-6* and *C22:4n-6*. [4]Total *n-3* includes *C18:3n-3, C20:3n-3, C20:5n-3, C22:5n-3* and *C22:4n-6*.

**Table 3.** Growth performance* of darkbarbel catfish *P. vachelli* fed diets with different lipid sources.

| Growth performance[2] | Diets[1] | | | |
|---|---|---|---|---|
| | FO | MO1 | MO2 | SO |
| IBW(g) | 1.00±0.12 | 1.00±0.12 | 1.00±0.13 | 1.00±0.17 |
| FBW(g) | 7.68±1.46 | 8.24±1.70 | 8.31±0.64 | 7.47±0.83 |
| WG | 6.51±1.44 | 7.06±1.66 | 7.14±0.65 | 6.32±0.82 |
| SGR | 2.50±0.22 | 2.59±0.24 | 2.62±0.11 | 2.48±0.14 |
| HSI | 2.66±0.42 | 3.04±0.76 | 2.93±0.62 | 2.54±0.60 |
| IPF | 3.52±0.39 | 3.70±0.42 | 3.92±0.17 | 4.49±0.22 |

*Values are means ± S.D. of three replicates. [1]Diet abbreviations refer to Table 1. IBW (g), initial mean body weight; FBW (g), final mean body weight; WG, weight gain; SGR, specific growth rate ; IPF, intraperitoneal fat ratio; HSI, hepatosomatic index.

(w/v) solution of polyethyleneglycol (Sigma). The difference in the protein contents was considered the IgM content.

#### *Serum complements C3 and C4 contents*

The kit of immunoturbidimetry (Nanjing Jiancheng Bioengineering Institute, China) was adopted for the detection of serum complement C3 and C4 contents as described by Tang et al. (2008). The serum was mixed with the antibody from the kit, and then an antigen-antibody complex was produced. The optical density (OD) was measured at 340 nm with UV-visible spectrophotometer. Compared with the values of the standards from the kit, the C3 and C4 contents were calculated as mg/L.

#### Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests using software SPSS 16.0. The results are presented as mean ± SD, and probabilities of $P < 0.05$ were considered significant.

## RESULTS

In the 80-day growth trial, no significant differences were found in weight gain (WG), special growth rate (SGR), hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF) of fish fed different diets ($P > 0.05$, Table 3). But WG, SGR and HSI were slightly higher in fish fed MO1 or MO2 than the control, while IPF increased progressively with the increase of soybean oil in feed.

Total saturated fatty acid (SFA) in the muscle of fish fed FO and MO1 were significantly higher than those in fish fed SO and MO2 ($P < 0.05$, Table 4). Total monounsaturated fatty acid (MUFA) in fish fed FO was significantly higher than that in fish fed other diets ($P < 0.05$, Table 4). Similarly, MUFA in fish fed MO1 was significantly higher than that in fish fed MO2 and SO ($P < 0.05$). The highest total n-6 fatty acids was found in fish fed SO and lowest in fish fed FO. In contrast, the total n-3 fatty acids in fish fed FO were highest, but lowest in fish fed SO ($P < 0.05$). Inclusion of soybean oils in feed significantly increased the content of linoleic acid (18: 2n-6, $P < 0.05$) and thus significantly modified the content of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA ) and the ratio of n-3/n-6 fatty acids in the muscle ($P < 0.05$).

Table 5 presents the significant effects of the inclusion of soybean oil in feed on the activities or contents of some serum immune parameters. The lysozyme activity, contents of complement C3, C4 and total IgM in fish fed MO1 were higher than those in fish fed other diets ($P < 0.05$), but were lower in fish fed SO than those in fish fed other diets ($P < 0.05$). However, there was no significant difference between the treatments of FO and MO2 ($P > 0.05$).

## DISCUSSION

In this study, juvenile darkbarbel catfish fed diets with different inclusions of soybean oil showed no significant differences in growth after 80 days. This is in agreement with the results previously reported in *Acanthopagrus schlegeli* (Peng et al., 2008), *Diplodus puntazzo* (Piedecausa et al., 2007), *E. malabaricus* (Lin and Shiau, 2007) and *Oncorhynchus mykiss* (Caballero et al., 2002), representing successful replacement of dietary fish oil with vegetable oils in fish species without compromising their growth. Despite statistical insignificance obtained in the study, fish fed the diets with partial inclusion of soybean oil (MO1 and MO2) showed numerically higher growth rate (WG, SGR, HSI and IPF) than fish fed FO or SO. This pattern of growth may probably resulted in from 11% lipid content in formulation of the experimental diets satisfying fish growth and development based on the previous studies of dietary lipid requirement for darkbarbel catfish (Han et al., 2005; Huang et al., 2009). In addition, the higher growth performance was observed on juvenile darkbarbel catfish fed diets MOs (MO1 and MO2), probably due to the n-3 to n-6 ratio of 1.72-0.69 from diets MOs was suitable for improving growth of darkbarbel catfish. The fatty acid (FA) ratio can affect fish growth by the interaction between dietary n-3 and n-6 fatty acids on endogenous FA elongation and

