

The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from January 2010 The Israeli Journal of Aquaculture - Bamidgeh (IJA) has been published exclusively as an **online Open Access** scientific journal, accessible by all.

Please visit our [IJA Website](http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija)

<http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija>

for free publications and to enable you to submit your manuscripts.

This transformation from a subscription printed version to an online Open Access journal aims at supporting the concept that scientific peer-reviewed publications and thus the IJA publications should be made available to all for free.

Editor-in-Chief

Dan Mires

Editorial Board

Rina Chakrabarti	University of Delhi India
Angelo Colorni	National Center for Mariculture Israel
Daniel Golani	The Hebrew University of Jerusalem Israel
Sheenan Harpaz	Agricultural Research Organization, Israel
David Haymer	University of Hawaii at Manoa USA
Gideon Hulata	Agricultural Research Organization, Israel
Ingrid Lupatsch	AB Agri Ltd, UK
Constantinos Mylonas	Hellenic Centre for Marine Research, Greece
Jaap van Rijn	The Hebrew University of Jerusalem, Israel
Amos Tandler	National Center for Mariculture, Israel
Emilio Tibaldi	Udine University Italy
Zvi Yaron	Tel Aviv University Israel

Copy Editor

Miriam Klein Sofer

Published by the
**The Society of Israeli Aquaculture and
Marine Biotechnology (SIAMB)**
in partnership with the
University of Hawaii at Manoa Library
and the
AquacultureHub

A non-profit organization 501c3

<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII[®]
MĀNOA
LIBRARY



AquacultureHub.org

AquacultureHub
educate • learn • share • engage

ISSN 0792 - 156X

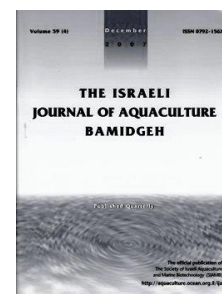
© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:

**The Society of Israeli Aquaculture and
Marine Biotechnology (SIAMB)**



Published as an open-access journal by the Society of Israeli Aquaculture & Marine Biotechnology (SIAMB). To read papers free of charge, please register online at <http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija>
The sale of IJA papers is strictly forbidden



Dietary Lipid Level Affects Growth Performance, Antioxidant Capacity, Hematological Characteristics and Lipid Metabolism in Juvenile Black Seabream (*Acanthopagrus schlegelii*)

Ting-ting Pan, Hong Qiu, Ting-ting Zhu, You Lu, Ye Yuan, Qicun Zhou, Min Jin*

Laboratory of Fish and shellfish Nutrition, School of Marine Sciences, Ningbo University, Ningbo 315211, China

Keywords: Antioxidant capacity; black seabream; dietary lipid level; hematological characteristics; lipid metabolism

Abstract

An 8-week feeding trial was conducted to investigate the effects of dietary lipid levels on growth, antioxidant capacity, hematological characteristics, and lipid metabolism of juvenile black seabream (*Acanthopagrus schlegelii*) of initial weight 4.33 ± 0.01 g. Three isonitrogenous diets were formulated to contain lipid levels of 36.8 (Diet 1, control group), 82.9 (Diet 2) and 136g/kg (Diet 3). After the feeding trial, fish fed high lipid levels (Diet 2 and Diet 3) showed higher growth performance and feed efficiency compared to low level (Diet 1) group. The hematological characteristics were significantly higher in Diet 3 than Diet 1. Fish fed Diet 3 had significantly higher malondialdehyde (MDA) content and catalase (CAT) activity in serum than Diet 1 group. Conversely, the hepatic glutathione peroxidase (GSH-Px) and fatty acid synthase (Fas) activity were significantly lower in fish fed Diet 3. Overall, this study indicated that juvenile black seabream could adapt high lipid diet (82.9~136g/kg) by regulating growth, hematological characteristics, and lipid metabolism.

* Corresponding author. Min Jin, e-mail: jinmin@nbu.edu.cn

Introduction

Dietary lipids play a vital role in the energy production processes of animal tissues. As a source of essential fatty acids and phospholipids they contribute to the maintenance of normal cell structure and biological function (NRC, 2011). Feed conversion could improve in fish, and the amount of waste production could decrease by increasing the lipid content in diets to spare protein (Vergara et al., 1999). Furthermore, appropriate levels of non-protein energy sources determine the efficiency of protein utilization (NRC, 2011). Thus, in the past few decades, dietary lipids have been widely used in aquaculture as a cheap energy source to save dietary protein and increase feed efficiency (Li et al., 2016). Nevertheless, the study of dietary lipid level requirements in black seabream is scarce.

