

# Effects of Dietary Protein and Lipid Levels on Growth, Feed Utilization and Body Composition of Adult Starry Flounder (Platichthys stellatus)

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A 25-week feeding trial of two dietary protein (47 and 52%) and three dietary lipid level (7, 12 and 17%) factorial design with three replications were conducted to determine effects of dietary protein and lipid levels on growth, feed utilization and body composition of adult starry flounder (*Platichthys stellatus*), average initial weight 332 g, during the winter season. Survival of fish was not affected by either dietary protein or dietary lipid level. Weight gain, feed efficiency and protein efficiency ratio improved with dietary protein and lipid levels except for those of fish fed the 52% protein diet with 17% lipid. The best growth and feed utilization were observed in the 52% protein diet with 12% lipid, but were not significantly different from those of fish fed the 52% protein diet with 17% lipid or the 47% protein diets with 17% lipid levels. Hepatosomatic and visceralsomatic indexes were significantly influenced by dietary protein level, but not by dietary lipid level. None of moisture, crude protein, crude lipid, or glycogen contents of dorsal muscle or liver in starry flounder except for crude lipid in dorsal muscle was significantly influenced by either dietary protein or dietary lipid level. Plasma cholesterol concentration was significantly influenced by both dietary protein and dietary lipid levels. The results of this study suggest that the diets containing 47% protein with 17% lipid or 52% protein with 12-17% lipid are optimal for growth and feed utilization of adult starry flounder under these experimental conditions.

Key words; Starry flounder, Platichthys stellatus, Dietary protein, Lipid

#### Introduction

Dietary protein content is the most important factor affecting growth performance of fish and feed cost (NRC, 1993). Generally, an increase of protein level in diet improves fish production, especially for carnivorous fish, but proportionally increases feed cost. Protein utilization for growth of fish can be improved by partially replacing protein with lipid and/or carbohydrate in diet. However, excessive energy in diet can lead to increase body lipid deposition and reduce growth of fish due to lacking of necessary nutrients for growth resulted from reduction in feed consumption by fish (Daniels and Robinson, 1986). On the other hand, insufficient energy in diet can lead

to waste protein because dietary protein is utilized for energy source and ammonia excreted deteriorates water quality, and eventually elevates fish production cost (Shyong et al., 1998). Therefore, it is important to supply proper protein and energy levels in feed for body protein synthesis of fish. A protein-sparing effect associated with increasing dietary energy level in terms of supplementation of lipid and carbohydrate source into feed has been reported for several species of fish (Cho and Kaushik, 1990; Vergara et al., 1996; Company et al., 1999; Harpaz et al., 1999; Lee et al., 2002a). However, supplementation of lipid rather than carbohydrate as an energy source is generally more effective method to increase dietary energy level because lipid is an energy-dense nutrient and readily metabolized by fish, especially by most carnivorous

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fish comparing to omnivorous or herbivorous fish (NRC, 1993).

Starry flounder *Platichthys stellatus* is known to be adapted for wide ranges of temperature and salinity, and highly resistive against disease. Therefore, starry flounder has high potential as a candidate for aquaculture and is recently receiving much attention in Korea, and its seedling production technique has been developed. Nutritional studies have been conducted on dietary essential fatty acids requirement (Lee et al., 2003a) and availability of dietary carbohydrate sources (Lee and Lee, 2004) for starry flounder. The present study determined the effects of dietary protein and lipid (energy) levels on growth, feed utilization and body composition of sub-adult starry flounder during low water temperature season.

#### Materials and Methods

A 2×3 factorial experimental design with 3 replicates was used in this study. Six experimental diets were formulated to contain 2 levels of protein (47 and 52%) and 3 levels of lipid (7, 12 and 17%). Ingredients and proximate composition of the experimental diets are presented in Table 1. Pollack fish and anchovy meals were used as main protein

source, and dietary protein level proportionally increased with an increase in both ingredients. To determine the effect of various levels of lipid at different protein levels, the amount of pollack fish meal, anchovy meal and squid liver oil in the experimental diets were adjusted mainly at the expense of both alpha-potato starch and cellulose. All ingredients of the experimental diets were mixed with freshwater at the ratio of 6:4 and pressure-pelleted. The experimental diets were stored in freezer at -30 °C until use.