**Table 4.** Muscle fatty acid composition* of darkbarbel catfish *P. vachelli* fed diets with different lipid sources (% total detectable FA).

| Fatty acid | Diets[1] | | | |
|---|---|---|---|---|
| | FO | MO1 | MO2 | SO |
| C14:0 | 3.49±0.04$^{a}$ | 2.94±0.03$^{b}$ | 2.03±0.16$^{c}$ | 1.79±0.02$^{c}$ |
| C15:0 | 0.42±0.05$^{a}$ | 0.39±0.08$^{ab}$ | 0.25±0.04$^{bc}$ | 0.21±0.03$^{c}$ |
| C16:0 | 17.45±0.71$^{a}$ | 17.05±0.49$^{a}$ | 15.29±0.26$^{b}$ | 15.02±0.68$^{b}$ |
| C17:0 | 0.63±0.08 | 0.60±0.12 | 0.40±0.07 | 0.37±0.10 |
| C18:0 | 4.43±0.20a | 4.97±0.36b | 4.80±0.11b | 4.85±0.23b |
| ∑SFA | 26.89±0.39$^{a}$ | 26.52±0.41$^{a}$ | 23.40±0.27$^{b}$ | 22.94±0.33$^{b}$ |
| C16:1 | 6.44±0.19$^{a}$ | 4.88±0.12$^{b}$ | 3.22±0.30$^{b}$ | 3.12±0.28$^{b}$ |
| C18:1n-9 | 27.71±0.29$^{b}$ | 28.64±0.37$^{a}$ | 28.03±0.46$^{b}$ | 28.02±0.15$^{b}$ |
| C20:1n-9 | 4.17±0.02$^{a}$ | 3.30±0.02$^{ab}$ | 2.15±0.04$^{bc}$ | 2.41±0.03$^{c}$ |
| ∑MUFA | 38.32±0.82$^{a}$ | 36.82±0.65$^{b}$ | 33.41±0.91$^{c}$ | 33.55±0.47$^{c}$ |
| C18:2n-6 | 9.9±0.12$^{a}$ | 12.79±0.06$^{b}$ | 25.84±0.47$^{c}$ | 27.39±0.61$^{d}$ |
| 20:4n-6 | 0.51±0.01$^{a}$ | 0.57±0.08$^{a}$ | 0.74±0.10$^{b}$ | 0.87±0.23$^{b}$ |
| ∑n-6 | 10.47±0.15$^{a}$ | 13.80±0.14$^{b}$ | 27.22±0.62$^{c}$ | 28.91±0.83$^{d}$ |
| C18:3n-3 | 1.70±0.10$^{a}$ | 1.96±0.20$^{a}$ | 2.92±0.22$^{b}$ | 3.21±0.19$^{b}$ |
| C20:5n-3 | 4.85±0.08$^{a}$ | 4.48±0.11$^{b}$ | 2.52±0.43$^{c}$ | 2.14±0.23$^{d}$ |
| C22:6n-3 | 13.45±1.06$^{a}$ | 12.68±1.21$^{b}$ | 8.18±0.97$^{c}$ | 7.21±0.75$^{d}$ |
| ∑n-3 | 23.63±1.24$^{a}$ | 22.16±1.52$^{b}$ | 15.78±1.33$^{c}$ | 13.81±1.09$^{d}$ |
| ∑PUFA | 34.80±0.79$^{a}$ | 36.67±0.95$^{b}$ | 43.2±1.58$^{c}$ | 43.51±0.88$^{c}$ |
| n-3/ n-6 | 2.26±0.05$^{a}$ | 1.61±0.02$^{b}$ | 0.58±0.04$^{c}$ | 0.48±0.06$^{c}$ |

*Values are means of three replicates ± SD. Means in a row with the same letter superscript are not significantly different ($P < 0.05$).

**Table 5.** Serum immune parameters* of darkbarbel catfish *P. vachelli* fed diets with different lipid sources.

| Immune parameter | Diets | | | |
|---|---|---|---|---|
| | FO | MO1 | MO2 | SO |
| Lysozyme activity (U/ mL) | 97.65±14.02$^{b}$ | 132.72±16.16$^{a}$ | 106.40±22.32$^{b}$ | 69.43±16.16$^{c}$ |
| Complement C3 content (mg/L) | 0.22±0.03$^{b}$ | 0.33±0.08$^{a}$ | 0.23±0.09$^{b}$ | 0.13±0.05$^{c}$ |
| Complement C4 content (mg/L) | 0.08±0.01$^{b}$ | 0.16±0.01$^{a}$ | 0.10±0.03$^{b}$ | 0.06±0.01$^{c}$ |
| Total IgM level (mg/L) | 0.28±0.02$^{b}$ | 0.47±0.07$^{a}$ | 0.25±0.08$^{b}$ | 0.22±0.01$^{b}$ |

*Values are means ± S.D. of three replicates and values with different letter superscripts within the same row are significantly different at $P < 0.05$.

desaturation enzyme systems, especially by the Δ6 and Δ5 desaturase enzymes (Tan et al., 2009). Therefore, the higher HSI and IPF in fish fed MOs with n-3 to n-6 ratio of 1.72 and 0.69, probably resulted in from the increasing activities of elongase and desaturase in hepatocytes of fish due to their higher affinity towards the n-3/n-6 fatty acids (Tocher et al., 2002; Stubhaug et al., 2005; Blanchard et al., 2008). Our result indicated that inclusion of soybean oil in feed does not compromise the species growth, suggesting the possible replacement of fish oil with soybean oil in diet formulation for darkbarbel catfish.