Lipid metabolism including anabolism (biosynthesis) and catabolism, involves many biochemical reactions, as well as many rate-limiting enzymes. In terms of lipid anabolism, fatty acid synthetase (Fas) catalyzes de novo fatty acid synthesis, which a key pathway in lipogenesis that occurs and has been characterized in fish (NRC, 2011). Carnitine palmitoyltransferase (Cpt1) is regarded as a main regulatory enzyme in fatty acid β -oxidation catalyzing the conversion of cytosolic fatty acyl-CoA to fatty acyl-carnitine for entry into mitochondria (Yang, 2010). However, the lipid metabolism regulation mechanisms by dietary lipid levels in black seabream is still unknown.

Black seabream (*Acanthopagrus schlegelii*) is a very popular and commercially important marine fish species cultured in China, Japan, Korea, and other countries in Southeast Asia. It has been regarded as an excellent aquaculture species for intensive culture since it exhibits rapid growth, has high disease resistance, and has the ability to tolerate a wide range of environmental conditions. However, black seabream farming foods are mainly trash fish, leading to high feed costs and environmental pollution (Zhou et al., 2011). To date, numerous studies have reported on nutritional requirements of black seabream; these include protein, amino acid, essential fatty acid, vitamins, etc. (Ma et al., 2013; Zhou et al., 2011), but there are no studies concerning dietary lipid level requirements of juvenile black seabream. Thus, the objective of present study aimed to clarify the effects of dietary lipid levels on growth performance antioxidant capacity, hematological characteristics, and lipid metabolism in juvenile black seabream. This study also aimed to investigate the way in which black seabream adapt to high dietary lipid levels as well as their use of high-fat formulation in commercial diets.

Materials and Methods

Three isonitrogenous ($\sim 45\%$ crude protein) diets were formulated to contain different lipid levels: 3.7% (Diet1), 8.3% (Diet2) and 13.6% (Diet3), respectively (Table 1). White fishmeal, soybean protein concentrate, wheat gluten meal, and kill meal were used as protein sources, whereas soybean lecithin and fish meal were used as the main lipid sources. All ingredients were purchased from Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China. All dry ingredients were ground into fine powder, particle size $< 177\ \mu\text{m}$, micro components such as minerals and vitamins premix were added, followed by lipids and distilled water (35%, w/w). The ground ingredients were mixed in a Hobart type mixer and cold-extruded pellets produced (F-26, Machine factory of South China University of Technology) with pellet strands cut into uniform sizes of 2 mm and 4 mm diameter pellets, (G-250, Machine factory of South China University of Technology). Pellets were steamed for 30 min at 90°C , and then air-dried to approximately 10% moisture, sealed in vacuum-packed bags and stored at -20°C until use in the feeding trial.

Table 1. Formulation and proximate composition of the experimental diets (g/kg dry diet).

Ingredient	Experimental diets		
	Diet1	Diet2	Diet3
White fish meal	230	230	230
Peru fish meal	150	150	150
Soybean protein concentrate	143	143	143
Wheat gluten meal	40.0	40.0	40.0
Wheat flour	215	215	215
Kill meal	20.0	20.0	20.0
α -starch	49.0	49.0	49.0
Cellulose	100	50.0	-
Soybean lecithin	10.0	10.0	10.0
Fish oil	-	50.0	100
Vitamin premix ¹	10.0	10.0	10.0
Mineral premix ¹	15.0	15.0	15.0
Ca(H ₂ PO ₄) ₂	15.0	15.0	15.0
Choline chloride	3.0	3.0	3.0
<i>Proximate composition</i> ²			
Dry matter	889	918	898
Crude protein	455	457	461
Crude lipid	36.8	82.9	136
Ash	111	111	112

¹. Vitamin premix and mineral premix were supplied by Ningbo Tech-bank Aqua feed company, Ningbo, China.

². Means of three replicate analyses.