Starry flounder were obtained from Finfish Research Center, National Fisheries Research and Development Institute (Gyungbook, Korea). They were fed a commercial flounder feed (50% crude protein) for two weeks while being acclimated to the experimental conditions. Adult fish (an average body weight: 332±4.8 g) were randomly distributed into each of 18 green circular fiberglass-reinforced plastic tanks (300 L water volume) with 13 fish to each tank. Fish were hand-fed to visual satiety twice daily at 0900 and 1700 for 25 weeks. Weight gain of fish was measured in 8-week interval. Pellet size was adjusted and an appropriately sized pellet was supplied as fish grew. Filtered seawater (34±0.2%, mean±SD)

Table 1. Ingredients and nutrients composition of the experimental diets

		-	Die	ets		Tarina (1
	P <sub>47</sub> L <sub>7</sub>	P <sub>47</sub> L <sub>12</sub>	P <sub>47</sub> L <sub>17</sub>	P <sub>52</sub> L <sub>7</sub>	P <sub>52</sub> L <sub>12</sub>	P <sub>52</sub> L <sub>17</sub>
Ingredients (%)						
Pollack fish meal <sup>1</sup>	23.0	23.0	23.0	26.0	26.0	26.0
Anchovy rneal <sup>2</sup>	42.0	42.0	42.0	46.0	46.0	46.0
Alpha-potato starch	21.0	13.0	5.0	21.0	13.0	5.0
Squid live oil <sup>3</sup>	2.0	7.0	12.0	1.5	6.5	11.5
Vitamin premix <sup>4</sup>	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix <sup>4</sup>	3.0	3.0	3.0	3.0	3.0	3.0
Carboxymethyl cellulose		3.0	6.0		3.0	6.0
$\alpha$ -Cellulose	6.5	6.5	6.5			
Choline salt	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient contents (dry matter basis)						
Crude protein (%)	47.1	46.4	47.0	52.9	53.4	52.0
Crude lipid (%)	7.0	12.5	17.4	7.2	11.9	17.0
Ash (%)	12.5	13.2	15.6	13.6	15.4	16.3
Crude fiber (%) <sup>5</sup>	8.1	8.1	8.1	1.1	1.1	1.1
NFE (%) <sup>6</sup>	25.3	19.8	11.9	25.1	18.1	13.6
Energy (kcal/g) <sup>7</sup>	3.53	3.77	3.92	3.77	3.93	4.15
n-3 HUFA (%) <sup>8</sup>	8.0	1.8	2.9	0.7	1.8	2.8
E/P ratio	7.5	8.1	8.4	7.1	7.4	8.0

¹Produced by steam dry method. ²Imported from Chile. ³Provided by E-wha Oil & Fat Ind. Co., Busan, Korea. ⁴Same as Lee et al. (2003c). ⁵Calculated based on dietary ingredients. ⁶Calculated by the difference (=100-crude protein-crude lipid-ash-crude fiber). ¹Based on 4 kcal/g protein, 9 kcal/g lipid and 4 kcal/g carbohydrate (Cho et al., 1982). ⁵Highly unsaturated fatty acids (C=20).

was supplied at a flow rate of 10 L/min to each tank. Fish were held under natural photoperiod condition and water temperature ranged from 9.2 to 18.4°C (12.3±0.18°C) throughout the feeding trail.

At the end of the feeding trial, blood were sampled from the caudal vein of 3 fish in each tank by using heparinized syringe after fish were starved for 36 h and anesthetized with MS222 at the concentration of 100 ppm. Blood sample was centrifuged at 7,500 rpm for 5 min, and plasma was separated and stored in freezer at -75 °C. Before being frozen, plasma was divided into the separate aliquots for analysis of protein, glucose, cholesterol and triglyceride concentrations. For chemical analysis, 10 fish at the initiation and 5 fish from each tank at the termination of the feeding trial were sacrificed and stored in freezer at -30 °C.

Chemical composition of the experimental diets and fish carcass were determined by AOAC (1990) method: dry matter by drying in an oven at 105°C for 24 h, crude protein (N×6.25) by the Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland), crude lipid by ether extraction after acid hydrolysis, ash by incineration in a muffle furnace at 550°C for 6 h, and crude fiber by Fibertec automatic analyzer (Tecator, Hoganas, Sweden). Liver glycogen was measured by enzymatic method using amyloglucosidase (Fluka, EC 3.2.1.3) as described by Murat and Serfaty (1974). Lipid was extracted by the method of Folch et al., (1957) and fatty acid methyl esters were prepared by transesterification with 14% BF<sub>3</sub>-MeOH (Sigma, USA). Fatty acid methyl esters were analyzed by using a gas chromatography (HP-5890 II; Hewlett-Packard,

Palo Alto, USA) with a flame ionization detector, equipped with capillary column (HP-INNOWax; 30 m×0.32 mm×0.5  $\mu$ m, USA). Injector and detector temperatures were 250 and 270 °C, respectively. The column temperature was programmed from 170 to 225 °C at a rate of 1 °C/min. Helium was used as the carrier gas. The plasma protein, glucose, cholesterol and triglyceride concentrations were measured using a commercial clinical investigation kits (Wako Pure Chemical Industries, Ltd., Japan).