The positive relationship between tissue fatty acid composition and dietary fatty acid contents has been well demonstrated in previous studies (Bransden et al., 2005; Zia-Ul-haq et al., 2007a, b, 2008, 2010, 2011a, b; Luo et al., 2008; Rezek et al., 2010). In this study, the muscle fatty acid composition in fish generally reflected the lipid composition in diets similar to the report of Ng et al. (2003) that the high fatty acids in feed can lead to high fatty acids in muscle. Moreover, there were differences in fatty acid composition between fish muscle and the diets in our study.

For instance, lower content of SFA in muscle relative to the diets indicates that darkbarbel catfish has limited ability to deposit SFA into the tissue and metabolize it to meet up energy demands. This claim is supported by the research in *Mystus nemurus* (Ng et al., 2000) and *Pelteobagrus fulvidraco* (Tan et al., 2009). Freshwater fish has an innate ability to convert these C18 fatty acids to the long-chain n-3 and n-6 fatty acids (e.g.

EPA, DHA and ARA) *in vivo* by an alternating sequence of desaturation and elongation (Garg et al*.,* 1988; Blanchard et al., 2008), which have been observed in some freshwater fish species such as *M. nemurus* (Ng et al., 2000), *Oncorhyncus mykiss* (Buzzi et al., 1996), *Perca fluviatilis* (Blanchard et al., 2008) and *Pelteobagrus fulvidraco* (Tan et al*.,* 2009). Therefore linolenic acid (LnA, C18:3n-3) and linoleic acid (LA, C18:2n-6) are considered as the essential fatty acids for freshwater fish (Turchini et al., 2006). Darkbarbel catfish probably possesses the similar ability to convert LnA and LA to 20:5n-3 (EPA), 22:6n-3 (DHA) and 20:4n-6 (ARA), respectively (Nakamura and Nara, 2004; Sprecher et al., 1995). Therefore in this study, the dietary inclusion of soybean oil (rich in C18:2n-6) finally led to accumulation of n-6 fatty acids and reduction of n-3 fatty acids in fish muscle. Piedecausa et al. (2007) also reported the similar result that the higher level of 18:2n-6 fatty acid in soybean oil can promote lipid accumulation in fish body. Therefore, an optimal replacement (25 to 75%) of fish oil by soybean oil in feed with the n-3 to n-6 PUFA ratios of 0.69-1.72 may provide better growth for juvenile darkbarbel catfish.

The change of dietary fatty acid composition and the ratio of n-6/n-3 fatty acids can also affect fish immunity and disease resistance (Blazer, 1992; Kiron et al., 1995; Yildirim-Aksoy et al., 2007; Montero et al., 2008). In this study, the MO1 diet with the n-3/n-6 fatty acid ratio of 1.72 significantly increased the activity of serum lysozyme, the contents of serum complement C3, C4 and total IgM, suggesting that such an n-3/n-6 fatty acid ratio in diet was appropriate to darkbarbel catfish and stimulated its immune function. This result was probably attributed to, on the one hand, the ratio of n-3 and n-6 PUFA can affect immune cell functions, cell signaling and humoral immunological processes by regulating the production of eicosanoids, derived from DHA, EPA and ARA as their precursors (Calder, 2006; Yaqoob and Calder, 2007; Montero et al., 2010). On the other hand, the balanced n-3/n-6 fatty acid ratio is a requirement for fish health and good immunity (Simopoulos, 2008). An excess of n-6 PUFA adversely affects antibody production and serum immune response parameters, whereas excessive level of n-3 PUFA reduce macrophage killing ability and disease resistance in fish (Bell et al., 1996; Yildirim-Aksoy et al., 2007). In this study, in comparison to the control FO (total fish oil) and SO (total soybean oil), a well-balanced n-3/n-6 fatty acids ratio of 1.72 (MO1) significantly improved the immunity of the fish.

However, despite the crucial correlation between dietary lipid composition and fish immunity, the functional effects of dietary lipid on fish immune response are controversial. Positive effects of n-3 fatty acids on the immune response were found in *O. mykiss* (Kiron et al., 1995) and *S. aurata* (Montero et al., 2008), whereas negative effects of high levels of dietary n-3 PUFAs on immunity are found in *I. punctatus* (Fracalossi and Lovell, 1994) and *O. mykiss* (Ashton et al., 1994). In this study, although we found that partial inclusion (25%) of soybean oil in diet can enhance immunity, it still warrants further study to investigate the mechanism why inclusion of vegetable oil can improve fish immunity.

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