Maize Oil Can Replace Fish Oil in The Diet of Grouper Postlarvae (Epinephelus Coioides) Without Adversely Affecting Growth or Fatty Acid Composition

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Abstract: This experiment was conducted to evaluate the effects of dietary lipid sources on growth, survival and body composition of 40 day post hatch larval grouper, Epinephelus coioides. Fish were fed fish meal and protein hydrolysate based diets for 32 days with either 100% maize oil or 100% fish oil in triplicate from 40-day after hatching to slaughter size (fish weight: 0.32 g to 11 g). Final body wet weight (FBW: 11.8±0.7 and 11.1±0.1, respectively), weight gain (WG: 3556±251 and 3360±189, respectively), specific growth rate (SGR: 11.2±0.2 and 11.1±0.2, respectively) and survival rate (80±5 and 79±4, respectively) were not significantly affected by dietary lipid sources ($P>0.05$). The effect of different oil sources on the composition of tissues was significant only for dorsal muscle lipid. In dorsal muscle, lipid content was significantly higher in fish oil group. The fatty acids composition of the muscle lipids well reflected the fatty acids composition of the experimental diets. The growth performance showed that a balance is required between growth-promoting essential fatty acids (EFA) qualities of dietary n-3 highly unsaturated fatty acid (n-3 HUFA) and their potentially growth-inhibiting (pro-oxidant) qualities. Results indicated when EFA of the diet are sufficient for the development of postlarvae, there is no difference whether use fish or maize oil in the formulated diets, 2.87% n-3 HUFA is sufficient for grouper postlarvae development and more n-3 HUFA are not necessarily beneficial to fish performance.

Key words: Grouper (Epinephelus coioides), lipid source, growth, fatty acid

INTRODUCTION

Lipid nutrition are important for fish growth because they are source of energy, essential fatty acids (EFA) and other lipid classes like phospholipids, sterols and fat-soluble vitamins. Several studies have shown that the source and content of the lipid of the feed have a major impact on larval development [1]. Most marine fish require n-3 HUFA (such as docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA)) for normal growth and development [2]. Fish oil is the only commercially available source of EPA and DHA [3], and it is widely used in the formulation of commercial aquaculture feeds. These fatty acids, function as critical structural and physiological components of the cell membranes of most tissues [4]. Fish oil contains the long chain n-3 HUFA and thus it is considered to have superior quality than maize oil [3]. However, global fisheries are now stagnating and the current yield of fish oil from industrial fisheries, circa 1.4 million tons in 1996, is unlikely to be significantly exceeded in future [6]. Fish farming consumed a total of 560,000 tons of fish oil in 1996 [5]. Global aquaculture has grown at 11.6% per annum compound growth since 1984 [7] and is continuing to grow at a similar rate. It is clear that demand for fish oil from aquaculture must, before long, exceed supply. This problem will be exacerbated by growing environmental pressure to decrease exploitation pressure on finite marine resources and by increasing consumer perception that levels of pollutants such as dioxin in fish oils have now reached unacceptable levels [8]. Moreover, it has been estimated that aquaculture feeds currently use about 60% of the global supply of fish oil, and by the year 2010, fish oil used in aquaculture will be about 75% of the world supply [9]. For these reasons, finding alternatives to fish oils in farmed aquaculture feeds is becoming an increasingly urgent issue. The use of alternate lipids in aquaculture needs to be addressed not only to possibly reduce diet cost, but also to potentially...
reduce the current pressure on fisheries resources. On the other hand, many aquaculturist and nutritionists are interested in lipid nutrition of fish, such as lipid source, because this dietary component has great effect on growth and heath of the organism [10], as well as on the quality of resulting products [11].

