

Effects of Dietary Nutrient on the Biological Index and Serum Chemistry of Juvenile Olive Flounder *Paralichthys olivaceus* Achieving Compensatory Growth

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Abstract

Effects of dietary nutrient content on the biological index and serum chemistry of olive flounder *Paralichthys olivaceus* achieving compensatory growth were investigated. Six treatments were prepared in triplicate. Fish were hand-fed with the control (C) diet twice daily for 8 weeks (8W-C) or fish were starved for 2 weeks and then hand-fed with the C, high protein (HP), high carbohydrate (HC), high lipid (HL), or intermediate protein, carbohydrate and lipid (IPCL) diets for 6 weeks, referred to as 6W-C, 6W-HP, 6W-HC, 6W-HL, and 6W-IPCL, respectively. Weight gain of fish in the 8W-C, 6W-HP, and 6W-IPCL treatments was higher than that of fish in the 6W-C treatment. Condition factor (CF) of the fish in the 6W-HP, 6W-HC and 6W-IPCL treatments was higher than that of fish in the 8W-C and 6W-C treatments. The hepatosomatic index (HSI) of fish in the 6W-HC, 6W-HL and 6W-IPCL treatments was higher than that of the fish in the 8W-C, 6W-C and 6W-HP treatments. Serum chemistry except triiodothyronine (T₃) was not significantly different among the treatments. In conclusion, CF and HSI of the fish could be indices reflecting compensatory growth, whereas T₃ seemed to play a partial role in achieving compensatory growth.

Key words: Olive flounder, *Paralichthys olivaceus*, Compensatory growth, Serum chemistry, T₃ (Triiodothyronine)

Introduction

Juvenile olive flounder *Paralichthys olivaceus* achieved full compensatory growth when they were fed daily for 6 weeks after a 2-week feed deprivation period in the earlier studies (Cho, 2005; Cho et al., 2006). Therefore, applying a feeding strategy to achieve full compensatory growth is efficacious in fish farming, particularly when fish should be starved under unfavorable conditions such as a coldwater mass, red tide or handling stress resulting from grading, transporting, and medicating. Compensatory growth has been reported in several fish species including coldwater fish (Jobling, 1988; Riley et al., 1993; Nanton et al., 2003), temperate water fish (Cho, 2005; Cho et al., 2006; Oh et al., 2008; Cho and Heo, 2011), and tropical water fish (Xie et al., 2001; Tian and Qin,

2004).

Biological indices of fish such as the hepatosomatic index (HSI) and condition factor (CF) have been used to identify compensatory growth. HSI is a good compensatory growth index in rainbow trout *Oncorhynchus mykiss*, channel catfish *Ictalurus punctatus*) and olive flounder (Farbridge and Leatherland, 1992; Gaylord and Gatlin, 2000; Cho, 2005). Furthermore, Bavevi et al. (2010) proposed that body length (or some other measure that incorporates length, such as condition) should always be assessed when characterizing the compensatory growth of gilthead sea bream *Sparus aurata*.

A relationship between hormonal changes in fish after fasting or refeeding has been reported (Eales et al., 1992; MacK-

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enzie et al., 1998; Gaylord and Gatlin, 2000). Additionally, MacKenzie et al. (1993) and Gaylord et al. (2001) reported that increase of plasma thyroid hormone is associated with an increase in growth rate of red drum *Sciaenops ocellatus* and channel catfish that achieved compensatory growth. Because the quality and/or quantity of the diet largely affect the proximate composition of fish, manipulation of the diet could affect thyroid hormones of fish undergoing compensatory growth.

In the present we investigated the effects of dietary nutrients on the biological indices and serum chemistry of olive flounder achieving compensatory growth.

Materials and Methods

Fish and experimental conditions

Juvenile olive flounder were purchased from a private hatchery and transferred to the laboratory. Before initiation of the feeding trial, the fish were acclimated to the experimental conditions for 2 weeks. In total, 450 fish averaging 16.0 g were randomly chosen and distributed into 18 180-L flow-through tanks (25 fish/tank). The flow rate in each tank was 6.5 L/min, and the water temperature was 16.0-25.5°C.