The data were subjected to One-way and Two-way ANOVA to test the effect of dietary protein and lipid levels on fish performance. If significant (P<0.05) differences were found in one-way ANOVA test, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. All statistical analyses were carried out by using the SPSS program Version 10.0 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

#### Results and Discussion

Survival, weight gain, feed efficiency and protein efficiency ratios of starry flounder fed the experimental diets are presented in Table 2. Survival of fish was above 90% at the end of the feeding trial in all groups and was not significantly (P>0.05) affected by either dietary protein level or dietary lipid level. Weight gain (g/fish) of starry flounder was significantly (P<0.05) affected by both dietary protein and lipid levels. Reduction of weight was observed in starry flounder fed the 47% protein diet with 7% lipid level in any measurement at 4-week intervals throughout the feeding trial. This was resulted from

Table 2. Survival, weight gain, feed efficiency and protein efficiency ratios of starry flounder fed the experimental diets containing various protein and lipid levels

Diets	Initial weight (g/fish)	Survival (%)	Weight gain (g/fish)	Feed efficiency (%) <sup>2</sup>	Protein efficiency ratio <sup>3</sup>
P <sub>47</sub> L <sub>7</sub>	332±1.2	92±7.7 <sup>ns</sup>	-7.9±8.45 <sup>a</sup>	-6.2±5.76 <sup>a</sup>	-0.13±0.123 <sup>a</sup>
P <sub>47</sub> L <sub>12</sub>	331±1.5	100±0.0	61.3±14.71 <sup>b</sup>	48.3±10.04 <sup>bc</sup>	1.04±0.217 <sup>bc</sup>
P <sub>47</sub> L <sub>17</sub>	335±3.7	100±0.0	81.9±9.20 <sup>bc</sup>	64.1±6.60 <sup>cd</sup>	1.36±0.140 <sup>c</sup>
P <sub>52</sub> L <sub>7</sub>	331±3.7	100±0.0	51.9±12.74 <sup>b</sup>	37.1±7.38 <sup>b</sup>	0.70±0.140 <sup>b</sup>
P <sub>52</sub> L <sub>12</sub>	333±3.5	97±2.7	118.3±10.19 <sup>c</sup>	76.9±5.63 <sup>d</sup>	1.44±0.106°
P <sub>52</sub> L <sub>17</sub>	329±1.8	92±7.7	89.5±19.26 <sup>bc</sup>	54.1±9.05 <sup>bcd</sup>	1.04±0.174 <sup>bc</sup>
Two-way ANOVA					
Dietary protein (DP)		P<0.9	P<0.002	P<0.01	P<0.03
Dietary lipid (DL)		P<0.9	P<0.001	P<0.001	P<0.001
DP×DL		P<0.3	P<0.001	P<0.01	P<0.01

<sup>&</sup>lt;sup>1</sup>Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05). <sup>2</sup>Body wet weight gain×100/feed intake (dry matter). <sup>3</sup>Body wet weight gain/protein intake. <sup>ns</sup>Not significant (P>0.05).

that essential nutrients in the 47% protein diet with 7% lipid were utilized as energy source instead of growth due to low energy content. Since dietary energy requirement for basal metabolism and maintenance of fish must be satisfied before essential nutrients are able be utilized for growth and reproduction, poor fish performance with a low energy diet was commonly obtained in other fish (Company et al., 1995; McGoogan and Gatlin, 1999). Weight gain of starry flounder improved with increasing dietary protein and lipid levels except for that of fish fed the 52% protein diet with 17% lipid in this study. The best weight gain was observed in starry flounder fed the 52% protein diet with 12% lipid. but was not significantly (P>0.05) different from that of fish fed the 47 and 52% protein diets with 17% lipid. Significant (P<0.001) interaction between the effects of cietary protein and lipid levels on weight gain was observed.