Black seabream juveniles (initial weight 4.33 ± 0.01 g) were obtained from a local commercial hatchery at Xiangshan Bay, Ningbo, China. Prior to the experiment, the black seabream juveniles were acclimated for two weeks and fed on a commercial diet (45 % dietary protein, 10% crude lipid, Ningbo Tech-Bank Corp.). A completely randomized trial design was implemented. A total of 360 black seabream juveniles were randomly allocated to 9 floating net cages (1.5 m \times 1.5 m \times 2.0 m) corresponding to triplicate cages of the three dietary treatments. Fish were hand-fed twice daily at 5:00 am and 17:00 pm for eight weeks. During the experimental period, physico-chemical conditions including temperature 26.5–31.5°C, salinity (19–25 ‰), ammonia nitrogen (< 0.05 mg/L), and dissolved oxygen (> 7.0 mg/L) were monitored daily (YSI Proplus, YSI, Yellow Springs, Ohio, USA).

At the end of the feeding trial, fish were anesthetized with tricaine methane sulfonate (MS-222). Five fish from each cage (15 per treatment) were pooled (n = 3) and used for analyzing the proximal composition of whole body, where three fish (nine per treatment, n=9) were used to determine morphological parameters including condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and intraperitoneal fat (IPF) ratio. Muscle and liver samples were also collected and stored at -80°C until further analysis of proximal composition (pools of 3 fish per cage, n = 3) and enzyme activity (pools of 3 fish per cage, n = 3). Blood samples were taken from the caudal vasculature of 8 fish per cage by using 2 ml syringes.

The crude protein, crude lipid, moisture, and ash contents of diets as well as whole fish and muscle were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2006). Briefly, moisture content was determined by drying the samples to a constant weight at 105°C. Crude protein (N \times 6.25) was determined via the Dumas combustion method with a protein analyzer (Leco FP528, St. Joseph, USA). Crude lipid was determined by the ether extraction method using the Soxhlet Method (Soxtec System HT6, Tecator, Sweden), and ash content was determined using a muffle furnace at 550°C for 8 h.

Blood was assayed within 24 h from collection after storage at 4°C, with serum collected by centrifugation at 956 g for 10 min at 4°C (Eppendorf, Centrifugal 5810 R, Hamburg, Germany). Liver samples were homogenized in nine volumes (w/v) of ice-cold physiological saline 0.89 % (w/v), and then centrifuged as described above. The contents of malondialdehyde (MDA) and protein, as well as enzymatic activities of glutathione peroxidase (GSH-Px), superoxidedismutase (SOD) and catalase (CAT), were determined

in serum and liver homogenates using assay kits (Nanjing Jiancheng Bioengineering Institute, China). All the content of MDA and protein, as well as enzymatic activities of GSH-Px, SOD and CAT, were calculated according to the manufacturer's instructions.

Carnitine palmitoyl transferase (Cpt1) and fatty acid synthase (FAS) are key enzymes for lipid metabolism in fish. Liver samples were processed as described before. The supernatant was used to determine the activities of Cpt1 and FAS by using Elisa assay kit for fish from Yuan Ye Biological Company (Shanghai, China). These hepatic lipid metabolism activities were analyzed according to the manufacturer's instructions.

Cholesterol (CHOL), glucose (GLU), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein (TP) and triacylglycerol (TAG) contents were measured in serum samples. These analyses were carried out with an automatic blood analyzer (Hitachi 7600-110 Ltd, Japan) at Ningbo University Hospital.

Results are presented as means \pm SEM (number of replicates as indicated). The homogeneity of variances (Levene's test) were checked prior ANOVA tests. Effects of dietary lipid levels were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test at a significance level of $P \leq 0.05$ (IBM SPSS Statistics 20).

Results

The impact of dietary lipid levels on growth performance, feed utilization, and morphologic index are presented in Table 2. Weight gain (WG) and specific growth rate (SGR) in Diet1 group were significantly lower than Diet2 and Diet3 ($P < 0.05$). Moreover, the highest values of feed efficiency (FE) and protein efficiency ratio (PER) were all found in fish fed with Diet3 ($P < 0.05$). Nevertheless, survival rates did not show any statistical differences among dietary treatments ($P > 0.05$). The intraperitoneal fat ratio (IPF), hepatosomatic (HSI) and viscerosomatic (VSI) indices were significantly higher in fish fed higher dietary lipid level (Diet3) ($P < 0.05$). In contrast, dietary lipid level had no effect on condition factor (CF) ($P > 0.05$).