The effects of different lipid sources of dietary on different fish species have been demonstrated: *Oncorhynchus mykiss* [12], *Sciaenops ocellatus* [13], *Salvelinus fontinalis* [14], *Anguilla australis* [15]. Fish oil replacement with unconventional lipid sources seems to be possible if the essential fatty acid (EFA) requirements are satisfied [16, 17, 18]. But to date, the works on grouper are relatively limited. The nutrient requirement studies of grouper (*Epinephelus coioides*) have been addressed on calcium and phosphorus [19], L-methionine [20], tryptophan [21] and replacement of fish oil by meal by-product meals [22]. No research has been reported the effect of dietary lipid sources on grouper postlarvae (*Epinephelus coioides*).

Consequently, this study was conducted to determine the influence of dietary source of oil on growth, survival and body composition of grouper postlarvae, *Epinephelus coioides*.

**MATERIALS AND METHODS**

**Experimental diets and diet preparation:** Two isonitrogenous (59% protein) and isolipidic (16% lipids) diets were formulated (Table 1). The two lipid sources were fish oil and maize oil and they were from New Zealand (Bakels Edible Oils Ltd., Mt Macnagui). Diet preparation was as described by previous study [23]. Briefly, all dry ingredients for each of the experimental diets were weighed, combined and thoroughly mixed until homogenous in a Hobart-type mixer, and then distilled water (20%, v/w) and oils were added and thoroughly mixed. Pellets were obtained using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, PR China), air-dried to about 100g kg⁻¹ moisture and ground using a mortar and pestle and graded via a series of different-sized metal sieves. Diets must be prepared as microparticles whose sizes must be adapted to the size of the postlarvae mouth. Size of the microparticulate diets used in the experiment was 300 um for grouper postlarvae at the initial feeding period, then 500 to 800 um from day 1 to 6, 1.0 to 1.2 mm from day 7 to 12, 1.5 mm from day 13 to 18 and 1.5 to 2.5 mm to day 32. Diets were sealed in vacuum-packed plastic bags and stored in -20°C until feeding. The available dietary energy was calculated using physiological fuel value of 4.0, 4.0 and 9.0 kcal g⁻¹ for protein, carbohydrate and lipid, respectively [24].

| Ingredients          | White fish meala | Fish protein hydrolysateb | Soybean lecithinc | Fish oild | Maize oile | a-Starchf | Mineral premix | Vitamin premixg | Lysine, HCLh | Othersi |
|----------------------|------------------|--------------------------|------------------|-----------|-----------|----------|--------------|----------------|-------------|---------|
|                      | 51.8             | 20                       | 5                | /         | 8         | 5        | 1            | 1.6           | 4          | 2.6     |
|                      | 51.8             | 20                       | 5                | /         | 8         | 5        | 1            | 1.6           | 4          | 2.6     |

Table 1: Ingredients and proximate composition of the experimental diets (g per 100g dry matter)

| Ingredients | Moisture | Crude protein | Crude lipid | Ash | n-3 HUFA | Crude energy (Kcal/100g) |
|-------------|----------|---------------|-------------|-----|----------|-------------------------|
|             | 16.1     | 59.0          | 16.0        | 9   | 2.87     | 400                     |
|             | 15.9     | 59.1          | 16.2        |     | 6.12     | 402.2                   |

**Fish culture:** The system consisted of six aquaria (150 L × 70 W × 60 H cm), each supplied with 500 l continuously filtered seawater circulating all the time. Each aquarium was connected with a separate recirculating system, and could modulate the flow rate of water based on the fish developmental condition. Each recirculating system was equipped with a sand filter and packed-column biological filter. During the trial, the diurnal cycle was 12-h light/12-h dark. Water
Sampling and dissection: After the final weighing and fasting for 24 h, twelve fish per tank were randomly collected and used for analysis of body composition and dorsal muscle fatty acids composition. Fish were randomly captured and killed by immediate spinal destroying for measure and dissection. Wet weight (WW) of the intact fish and corresponding organs was determined and the hepatosomatic index (HSI =100×liver WW/fish WW), visceralosomatic index (VSI =100×viscera WW/fish WW) were calculated. Livers and dorsal muscle dissected were frozen immediately in liquid nitrogen and stored at -70°C until in need.