Feed efficiency and protein efficiency ratio were significantly (P<0.05) influenced by both dietary protein and lipid levels. Feed efficiency and protein efficiency ratio improved with dietary protein and lipid levels except for those of fish fed the 52% protein diet with 17% lipid in this study, partially agreeing with other studies showing that feed efficiency and protein efficiency ratio generally improved up to dietary protein or lipid level being optimum (Lee et al., 2000; 2002a). The poorest feed efficiency and protein efficiency ratios were observed in starry flounder fed the 47% protein diet with 7% lipid and the 52% protein diet with 12% lipid produced the highest ones. Significant (P<0.05) interaction of dietary pretein and lipid levels on feed efficiency and protein efficiency ratio was observed in this study.

Based on weight gain, feed efficiency and protein efficiency ratio, the diets containing 47% protein with 17% lipid or 52% protein with 12-17% lipid could be used for adult starry flounder under low water temperature conditions. Dietary protein levels used for starry flounder was similar to dietary protein requirements estimated in other flatfish: about 50% for plaice *Pleuronectes platessa* (Cowey et al., 1972), Atlantic halibut *Hippoglossus hippoglossus* (Helland and Grisdale-Helland, 1998), flounder *Paralichthys olivaceus* (Lee et al., 2000) and young turbot *Scophthalmus maximus* (Lee et al., 2003b).

Improvement in weight gain, feed efficiency and protein efficiency ratio for starry flounder in the 47% protein diet with 12 and 17% lipid compared to 7% lipid or the 52% protein diet with 12% lipid compared to 7% lipid in this study indicated protein-sparing effect of lipid agreeing with other studies (Vergara et al., 1996; Company et al., 1999; Harpaz et al., 1999). However, protein-sparing effect of excessive lipid (17%) in the 52% protein diet was not observed in this study. Also, no beneficial effects of excessive lipid in feed on performance of Atlantic croaker (Micropogonias undulates), Mediterranean yellowtail (Seriola dumerilii) and flounder (P. olivaceus) was observed (Davis and Arnold, 1997; Jover et al., 1999; Lee et al., 2003c). Unlike starry flounder in this study, however, flounder (P. olivaceus) res- ponded better on the lower lipid diet at all tested protein levels (Lee et al., 2000).

Daily feed consumption by starry flounder throughout the feeding trial is given in Table 3. Daily feed intake was significantly (P<0.05) affected by dietary lipid level, but not by dietary protein level. The least daily feed intake was observed in fish fed the 47%

Table 3. Daily feed consumption of starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

Diets	Daily feed intake (%) <sup>2</sup>	Daily lipid intake (%)	Daily protein intake (%)
P <sub>47</sub> L <sub>7</sub>	0.266±0.001°	0.019±0.000 <sup>b</sup>	0.126±0.003°
P <sub>47</sub> l <sub>-12</sub>	0.192±0.002 <sup>a</sup>	0.024±0.000°	$0.090 \pm 0.000^{a}$
P <sub>47</sub> L <sub>17</sub>	0.188±0.001 <sup>a</sup>	0.033±0.000 <sup>e</sup>	$0.088 \pm 0.000^{a}$
$P_{52}L_7$	0.214±0.004 <sup>b</sup>	0.015±0.000 <sup>a</sup>	0.113±0.003 <sup>b</sup>
P <sub>52</sub> '12	0.215±0.001 <sup>b</sup>	$0.026\pm0.000^{d}$	0.114±0.003 <sup>b</sup>
P <sub>52</sub> 17	0.218±0.001 <sup>b</sup>	0.037±0.000 <sup>f</sup>	0.113±0.000 <sup>b</sup>
Two-way ANOVA			
Dietary protein (DP)	P<0.9	P<0.001	P<0.001
Dietary lipid (DL)	P<0.001	P<0.001	P<0.001
DP×DL	P<0.001	P<0.001	P<0.001

<sup>1</sup>Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05). <sup>2</sup>Feed intake (dry matter)×100/[(initial fish wt.+final fish wt.+dead fish wt.)/2×days fed].

protein diets with 12 and 17% lipids. This result was similar to other studies showing that consumption by fish fed low-energy diets was more than that by fish fed high-energy diets (El-Dahhar and Lovell, 1995; Lee et al., 2000; 2002b). However, daily feed intake was not influenced by lipid levels in the 52% protein diets. Significant (P<0.05) interaction of dietary protein and lipid levels on daily feed intake was observed. Daily lipid and protein intakes were significantly (P<0.05) influenced by both dietary protein and lipid levels. Daily lipid intake of starry flounder proportionally increased with dietary lipid level. The lowest daily protein intake was observed in starry flounder fed the 47% protein diet with 17% lipid level, and this was probably resulted from reduction of feed consumption by fish fed the highenergy diet. Significant (P<0.05) interaction of dietary

protein and lipid levels on daily lipid and protein intakes was observed in this study.