Table 2. Growth performance, feed utilization and morphologic index of black sea bream fed diets containing different lipid levels.

Parameters	Experimental diets			ANOVA P
	Diet1	Diet2	Diet3	
IBW (g) ¹	4.34 \pm 0.01	4.33 \pm 0.01	4.33 \pm 0.01	0.145
FBW (g) ²	41.18 \pm 1.10 ^a	43.57 \pm 0.75 ^b	44.49 \pm 0.56 ^b	0.007
WG (%) ³	849.10 \pm 26.79 ^a	907.18 \pm 16.28 ^b	928.23 \pm 10.62 ^b	0.006
SGR (%/d) ⁴	4.02 \pm 0.05 ^a	4.12 \pm 0.03 ^b	4.16 \pm 0.02 ^b	0.006
FE ⁵	0.95 \pm 0.04 ^a	0.99 \pm 0.02 ^{ab}	1.04 \pm 0.01 ^b	0.009
PER ⁶	2.09 \pm 0.08 ^a	2.17 \pm 0.04 ^a	2.52 \pm 0.05 ^b	0.000
Survival (%) ⁷	98.89 \pm 1.92	100 \pm 0.00	100 \pm 0.00	0.422
HSI (%) ⁸	1.76 \pm 0.01 ^{ab}	1.63 \pm 0.06 ^a	1.82 \pm 0.11 ^b	0.040
CF (g/cm ³) ⁹	3.46 \pm 0.20	3.36 \pm 0.36	3.51 \pm 0.46	0.877
VSI (%) ¹⁰	6.81 \pm 0.27 ^{ab}	6.42 \pm 0.43 ^a	7.68 \pm 0.37 ^b	0.014
IPF (%) ¹¹	1.79 \pm 0.23 ^a	2.42 \pm 0.04 ^a	3.25 \pm 0.47 ^b	0.003

Data are reported as the mean \pm SD (n = 3 for IBW, FBW, SGR, FE and Survival; n = 9 for CF, VSI, HSI and IPF). Values in the same line with different superscripts are significantly different ($P < 0.05$).

¹. IBW, initial body weight

². FBW, final body weight

³. Weight gain (WG, %) = 100 \times ((final body weight – initial body weight) / initial body weight).

⁴. Specific growth ratio (SGR, % day⁻¹) = 100 \times ((Ln final body weight (g) – Ln initial body weight (g)) / days).

⁵. Feed efficiency (FE) = weight gain (g, wet weight) / feed consumed (g, dry weight).

⁶. Protein efficiency ratio (PER) = weight gain (g, wet weight)/protein fed (g, dry weight)

⁷. Survival (%) = 100 \times (final fish number / initial fish number).

⁸. Hepatosomatic index (HSI, %) = 100 \times (liver weight / wet body weight).

⁹. Condition factor (CF, g/cm³) = Body weight (g) \times 100 / body length (cm)³.

¹⁰. Viscerosomatic index (VSI, %) = 100 \times (visceral weight / wet body weight).

¹¹. Intraperitoneal fat ratio (IPF, %) = 100 \times (intraperitoneal fat weight / wet body weight).

The effects of dietary different lipid levels on proximate composition of whole body and muscle (% wet basis) after the feeding trial are shown in Table 3. The dry matter and crude lipid contents in the whole body in fish fed Diet3 were significantly higher than other treatments ($P < 0.05$). On the contrary, the highest crude protein content was

found in fish fed with Diet1 ($P < 0.05$). Similar results were found in muscle composition, fish fed Diet3 showed the highest contents of dry matter and crude lipid ($P < 0.05$). Nevertheless, the crude protein and ash contents in muscle as well as the ash in whole body did not show any significant differences among dietary groups ($P > 0.05$).

Table 3. Proximate composition in whole body and muscle of black sea bream (*Acanthopagrus schlegelii*) (g/kg wet weight) fed diets containing different lipid levels.