Preparation of the experimental diets

Five experimental diets were prepared: control (C), high protein (HP), high carbohydrate (HC), high lipid (HL), and intermediate protein, carbohydrate and lipid (IPCL) diets. The ingredient and nutrient composition of the diets are given in Table 1. Crude protein (48.6%) and lipid (7.3%) levels in the C diet satisfied the requirements for juvenile olive flounder (Lee et al., 2000, 2002). Protein, carbohydrate (nitrogen-free

extract), and lipid levels in the HP, HC, and HL diets were increased to 56.3%, 31.6% and 12.4%, respectively, at the expense of cellulose and/or wheat flour. Finally, protein and lipid levels of the IPCL diet were increased to 53.1% and 9.2%, respectively, at the expense of cellulose and wheat flour.

Experimental design

Six treatments were prepared in triplicate. Fish were hand-fed with the C diet to apparent satiation twice daily (09:30 and 17:00), 6 days per week, for 8 weeks (8W-C), or fish were starved for 2 weeks and then hand-fed with the C, HP, HC, HL, or IPCL diets to apparent satiation twice per day for 6 weeks, referred to as 6W-C, 6W-HP, 6W-HC, 6W-HL, and 6W-IPCL, respectively.

Analysis of biological indices and fish serum

Total length and body weight were measured in five randomly chosen fish from each tank at the end of the 8-week trial. Fish were dissected to measure liver weight to determine the biological indices: condition factor (CF) = fish weight (g)/total length (cm)³, and hepatosomatic index (HSI) = liver weight (g) × 100/fish weight (g).

Blood samples were obtained from the caudal vein of 5 randomly chosen 24-h starved fish from each tank by syringes. Serum was collected after centrifugation (3,000 rpm for 10 min) and stored at -70°C in separate aliquots to analyze total protein, glucose, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and triglyceride (TG) using automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II Chemistry System; Johnson and Johnson Clinical Diagnostics Inc., New York, NY, USA). Additionally, triiodothyronine (T₃) and thyroxine (T₄) hormones were also analyzed by radioimmunoassay and a gamma counter (Cobra II; Packard, Dallas, TX, USA).

Statistical analysis

A one-way analysis of variance and Duncan's multiple range test (Duncan, 1955) were performed to analyze differences among the means of treatments using SAS program version 9.1 (SAS Institute, Cary, NC, USA). A $P < 0.05$ was considered significant.

Results and Discussion

Weight gain (%) of olive flounder in the 8W-C, 6W-HP and 6W-IPCL treatments was significantly ($P < 0.05$) higher than that of fish in the 6W-C treatment, but not significantly ($P > 0.05$) different from that of fish in the 6W-HC and 6W-HL treatments (Table 2). Poorer growth of olive flounder in the 6W-C treatment compared to that of fish in the 8W-C treat-

Table 1. Ingredients and nutrient composition of the experimental diets

	Experimental diets				
	C	HP	HC	HL	IPCL
Ingredients (%)					
Fishmeal	60	74	60	60	68
Cellulose	10		2	6.3	
α -Starch	5	5	13	5	9
Wheat flour	19.3	16.6	19.3	19.3	15.3
Squid liver oil	2	0.7	2	2	2
Soybean oil				3.7	2
Others	3.7	3.7	3.7	3.7	3.7
Nutrient (% DM basis)					
Crude protein	48.6	56.3	48.1	48.1	53.1
Crude lipid	7.3	7.3	7.4	12.4	9.2
Carbohydrate	34.1	24.3	34.6	29.6	26.7
Ash	10.0	12.1	9.8	9.9	11.0

C, control; HP, high protein; HC, high carbohydrate; HL, high lipid; IPCL, intermediate protein, carbohydrate and lipid.

ment contradicted results from earlier studies (Cho, 2005; Cho et al., 2006).