Analytical methods: Diets and dissected tissues were analyzed in triplicate for proximate composition. Moisture, crude protein, crude lipid and ash were determined using standard methods [25]. Moisture was determined by drying in an oven at 105°C for 24 h. Crude protein (N×6.25) was analyzed by the Kjeldahl method (1030-Auto-analyzer, Tectator, Sweden); Crude ash by incineration in a muffle furnace at 550°C for 24 h. Crude lipid was determined and the ether-extraction method by Soxtec System HT (Soxtec System HT6, Tectator, Sweden).

Total lipid of dorsal muscle was extracted according to previous method [26]. Fatty acids from lipid samples were methylated using 10% potassium hydroxide in methanol for 1 h at room temperature [27]. Fatty acid methyl esters were then analysed and quantified using a Hewlett-Packard HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) in a cross-linked 5% phenylmethyl silicone gum phase column (length 25 m; internal diameter 0.32 mm; film thickness 0.25 mm; N2 as the carrier gas; HP-Ultra 2), equipped with flame ionisation detection. The injector and detector temperatures were 280 and 300°C, respectively. The column temperature set at 190°C was then increased to 260°C (2°C/min) and held for 5 min. Results are expressed as the percentage of each fatty acid with respect to total fatty acids.

Statistical analyses: Statistical analysis of the result was done by Student t-test using the software of the SPSS for Windows (ver10.0, U.S.A.).

RESULTS

There were no significant differences among dietary groups for feed conversion ratio (FCR), WG, SGR, survival and FBW (Table 2). There were also no significant differences in HSI and VSI between treatments (Table 2). In the present study, FCR was probably overestimated because feed waste for the first six days can not be determined accounts.

No significant differences were found between treatments for whole body or liver compositions (Table 3). No difference in dorsal muscle moisture and protein contents were found. However, fish fed fish oil had significantly higher lipid content in dorsal muscle than fish fed maize oil (P<0.05).

The fatty acids content of the experimental diets and dorsal muscle and liver are shown in Table 4. The FA composition of dorsal muscle well reflected the fatty acids composition of the experimental diets, as levels of SAT, MONO, n-3 HUFA and n-6 HUFA all reflected the composition of each respective dietary lipid. Fish fed the diet containing maize oil had the highest levels of MONO, and fish fed the fish oil diet had the highest levels of n-3 HUFA.

Table 2: Effect of diet on growth performance, tissue indices and feed conversion ratio of the fish.

| Treatment Performance | Maize oil | Fish oil |
|-----------------------|-----------|----------|
| IBW (g)               | 3.2±0.02  | 3.2±0.01 |
| FBW (g)               | 11.8±0.7  | 11.1±0.1 |
| WG (%)                | 3556±251  | 3360±189 |
| SGR (%)               | 11.2±0.2  | 11.1±0.2 |
| Survival (%)          | 80.2±5.1  | 78.8±4.3 |
| FCR (%)               | 1.27±0.09 | 1.34±0.04 |
| HSI (%)               | 4.23±0.21 | 4.19±0.12 |
| VSI (%)               | 12.4±0.2  | 12.6±0.1 |

Data represent mean ± S.E.M. (n=3).

Values in the same row with different letters are significantly different (P<0.05).

1IBW: Initial body wet weight (g).
2FBW: Final body wet weight (g).
3WG (%): weight gain=100×(final body weight- initial body weight)/ initial body weight (g).
4SGR: special growth ratio=100×(ln final wt.- In initial wt.)/32 days.
Table 3: Influence of maize oil diet and fish oil diet on whole body, dorsal muscle and liver composition (%) in larval grouper.  

| Composition          | maize oil | fish oil |
|----------------------|-----------|----------|
| Whole body           |           |          |
| Moisture             | 70.12±0.14| 69.65±0.13|
| Protein              | 16.09±0.08| 16.17±0.20|
| Lipid                | 8.95±0.13 | 8.95±0.06 |
| Ash                  | 3.84±0.05 | 3.88±0.01 |
| Dorsal muscle        |           |          |
| Moisture             | 77.23±0.14| 77.70±0.51|
| Protein              | 18.07±0.05| 17.81±0.24|
| Lipid                | 2.40±0.05  | 2.70±0.08 |
| Liver                |           |          |
| Moisture             | 59.25±0.94 | 57.29±0.34|
| Protein              | 8.32±0.58  | 7.34±0.27 |
| Lipid                | 12.92±1.03 | 13.62±0.87|

Data represent mean ± S.E.M. (n=3). Values in the same row with different letters are significantly different (P<0.05).