Parameter	Experimental diets			ANOVA P
	Diet1	Diet2	Diet3	
<i>Whole body composition</i>				
Dry matter	307±0.94 ^a	329±7.16 ^b	341±2.64 ^c	0.000
Protein	186±0.16 ^b	181±1.78 ^{ab}	176±3.76 ^a	0.008
Lipid	74.4±2.82 ^a	100±5.82 ^b	111±1.78 ^c	0.000
Ash	51.6±0.33	51.1±0.71	48.6±2.94	0.170
<i>Muscle composition</i>				
Dry matter	238±1.74 ^a	237±3.93 ^a	247±4.25 ^b	0.026
Protein	202±6.93	205±1.81	200±2.42	0.463
Lipid	27.4±1.11 ^a	26.9±0.31 ^a	36.8±1.57 ^b	0.000
Ash	14.9±0.98	14.9±0.38	14.6±0.09	0.840

Data are reported as the mean±SD (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$).

The activity of the antioxidant enzymes SOD and GSH-Px in serum as well as the levels of MDA and the activities of SOD and CAT in liver were not affected by dietary lipid levels ($P > 0.05$). In contrast, the content of MDA and activity of CAT in the serum increased as dietary lipid level increased ($P < 0.05$). The highest ($P < 0.01$) hepatic GSH-Px activity obtained in Diet1. (see Table 4)

Hepatic Cpt1 activity were not significantly affected ($P > 0.05$) by the dietary lipid levels (Table 4). Nevertheless, the hepatic FAS activity was significantly decreased ($P < 0.05$) with increased dietary lipid levels.

Table 4. Serum and hepatic parameters of black seabream fed diets containing different lipid levels.

Parameters	Experimental diets			ANOVA P
	Diet1	Diet2	Diet3	
<i>Serum oxidation and antioxidant parameters</i>				
MDA (nmol/ml) ¹	13.00±1.58 ^a	17.70±2.92 ^{ab}	18.67±0.68 ^b	0.025
SOD (U/ml) ²	4.56±0.15	4.78±0.30	5.01±0.08	0.081
CAT (U/ml) ³	8.19±0.72 ^a	9.87±0.93 ^{ab}	10.72±0.67 ^b	0.019
GSH-Px (U/ml) ⁴	606.92±62.31	586.15±87.69	761.54±80.07	0.062
<i>Hepatic oxidation and antioxidant parameters</i>				
MDA (nmol/mg/prot)	0.43±0.43	0.80±0.34	0.66±0.23	0.464
SOD (U/mg/prot)	9.44±0.48	11.30±1.06	10.22±1.14	0.128
CAT (U/mg/prot)	23.24±1.27	22.57±4.32	30.12±7.11	0.190
GSH-Px (U/mg/prot)	116.14±7.50 ^c	67.14±2.75 ^b	18.58±1.35 ^a	0.000
<i>Hepatic lipid metabolism activities</i>				
Fas (U/g/prot) ⁵	123.00±18.33 ^b	123.56±16.67 ^b	65.78±10.00 ^a	0.006
Cpt1 (U/g/prot) ⁶	59.22±3.09	53.04±7.93	52.55±2.50	0.060

Data are reported as the mean±SD (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$).

¹. MDA, methane dicarboxylic aldehyde

². SOD, superoxide dismutase;

³. CAT, catalase

⁴. GSH-Px, glutathione peroxidase

⁵. Fas, fatty acid synthase

⁶. Cpt1, carnitine palmitoyltransferase 1

Hematological characteristics in serum are shown in Table 5. CHO, HDL and LDL were significantly increased with increasing dietary lipid levels thus the highest contents were all found in Diet3 ($P < 0.05$). However, TP and GLU levels did not show any statistical differences among dietary treatments ($P > 0.05$).

Table 5. Hematological characteristics of black sea bream fed diets containing different lipid levels.

Parameters	Experimental diets			ANOVA P
	Diet1	Diet2	Diet3	
GLU (mmol/L) ¹	1.64±0.37	1.39±0.51	1.22±0.39	0.526
CHO (mmol/L) ²	9.05±0.91 ^a	10.60±1.28 ^{ab}	13.58±1.88 ^b	0.020
TAG (mmol/L) ³	4.42±0.19	3.99±0.74	5.50±1.97	0.359
HDL (mmol/L) ⁴	5.20±0.72 ^a	6.31±0.60 ^{ab}	7.70±1.08 ^b	0.028
LDL (mmol/L) ⁵	3.55±0.22 ^a	4.02±0.55 ^a	5.29±0.35 ^b	0.004
TP (g/L) ⁶	44.49±2.76	47.41±1.31	49.43±3.07	0.128

Data are reported as the mean±SD (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$).