However, no significant difference in weight gain of all fish groups experiencing 2-week feed deprivation (6W-HP, 6W-HC, 6W-HL, and 6W-IPCL treatments), except for fish in the 6W-C treatment compared to that of fish in the 8W-C treatment indicated that supplementing diets with nutrient such as protein, carbohydrate, and lipids and their combination resulted in compensatory growth. Moreover, a slight overcompensation of fish in the 6W-HP and 6W-IPCL treatments was observed suggesting that supplementing diets with protein and a combination of protein, carbohydrate, and lipids was effective to accelerate compensatory growth. Similarly, Cho and Heo (2011) showed that a combined HP (54.8%) and HL (14.0%) diet achieved overcompensation in juvenile olive flounder subjected to 1-week feed deprivation. Additionally, Gaylord and Gatlin (2001) showed that compensatory growth of channel catfish is primarily affected by dietary protein level rather than dietary energy level.

The CF of olive flounder in the 6W-HP, 6W-HC, and 6W-IPCL treatments was significantly ($P < 0.05$) higher than that

of fish in the 8W-C and 6W-C treatments, but not significantly ($P > 0.05$) different from that of fish in the 6W-HL treatment. This result suggests that CF could be a compensatory growth index and that fish fed the HP, HC, and IPCL diets after a 2-week food deprivation became fatter when compensatory growth was achieved. Similarly, Bavevi et al. (2010) demonstrated that gilthead sea bream *Sparus aurata* did not compensate length, but showed increased in condition. Those authors proposed that length (or some other measure that incorporates length, such as condition) should always be analyzed when characterizing compensatory growth.

The HSI of olive flounder in the 6W-HC, 6W-HL and 6W-IPCL treatments was significantly ($P < 0.05$) higher than that of fish in the 8W-C, 6W-C and 6W-HP treatments. In addition, the HSI of fish in the 6W-HP treatment was significantly ($P < 0.05$) higher than that of fish in the 8W-C and 6W-C treatments. The higher HSI of fish in the 6W-HP, 6W-HC, 6W-HL and 6W-IPCL treatments compared to that of fish in the 6W-C and 8W-C treatments indicated that HSI could be a compensatory growth index. Similarly, a high HSI is observed in fish that achieve compensatory growth (Farbridge and Leatherland, 1992; Gaylord and Gatlin, 2000; Cho, 2005).

Another reason for the high HSI in the 6W-HP, 6W-HC, 6W-HL, and 6W-IPCL treatments was probably due to the higher energy content of the diets (Table 1). This agreed with other studies showing that fish receiving higher energy diets have a higher HSI (Jobling, 1988; Nanton et al., 2003; Kjær et al., 2009). Kjær et al. (2009) demonstrated that a higher HSI in Atlantic cod (*Gadus morhua*) fed a high-fat diet was primarily due to an increase in liver cell size when fish were fed either a high-fat (30.5%) or low-fat (11.4%) diet for 112 days and then starved for 3 weeks.

Total protein, glucose, GOT, GPT, and T_4 were not significantly different among the treatments (Table 3). However, TG of olive flounder in the 6W-HL treatment was significantly ($P < 0.05$) higher than that of fish in all other treatments. Additionally, TG of fish in the 6W-IPCL treatment was significantly ($P < 0.05$) higher than that of fish in the 8W-C, but not significantly ($P < 0.05$) different from that of fish in the 6W-

Table 2. Weight gain (%) and biological index of olive flounder *Paralichthys olivaceus* fed the experimental diets containing various nutrient contents after 2-week feed deprivation

Treatments	Weight gain(%)	CF*	HSI†
8W-C	207.2 ± 29.43 ^a	0.93 ± 0.012 ^b	1.58 ± 0.069 ^e
6W-C	159.7 ± 14.38 ^b	0.93 ± 0.011 ^b	1.63 ± 0.021 ^e
6W-HP	238.3 ± 7.85 ^a	0.99 ± 0.023 ^a	1.96 ± 0.020 ^b
6W-HC	196.6 ± 5.60 ^{ab}	0.98 ± 0.009 ^a	2.50 ± 0.076 ^a
6W-HL	192.2 ± 9.67 ^{ab}	0.96 ± 0.010 ^{ab}	2.55 ± 0.022 ^a
6W-IPCL	213.4 ± 2.06 ^a	0.99 ± 0.003 ^a	2.64 ± 0.225 ^a

Values (mean of triplicate ± SE) in the same column with a same superscript are not significantly different ($P > 0.05$).