DISCUSSION

Most marine fish require the EFA (n-3 HUFA) for normal reproduction and growth. These essential FA have two main functions in fish the same as in vertebrate: to maintain the structural and functional integrity of the cell membranes of most tissues and act as the precursor of eicosanoids [28]. Recent investigations have indicated that DHA is necessary for development for the nervous system including brain and retina in larvae and juvenile. Marine fish larvae lack Δ⁹ desaturase rendering them unable to synthesize DHA and EPA from linolenic acid (18:3n-3) [29]. Thus most marine fish require pre-formed highly unsaturated long chain fatty acids (EPA; DHA) in their diets [1]. Fish oil offers a range of fatty acid classes, mainly contains the long chain n-3 HUFA and thus was considered more superior than maize oil in fish feed [3].

Experiments conducted using live prey [30, 31] or a compound diet [32] have shown that the optimal level of n-3 HUFA in diet for marine fish larvae is around 3% (dry matter). The present experiment was designed to have 2.87% and 6.12% (dry matter) n-3 HUFA in the maize oil diet and fish oil diet respectively, when fish were fed fish meal and protein hydrolysate based diets for 32 days, the fish oil did not improve the fish performance (WG, SGR and survival). The use of maize oil in diet of grouper resulted in substantial growth and excellent feed efficiency. Grouper seem able to efficiently utilize maize oil as the dietary lipid source as long as their requirement for EFA is met. The result indicated that n-3 HUFA content in the maize oil diet is sufficient for postlarvae development, the other more n-3 HUFA provided by fish oil is unnecessary.

The conclusion of our present experiment that superabundant n-3 HUFA are not necessarily beneficial to fish performance is similar with previous study [33]. In his experiment, maximal larval total length, individual larval dry weight and SGR were achieved with the treatment which contained 3.97% n-3 HUFA on a dry weight basis of diet. SGR in this treatment is 21.5±1.1, but the larval growth performance declined significantly when the amount of n-3 HUFA was 6.23% on a dry weight basis of diet. SGR in the treatment was 18.7±1.4. Study of Dentex dentex indicated the increasing oxidation of n-3 HUFA in larvae with increasing levels of dietary n-3 HUFA if the amount of n-3 HUFA exceeded the optimal level, and would have a poor performance [34].

In the present experiment, whole body and liver compositions were not to be related to dietary lipid source, but lipid content in muscle varied with lipid source. For example in dorsal muscle, fish oil diet groups had the higher crude lipid content than maize oil diet groups, the result in our experiment is similar with red drum study [35]. Previous research with red drum has indicated that certain diet formulations promote excessive lipid deposition in the peritoneal cavity and muscle, which may be indicative of inefficient utilization of dietary components [35]. Though total lipid in muscle was significantly affected by dietary lipid, the effect did not seem to detrimentally affect performance, feed efficiency or fatty acid composition of grouper in the present study, fish with high muscle lipid usually experience the greatest weight gain and feed efficiency. Previous research indicated that accumulation of lipid in liver has been identified as a fatty acid deficiency sign in some fish species [34, 35], but in our present experiment, there were no significant difference in liver lipid between the two diet groups, which indicated that the maize oil diet satisfied grouper postlarvae requirement for fatty acids. There was an increased level of carcass tissue lipid of P. monodon fed a diet containing cod liver oil [36]. It was also found that only n-3 HUFA significantly increased the total lipid content of shrimp tissue, but the n-6 HUFA did not significantly affect the total lipid content of shrimp tissue [37]. In our present study, the fish oil diet had more n-3 HUFA than the maize oil diet and the n-6 HUFA level was the same, this agrees with the conclusion of L. vannamei study [37].
Table 4. Selected fatty acid contents (% of total fatty acids) of the experimental diets and dorsal muscle. n.d.: non-detected. tr.: traces.