¹. GLU, glucose

². CHO, cholesterol

³. TAG, triglyceride;

⁴. HDL, high density lipoprotein

⁵. LDL, low density lipoprotein

⁶.TP, total protein

Discussion

Apart from lipids, protein and carbohydrates can also serve as a lipid source through lipogenesis with amino acids and pyruvate serving as the main carbon sources. However, with the development of aquatic feed, protein sources are regarded as the most expensive ingredients in diets formulated for commercial produce, thus the purpose is to minimize dietary protein that might be used as a source of energy. It is well known that dietary lipids play important roles as sources of essential fatty acids (EFA), needed for normal growth and survival, spare protein consume in fish (NRC, 2011), and it can also increase the tenderness of meat (Chen et al., 2012).

In the present study, higher growth performance and feed utilization were all obtained in fish fed higher dietary lipid levels (Diet2 and Diet3) but there was no statistical differences between Diet2 and Diet3 groups. These results suggest that high dietary lipid levels (8.29-13.59%) enhance growth performance of black seabream, as shown in other species, such as large yellow croaker (*Larimichthys crocea*) (Ai et al., 2008), coiba (*Rachycentron canadum*) (Wang et al., 2005) and white seabass (*Atractoscion nobilis*) (López et al., 2009). In this study, lower dietary lipid level suppressed growth performance. This might be attributed to essential fatty acid deficiency in the diet as reported in our previous study where optimal dietary n-3 HUFA could improve growth performance of black seabream (Jin et al., 2017a). Regarding feed utilization, fish fed the highest dietary lipid level (Diet3) showed significantly higher values of FE and PER than the other experimental groups in the present study. This suggests that dietary lipids can be utilized as an energy source by black seabream. Such protein sparing effects have been also demonstrated in many fish species fed diets containing lipid as a major energy source (López et al., 2009). In terms of growth performance and feed utilization, this study shows that black seabream can adapt to high dietary lipid level up to 136g/kg dry diet.

Lipid content in both whole body and muscle were significantly higher in Diet3 group. This is in agreement with some previous studies of marine fish (Wang et al., 2005; Yan et al., 2015). Likewise, the highest values of HSI, VSI and IPF were all observed in fish fed Diet3, which corresponds with cobia, *Rachycentron canadum* (Wang et al., 2005). In the present study, the lipid content was significantly higher than other treatments. Moreover, with the increasing of muscle lipid levels, the meat became more tender (Chen et al., 2012). Hence, we speculate that the tenderness of meat in black seabream could be improved by a high fat diet, but further studies are required. In the present study we confirmed that in tissues of black seabream, such as liver and muscle, fat deposition varies. Increased dietary lipid levels led to increased CHO, HDL, and LDL content in serum. Similar results were found in grass carp (*Ctenopharyngodon idellus*) and large yellow croaker (Li et al., 2016; Yan et al., 2015).

Lipids are an important structural and functional constituent of cells in biological systems, but lipid oxidation negatively affects the integrity of biological systems. Malondialdehyde (MDA), derived from the oxidation of fatty acids bearing more than two

methylene interrupted double bonds (Esterbauer et al., 1990), is an important metabolite derived from lipid peroxidation (Zuo et al., 2013). In this current study, the MDA content in the serum of fish fed Diet3 was significantly higher than the Diet1 group. Generally, high dietary lipid levels rather than low levels, tend to cause lipid peroxidation, since lipid peroxidation is caused by free radicals leading to oxidative destruction of PUFA constitutive of cellular membranes (Levent et al., 2006). Whereas, the hepatic MDA content did not show any statistical differences among all treatments, this result suggests that fish fed dietary lipid levels up to 136g/kg dry diet could not induce tissue peroxidation damage. On the other hand, to protect cells and tissues from oxidative damage, fish have endogenous antioxidant defense systems to help counteract the activity of free radicals (Jin et al., 2017b). SOD, GSH-Px and CAT are regarded as major antioxidant enzymes, since they have been shown to protect cells against lipid peroxidation (Kanter et al., 2004). In the present study, CAT activity increased with increasing dietary lipid levels, possibly responding to the high MDA concentration in the high dietary lipid level group, and consequently against lipid peroxidation in organisms.