C, control; HP, high protein; HC, high carbohydrate; HL, high lipid; IPCL, intermediate protein, carbohydrate and lipid.

*CF (condition factor) = Fish weight (g)/total length (cm)³, †HSI (Hepatosomatic index) = Liver weight (g) × 100/fish weight (g).

Table 3. Serum chemistry of olive flounder *Paralichthys olivaceus* at the end of the 8-week trial

Treatments	Total protein (g/dL)	Glucose (mg/dL)	GOT (IU/L)	GPT (IU/L)	TG (mg/dL)	T_3 (ng/dL)	T_4 (µg/dL)
8W-C	2.5 ± 0.03	294 ± 23.8	15.7 ± 2.91	4.3 ± 1.86	57 ± 5.5 ^d	189 ± 15.1 ^b	2.3 ± 0.10
6W-C	2.6 ± 0.07	289 ± 11.9	12.0 ± 1.00	2.7 ± 0.33	59 ± 2.3 ^{cd}	198 ± 16.0 ^b	2.5 ± 0.74
6W-HP	2.9 ± 0.07	285 ± 13.2	18.0 ± 5.51	2.7 ± 0.33	93 ± 14.0 ^{bcd}	275 ± 27.2 ^{ab}	2.1 ± 0.29
6W-HC	3.0 ± 0.27	256 ± 26.1	19.3 ± 2.19	3.0 ± 0.00	98 ± 9.8 ^{bc}	306 ± 67.2 ^{ab}	3.6 ± 1.88
6W-HL	3.0 ± 0.12	294 ± 18.9	19.5 ± 2.33	3.0 ± 0.33	163 ± 19.7 ^a	405 ± 37.4 ^a	2.0 ± 0.69
6W-IPCL	3.0 ± 0.15	298 ± 3.0	19.7 ± 0.50	3.0 ± 0.00	108 ± 1.0 ^b	380 ± 46.1 ^a	1.7 ± 0.31

Values (mean of triplicate ± SE) in the same column with a same superscript are not significantly different ($P > 0.05$).

GOT, glutamic oxaloacetic transaminase, GPT, glutamic pyruvic transaminase, TG, triglyceride; C, control; HP, high protein; HC, high carbohydrate; HL, high lipid; IPCL, intermediate protein, carbohydrate and lipid.

HP and 6W-HC treatments. The high TG in fish in the 6W-HL and 6W-IPCL treatments probably resulted from the HL (energy) content in the diets, which agreed with Lee and Kim (2005) who showed that increased dietary lipid levels increase serum TG and total cholesterol in fish. Additionally, Kjær et al. (2009) reported that increased dietary lipid levels increase serum triacylglycerol levels in Atlantic cod.

The T_3 levels of olive flounder in the 6W-HL and 6W-IPCL treatments were significantly ($P < 0.05$) higher than those of fish in the 8W-C and 6W-C treatments but not significantly different from those of fish in the 6W-HP and 6W-HC treatments. This result partially agreed with Riley et al. (1993) and MacKenzie et al. (1998) who showed that diet quality, particularly dietary protein levels largely affect thyroid hormone levels in fish, which seem to play a major role in achieving compensatory growth (Gaylord et al., 2001; Cho, 2009).

The results of this study demonstrated that CF and HSI of fish could be compensatory growth indices and that T_3 seemed to play a partial role in achieving compensatory growth in olive flounder.