| Fatty acids | Maize oil diet | Fish oil diet | Maize oil muscle | Fish oil muscle |
|-------------|----------------|---------------|------------------|----------------|
| 16:0        | 6.63±0.12<sup>a</sup> | 5.51±0.06<sup>b</sup> | 12.39±0.62       | 11.04±0.57     |
| 18:0        | 4.22±0.04<sup>b</sup>  | 5.03±0.06<sup>a</sup> | 5.22±0.14        | 5.58±0.16      |
| 22:0        | tr.             | tr.           | 0.50±0.08        | 0.57±0.11      |
| 16:1n-7     | 0.08±0.01<sup>b</sup> | 0.64±0.01<sup>a</sup> | 1.37±0.25        | 1.69±0.13      |
| 18:1n-9     | 46.43±0.54<sup>a</sup> | 25.93±0.62<sup>b</sup> | 27.55±0.55<sup>a</sup> | 21.26±0.72<sup>b</sup> |
| 18:2n-6     | 20.00±0.40<sup>a</sup> | 11.30±0.55<sup>b</sup> | 17.10±0.96<sup>a</sup> | 10.45±0.41<sup>b</sup> |
| 18:3n-3     | 0.67±0.03<sup>b</sup>  | 0.8±0.05<sup>a</sup>  | 0.78±0.02<sup>b</sup>  | 0.96±0.05<sup>a</sup>  |
| 18:4n-6     | 0.70±0.02<sup>b</sup>  | 0.73±0.03       | 1.42±0.30        | 1.34±0.28      |
| 22:5n-6     | n.d.            | tr.            | 0.63±0.06<sup>b</sup> | 0.88±0.03<sup>a</sup> |
| Saturated   | 10.85±0.09      | 12.04±0.12     | 18.11±0.56       | 17.19±0.78     |
| Monoenoic   | 47.04±0.54<sup>a</sup> | 27.98±0.70<sup>b</sup> | 31.37±0.64<sup>a</sup> | 26.45±0.44<sup>b</sup> |
| n-3 HUFA    | 17.83±0.37<sup>b</sup> | 37.70±1.00<sup>a</sup> | 25.22±1.02<sup>b</sup> | 31.07±1.17<sup>a</sup> |
| n-6 HUFA    | 1.37±0.03       | 1.53±0.03      | 2.83±0.34        | 3.18±0.32      |

Data represent mean ± S.E.M. (n=3). Values in the same row with different letters are significantly different (<i>P</i>&lt;0.05).

Generally speaking, the fish body fatty acid composition can reflect the fatty acid composition of the diet [38]. In the present experiment, the condition of the gross saturated and monounsaturated fatty acids in muscles just the same as the diets, saturated fatty acids had no difference and monounsaturated fatty acids had a significant difference (18.11±0.56, 17.19±0.78 and 31.37±0.64a, 26.45±0.44b respectively). The monounsaturated fatty acids are more from newly synthesized, only a few from the diet [39]. When the content of HUFA in the diet is beyond the requirement, monounsaturated fatty acids could be replaced by redundant HUFA [39]. It was considered that the diet HUFA could restrain the monounsaturated fatty acids synthesized ferment, which could lead to the reduction of these fatty acids amounts in fish tissues [60]. In the present study, the fish oil diet group had significantly lower monounsaturated fatty acids in dorsal muscle compared with the maize oil diet group (p<0.05). It indicated the fish oil diet had excessive HUFA again.

CONCLUSION

In conclusion, postlarval grouper were able to efficiently utilize maize oil as the dietary lipid source, once their requirement for EFA is met by a source rich in the long-chain (n-3) HUFA. Total lipid in muscle was significantly affected by dietary lipid, but this effect did not seem to detrimentally affect performance or fatty acid composition of grouper in the present study.

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