To investigate the mechanism of lipid metabolism regulated by dietary lipid levels in black seabream, we analyzed the activity of several key hepatic enzymes such as Cpt1 and Fas. Previous study has reported the key pathway in lipogenesis is catalyzed by the cytosolic FAS, which has been characterized in fish (NRC, 2011). In addition, the primary products of FAS are the saturated fatty acids (SFA) 16:0 (palmitic acid) and 18:0 (stearic acid), which can be biosynthesized *de novo* by all known organisms, including fish (Tocher, 2003). In this current study, hepatic FAS activity was significantly lower in fish fed Diet3 when compared to other groups. Similarly, the lowest values of 18:0 and 16:0 of tissues were all obtained in Diet3 group, which supported by previous studies (Tocher, 2003). These results showed that high dietary lipid levels could suppress the FAS activity to relieve excessive fat deposition in tissues of black seabream, since excessive fat deposition status have already been detected both in whole body and muscle in Diet3 in this study. Overall, this study indicated that high dietary lipid levels could significantly reduce the hepatic FAS activity and then decrease lipogenesis accordingly in black seabream. Furthermore, this might be an adaptation strategy to high dietary lipid levels in black seabream.

Mitochondrial fatty acid β -oxidation is the prime pathway for the degradation of fatty acids. Furthermore, mitochondrial β -oxidation is a key metabolic pathway for energy homeostasis in various organs such as liver, heart and muscle (Houten & Wanders, 2010). Lipid accumulation was found to occur because excess lipids that were consumed could not be oxidized (Lu et al., 2014). Cpt1 is the main regulatory enzyme in fatty acid β -oxidation and it can be inhibited by reducing the activity of Cpt1 (Lu et al., 2014; Yan et al., 2015; Zheng et al., 2013). To explore whether dietary lipid levels could alter the lipid accumulation by regulating Cpt1 activity, we analyzed Cpt1 activity. Results showed that hepatic Cpt1 activity did not show any statistical differences among all treatments. Similar results were obtained in other studies such as grass carp, large yellow croaker (Li et al., 2016; Yan et al., 2015).

Conclusion

In conclusion, the present study suggests that black seabream can obtain better growth performance and feed utilization with dietary lipid levels ranging from 8.3 ~ 13.6%. The results of this study indicate that dietary lipid levels impact tissue fatty acid profiles, antioxidant capacity, hematological characteristics and relative expression of lipid related genes in juvenile black seabream. Moreover, the study demonstrated that in order to decrease high fat deposition caused by high dietary lipid level, juvenile black seabream has coping strategies by decreasing lipogenesis and increasing lipolysis and mitochondrial fatty acid β -oxidation. further studies are needed.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant Nos. 31802303, 31272670 and 41476125), Key Research and Development Plan Project of Zhejiang Province (2018C02037), Zhejiang Aquaculture Nutrition & Feed Technology Service Team (ZJANFTST2017-2), Key Research Program of Zhejiang Province of China (No. 2017C02G1460834), Major Spark Plan Project of the National Ministry of Science and Technology (2014GA701001), the Open Fund of Zhejiang Provincial Top Key Discipline of Aquaculture in Ningbo University, K. C. Wong Magna Fund and K. C. Wong Education Foundation at Ningbo University. We would like to thank Li, Y. and Hou, Y. M., for their valuable help during feeding trial and sample analysis.

