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Effects of replacement of dietary fish oil by soybean oil on growth performance and liver biochemical composition in juvenile black seabream, *Acanthopagrus schlegeli*

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Abstract

A 9-week feeding experiment was carried out on juvenile black seabream *Acanthopagrus schlegeli* to evaluate the effects of dietary replacement of fish oil by soybean oil on fish growth and liver biochemical composition. Fish in triplicate were fed four diets, in which 0% (FO as control), 60% (60SO), 80% (80SO) and 100% (100SO) of fish oil was replaced by soybean oil. The weight gain of fish fed 60SO or 80SO diet was similar to that of fish fed the control diet, but a total replacement of fish oil by soybean oil significantly reduced fish growth. Although the inclusion of soybean oil resulted in an increase in the crude lipid content of the liver, the level of fish oil replacement did not significantly alter the hepatosomatic index, feed conversion ratio, condition factor and liver proximate composition. The inclusion of soybean oil in seabream diets increased hepatic α-tocopherol concentrations, but reduced thiobarbituric acid-reactive substances and plasma cholesterol. Linoleic acid and linolenic acid significantly increased in fish fed soybean oil diets, but docosahexaenoic acid, eicosapentaenoic acid and the ratio $n-3/n-6$ were significantly reduced by the inclusion of dietary soybean oil ($P<0.05$). Our results indicated that the inclusion of soybean oil increased the hepatic α-tocopherol content and reduced lipid peroxidation in fish. However, complete substitution of fish oil with soybean oil reduced growth efficiency. Thus, 60–80% replacement of fish oil by soybean oil is recommended in diet formulation for black seabream.

Keywords: Black seabream; *Acanthopagrus schlegeli*; Fish oil replacement; Soybean oil; Growth; Biochemical composition

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1. Introduction

Dietary lipids play an important role as a source of energy for fish growth and as carriers for fat soluble vitamins. Fish oil contains high quantities of $n-3$ HUFA and other essential fatty acids (EFA) necessary for marine fish (Sargent and Tacon, 1999). They serve as a functional element maintaining metabolism and contain attractants that enhance diet palatability. The demand for fish oils in aquafeeds has dramatically increased in the last decade (Barlow, 2000) and has placed unsustainable pressure on this finite resource (Tacon, 2004). Thus, the partial replacement of fish oils with vegetable oils in artificial feeds has gained increasing interest from aquaculturists (Caddy, 1999; Valdimarsson and James, 2001).

Aquaculture has continued to grow more rapidly than all other animal food-producing sectors with a global average growth rate of 8.8% per year since 1970 (FAO). Current projections anticipate that in a few years, global fish oil production may not be enough to supply the increasing demand of animal feed. On the contrary, production of global vegetable oil has steadily increased in recent years, reaching a volume of 100 times more than fish oil (Bimbo, 1990). Therefore, replacement

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of fish oil with vegetable oils appears to be a viable option given their availability, low cost and absence of dioxins and pollutants (Caballero et al., 2002; Izquierdo et al., 2003).

A key requirement for the replacement of fish oil in aquafeeds is to supply equivalent energy with balanced essential fatty acids. This is necessary in order to sustain high growth and survival, feed conversion efficiency, immune competence, disease resistance, and flesh quality. However, the lack of balanced fatty acid profiles (Sargent et al., 2002), low palatability (Guillou et al., 1995) and digestibility (Caballero et al., 2002) may hinder the practice of fish oil replacement by vegetable oils in fish diets. Despite this drawback, a total replacement of fish oil by vegetable oil has been successfully achieved in turbot *Psetta maxima* (Regost et al., 2003) and partial replacement has been reported in numerous fish species. For instance, up to 60% fish oil was successfully replaced by vegetable oil in juvenile European sea bass *Dicentrarchus labrax* L. (Montero et al., 2005; Mourente et al., 2005a) and gilthead sea bream *Sparus aurata* (Izquierdo et al., 2005). One side effect of including vegetable oils is that it alters the $n-3/n-6$ fatty acid ratio and interferes with eicosanoid synthesis (Fracalossi et al., 1994; Sargent et al., 2002). This can lead to decreased growth performance and has been observed as a result of 80% fish oil substitution in European sea bass (Montero et al., 2005) and gilthead sea bream diets (Montero et al., 2003; Izquierdo et al., 2005).

In the past, most studies on fish oil replacement have focused on the growth response to the addition of vegetable oil in various freshwater fish species such as rainbow trout *Oncorhynchus mykiss* (Fonseca-Madrigal et al., 2005) and Atlantic salmon *Salmo salar* L. (Bell et al., 2001; Torstensen et al., 2004), and marine fish species such as gilthead sea bream *S. aurata* (Caballero et al., 2003; Montero et al., 2003; Izquierdo et al., 2003, 2005), European sea bass *D. labrax* (Montero et al., 2005; Mourente et al., 2005a), grouper *Epinephelus malabaricus* (Lin and Shiau, 2007), sharpsnout seabream *Diplodus puntazzo* (Piedecausa et al., 2007) and turbot *P. maxima* (Bell et al., 1995; Regost et al., 2003). It is therefore necessary to investigate the functional response of fish to the supplement of dietary lipids.