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References

- Bavčević L, Klanjšček T, Karamarko V, Aničić I and Legović T. 2010. Compensatory growth in gilthead sea bream (*Sparus aurata*) compensates weight, but not length. *Aquaculture* 301, 57-63.
- Cho SH. 2005. Compensatory growth of juvenile flounder *Paralichthys olivaceus* L. and changes in biochemical composition and body condition indices during starvation and after refeeding in the winter season. *J World Aquac Soc* 36, 508-514.
- Cho SH. 2009. Effect of fasting and refeeding on growth and blood chemistry in juvenile olive flounder *Paralichthys olivaceus* L. *J Aquaculture* 22, 11-15.
- Cho SH and Heo TY. 2011. Effect of dietary nutrient composition on compensatory growth of juvenile olive flounder *Paralichthys olivaceus* using different feeding regimes. *Aquacult Nutr* 17, 90-97.
- Cho SH, Lee S, Park BH, Ji S, Lee J, Bae J and Oh S. 2006. Compensatory growth of juvenile olive flounder *Paralichthys olivaceus* L. and changes in proximate composition and body condition indexes during fasting and after refeeding in summer season. *J World Aquac Soc* 37, 1-7.
- Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics* 11, 1-42.
- Eales JG, MacLachy DL, Higgs DA and Dosanjh BS. 1992. The influence of dietary protein and caloric content on thyroid function and hepatic thyroxine 5'-monodeiodinase activity in rainbow trout, *Oncorhynchus mykiss*. *Can J Zool* 70, 1526-1535.
- Farbridge KJ and Leatherland JF. 1992. Temporal changes in plasma thyroid hormone, growth hormone and free fatty acid concentrations, and hepatic 5'-monodeiodinase activity, lipid and protein content during chronic fasting and re-feeding in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol Biochem* 10, 245-257.
- Gaylord TG and Gatlin DM. 2000. Assessment of compensatory growth in channel catfish *Ictalurus punctatus* R. and associated changes in body condition indices. *J World Aquac Soc* 31, 326-336.
- Gaylord TG and Gatlin DM. 2001. Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). *Aquaculture* 194, 337-348.
- Gaylord TG, MacKenzie DS and Gatlin DM. 2001. Growth performance, body composition and plasma thyroid hormone status of channel catfish (*Ictalurus punctatus*) in response to short-term feed deprivation and refeeding. *Fish Physiol Biochem* 24, 73-79.
- Jobling M. 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquaculture* 70, 1-19.
- Kjær MA, Vegusdal A, Berge GM, Galloway TF, Hillestad M, Krogdahl A, Holm H and Ruyter B. 2009. Characterisation of lipid transport in Atlantic cod (*Gadus morhua*) when fasted and fed high or low fat diets. *Aquaculture* 288, 325-336.
- Lee S and Kim KD. 2005. Effect of various levels of lipid exchanged with dextrin at different protein level in diet on growth and body composition of juvenile flounder *Paralichthys olivaceus*. *Aquac Nutr* 11, 435-442.
- Lee SM, Cho SH and Kim KD. 2000. Effects of dietary protein and energy levels on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). *J World Aquac Soc* 31, 306-315.
- Lee SM, Park CS and Bang IC. 2002. Dietary protein requirement of young Japanese flounder *Paralichthys olivaceus* fed isocaloric diets. *Fish Sci* 68, 158-164.
- MacKenzie DS, Moon HY, Gatlin DM and Perez LR. 1993. Dietary effects on thyroid hormones in the red drum, *Sciaenops ocellatus*. *Fish Physiol Biochem* 11, 329-335.
- MacKenzie DS, VanPutte CM and Leiner KA. 1998. Nutrient regulation of endocrine function in fish. *Aquaculture* 161, 3-25.
- Nanton DA, Lall SP, Ross NW and McNiven MA. 2003. Effect of dietary lipid level on fatty acid [beta]-oxidation and lipid composition in various tissues of haddock, *Melanogrammus aeglefinus* L. *Comp Biochem Physiol B* 135, 95-108.
- Oh SY, Noh CH, Kang RS, Kim CK, Cho SH and Jo JY. 2008. Compensatory growth and body composition of juvenile black rockfish *Sebastes schlegeli* following feed deprivation. *Fish Sci* 74, 846-852.
- Riley WW Jr, Higgs DA, Dosanjh BS and Eales JG. 1993. Influence of dietary amino acid composition on thyroid function of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 112, 253-269.
- Tian X and Qin JG. 2004. Effects of previous ration restriction on compensatory growth in barramundi *Lates calcarifer*. *Aquaculture* 235, 273-283.
- Xie S, Zhu X, Cui Y, Wootton RJ, Lei W and Yang Y. 2001. Compensatory growth of the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. *J Fish Biol* 58, 999-1009.