References

- Ai, Q. H., Zhao, J. Z., Mai, K. S., Xu, W., Tan, B. P., Ma, H. M., & Z. G. Liufu**, 2008. Optimal dietary lipid level for large yellow croaker (*Pseudosciaena crocea*) larvae. *Aquacult. Nutr.*, 14:515–522.
- AOAC**, 2006. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists. Arlington: VA.
- Chen, W. X., Liu, Q. Zh., & Z. Y. Fan**, 2012. Recent Advances in Research on Meat Quality Evaluation and Influencing Factor of Fish. *Meat Research.*, 26:234-40. (in Chinese, with English abstract).
- Esterbauer, H., Schaur, R.J.R, & Zollner, H**, 1990. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Bio Me.*, 11: 81-128.
- Houten, S. M., & R. J. Wanders**, 2010. A general introduction to the biochemistry of mitochondrial fatty acid β -oxidation. *J. Inherit. Metab. Dis.*, 33:469–477.
- Jin, M., Lu, Y., Yuan, Y., Li, Y., Qiu, H., Sun, P., ... & Q. C. Zhou**, 2017a. Regulation of growth, antioxidant capacity, fatty acid profiles, hematological characteristics and expression of lipid related genes by different dietary n-3 highly unsaturated fatty acids in juvenile black seabream (*Acanthopagrus schlegelii*). *Aquaculture*, 471:55–65.
- Jin, M., Monroig, Ó., Lu, Y., Yuan, Y., Li, Y., Ding, L., ... & Q. C. Zhou**, 2017b. Dietary DHA/EPA ratio affected tissue fatty acid profiles, antioxidant capacity, hematological characteristics and expression of lipid-related genes but not growth in juvenile black seabream (*Acanthopagrus schlegelii*). *PloS one*, 12:e0176216.
- Kanter, M., Coskun, O., Korkmaz, A., & S. Oter**, 2004. Effects of Nigella sativa on oxidative stress and β - cell damage in streptozotocin - induced diabetic rats. *Anat. Rec.*, 279:685–691.
- Levent, G., Ali, A., Ahmet, A., Polat, E.C., Aytaç, Ç., Ayşe, E., & S. Ahmet**, 2006. Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. *J. Transl. Med.*, 4:25.
- Li, A., Yuan, X., Liang, X. F., Liu, L., Li, J., Li, B., ... & J. Wang**, 2016. Adaptations of lipid metabolism and food intake in response to low and high fat diets in juvenile grass carp (*Ctenopharyngodon idellus*). *Aquaculture*, 457:43–49.
- López, L. M., Durazo, E., Viana, M. T., Drawbridge, M., & D. P. Bureau**, 2009. Effect of dietary lipid levels on performance, body composition and fatty acid profile of juvenile white seabass, *Atractoscion nobilis*. *Aquaculture*, 289:101–105.
- Lu, K. L., Xu, W. N., Wang, L. N., Zhang, D. D., Zhang, C. N., & W. B. Liu**, 2014. Hepatic β -oxidation and regulation of carnitine palmitoyltransferase (CPT) I in blunt snout bream *Megalobrama amblycephala* fed a high fat diet. *PloS one*, 9:e93135.
- Ma, J., Shao, Q., Xu, Z., & F. Zhou**, 2013. Effect of Dietary n-3 highly unsaturated fatty acids on Growth, Body Composition and Fatty Acid Profiles of Juvenile Black Seabream, *Acanthopagrus schlegelii* (Bleeker). *J. World Aquacult. Soc.*, 44:311–325.
- National Research Council (NRC)**, 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC, pp. 102–125.
- Tocher, D. R.**, 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.*, 11:107–184.

- Wang, J. T., Liu, Y. J., Tian, L. X., Mai, K. S., Du, Z. Y., Wang, Y., & H. J. Yang,** 2005. Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). *Aquaculture*, 249:439–447.
- Yang, J. H.,** 2010. Perfluorooctanoic acid induces peroxisomal fatty acid oxidation and cytokine expression in the liver of male Japanese medaka (*Oryzias latipes*). *Chemosphere*, 81:548–552.
- Yan, J., Liao, K., Wang, T., Mai, K., Xu, W., & Q. Ai,** 2015. Dietary lipid levels influence lipid deposition in the liver of large yellow croaker (*Larimichthys crocea*) by regulating lipoprotein receptors, fatty acid uptake and triacylglycerol synthesis and catabolism at the transcriptional level. *PLoS one*, 10:e0129937.
- Zheng, J. L., Luo, Z., Zhu, Q. L., Tan, X. Y., Chen, Q. L., Sun, L. D., & W. Hu,** 2013. Molecular cloning and expression pattern of 11 genes involved in lipid metabolism in yellow catfish *Pelteobagrus fulvidraco*. *Gene*, 531:53–63.
- Zhou, F., Song, W. X., Shao, Q. J., Peng, X., Xiao, J. X., Hua, Y., ... & W. K. Ng,** 2011. Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, *Acanthopagrus schlegelii*, juveniles. *J. World. Aquacult. Soc.*, 42:184–197.
- Zuo, R. T., Ai, Q. H., Mai, K. S., & W. Xu,** 2013. Effects of conjugated linoleic acid on growth, non-specific immunity, antioxidant capacity, lipid deposition and related gene expression in juvenile large yellow croaker (*Larimichthys crocea*) fed soyabean oil-based diets. *Br. J. Nutr.*, 110: 1220-1232.