The liver plays a central role in lipid metabolism including fatty acid synthesis and degradation through enzyme regulations, and it is also a sensitive organ reflecting dietary lipid change in fish (Kiessling and Kiessling, 1993; Henderson, 1996). The functional response of red drum, *Sciaenops ocellatus* to imbalanced dietary fatty acids has been detected through monitoring the change of biochemical composition in the liver (Craig et al., 1999). The liver cells can be functionally damaged by lipid peroxidation when the diet contains a high level of unsaturated animal fatty acids (Kanazawa, 1993; Mates et al., 1999). In contrast, the use of vegetable oils has been reported to reduce lipid peroxidation in mammals (Lopez-Bote et al., 1997) and fish (Stephan et al., 1995; Alvarez et al., 1998). For instance, 50% fish oil replacement by corn oil in grouper *E. malabaricus* diets reduced peroxidation in the liver (Lin and Shiau, 2007). Therefore, the response of fat deposition and peroxidation in the liver should be considered a physiological indicator when evaluating fish oil replacement by other lipid alternatives.

Black seabream *Acanthopagrus schlegeli* is a valuable commercial species for aquaculture in many parts of Asia (Chang and Yueh, 1990). The objective of this study was to investigate the response of black seabream to partial and total replacement of dietary fish oil with a soybean oil alternative. In this study, fish performance was evaluated through conventional variables such as growth, survival and food conversion ratio. In addition, the response of proximate body composition of juvenile black seabream to dietary lipid manipulation was also examined. More importantly, we further measured the responses of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation and oxidative stress, α-tocopherol, an antioxidant, in liver cells, and triglycerides and cholesterol in blood plasma to evaluate the possible oxidative and physiological stress of fish subjected to various levels of vegetable oil in the diet. This study will provide evidence and explanation for fish adaptation to dietary lipid manipulation.

2. Materials and methods

2.1. Experimental diets

Four iso-nitrogenous, iso-energetic, iso-lipidic experimental diets were formulated with 15% lipid derived from the following combinations: (1) 100% fish oil as control (FO), (2) 60% soybean oil and 40% fish oil (60SO), (3) 80% soybean oil and 20% fish oil (80SO), (4) 100% soybean oil (100SO) (Table 1). The fatty acid compositions of experimental diets were given in Table 2.

Table 1
Ingredient and proximate composition of the experimental diets

	Experimental diets			
	FO[a]	60SO	80SO	100SO
Ingredients (g/kg)				
Fish meal	350	350	350	350
Soybean meal	400	400	400	400
Wheat flour	129.3	129.3	129.3	129.3
L-methionine	2	2	2	2
L-lysine	8	8	8	8
Anchovy oil	90	36	18	0
Soybean oil	0	54	72	90
Vitamin premix[b]	10	10	10	10
L-ascorbic acid[b]	0.5	0.5	0.5	0.5
DL-α-tocopherol acetate[b]	0.2	0.2	0.2	0.2
Mineral premix[c]	10	10	10	10
Proximate composition				
Crude protein (% DM)	45.26	45.29	45.37	45.94
Crude fat (% DM)	14.95	15.36	15.16	15.22
Ash (% DM)	11.74	11.97	11.92	11.03
α-tocopherol (mg/kg)	107.41	106.03	105.59	105.27

[a] FO = 100% fish oil; 60SO = 60% soybean oil; 80SO = 80% soybean oil; 100SO = 100% soybean oil.

[b] Supplied (mg kg^{-1} diet): *myo*-inositol, 400; nicotinic acid, 150; calcium pantothenate, 44; riboflavin, 20; pyridoxine hydrochloride, 12; menadione, 10; thiamine hydrochloride, 10; retinyl acetate, 7.3; folic acid, 5; biotin, 1; cholecalciferol, 0.06; cyanocobalamin, 0.02. L-ascorbic acid: 93% AA activity; DL-α-tocopherol acetate: 50% vitamin E activity.

[c] Supplied (kg^{-1} diet): KH_2PO_4, 22 g; $FeSO_4 \cdot 7H_2O$, 1.0 g; $ZnSO_4 \cdot 7H_2O$, 0.13 g; $MnSO_4 \cdot 4H_2O$, 52.8 mg; $CuSO_4 \cdot 5H_2O$, 12 mg; $CoSO_4 \cdot 7H_2O$, 2 mg; KI, 2 mg.