Condition factor, hepatosomatic and viscerosomatic indexes of starry flounder at the end of the feeding trial are given in Table 4. Condition factor of fish was not significantly (P>0.05) affected by either dietary protein or dietary lipid level. Hepatosomatic and viscerosomatic indexes of starry flounder were significantly (P<0.05) affected by dietary protein level, but not by dietary lipid level. Similarly, hepatosomatic index of red drum *Sciaenops ocellatus* was significantly affected by dietary protein level, but not by dietary lipid level (McGoogan and Gatlin, 1999). Unlike this study, however, hepatosomatic and viscerosomatic indexes of rockfish were significantly affected by dietary lipid level, but not by dietary protein level (Lee et al., 2002a).

Table 4. Condition factor, hepatosomatic and viscerosomatic indexes of starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

Diets	Condition factor <sup>2</sup>	Hepatosomatic index <sup>3</sup>	Viscerosomatic index <sup>4</sup>
Initial	1.60	0.96	3.77
$P_{47}L_7$	1.34±0.078 <sup>ns</sup>	1.19±0.349 <sup>ns</sup>	3.89±0.383 <sup>ns</sup>
$P_{47}L_{12}$	1.45±0.049	1.59±0.254	3.95±0.238
$P_{47}L_{17}$	1.52±0.050	1.40±0.132	3.62±0.148
$P_{52}L_7$	1.55±0.065	1.88±0.436	4.41±0.393
$P_{52}L_{12}$	1.47±0.058	2.09±0.343	4.41±0.365
P <sub>52</sub> L <sub>17</sub>	1.51±0.083	1.72±0.324	4.45±0.247
Two-way ANOVA			
Dietary protein (DP)	P<0.2	P<0.02	P<0.02
Dietary lipid (DL)	P<0.3	P<0.8	P<0.9
DP×DL	P<0.2	P<0.9	P<0.9

<sup>&</sup>lt;sup>1</sup>Values are mean±SE of three replications. <sup>2</sup>Condition factor=(body weight/total body length<sup>3</sup>)×100. <sup>3</sup>Hepatosomatic index=(liver weight/body weight)×100. <sup>4</sup>Viscerosomatic index=(viscera weight/body weight) ×100. <sup>8</sup>Not significant (P>0.05).

Table 5. Proximate analysis of dorsal muscle of starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)
Initial	78.0	19.4	1.9
$P_{47}L_7$	80.0±2.03 <sup>ns</sup>	17.4±0.63 <sup>ns</sup>	0.05±0.05 <sup>ns</sup>
P <sub>47</sub> L <sub>12</sub>	79.8±0.49	18.8±0.67	0.18±0.13
P <sub>47</sub> L <sub>17</sub>	77.7±0.04	18.7±0.65	0.09±0.09
P <sub>52</sub> L <sub>7</sub>	77.5±0.79	20.2±0.24	0.48±0.12
$P_{52}L_{12}$	77.4±0.47	19.1±0.80	0.73±0.25
P <sub>52</sub> L <sub>17</sub>	80.0±0.77	18.6±0.84	0.34±0.22
Two-way ANOVA			
Dietary protein (DP)	P<0.3	P<0.1	P<0.01
Dietary lipid (DL)	P<0.9	P<0.9	P<0.3
DP×DL	P<0.05	P<0.1	P<0.7

<sup>&</sup>lt;sup>1</sup>Values are mean±SE of three replications. <sup>ns</sup>Not significant (P>0.05).

Proximate composition of dorsal muscle and liver in starry flounder are given in Tables 5 and 6, respectively. Moisture, crude protein, crude lipid or glycogen content of dorsal muscle and liver in starry flounder except for crude lipid in dorsal muscle was not significantly affected by either dietary protein or dietary lipid level. Proximate composition of fish was not relatively reflected from dietary nutrient composition in this study, conflicting with other studies (Helland and Grisdale-Helland, 1998; Lee et al., 2002a; b).

Blood chemistry of starry flounder at the end of the feeding trial is given in Table 7. Plasma cholesterol in blood of fish was significantly (P<0.05) affected by both dietary protein and lipid levels. Plasma triglyceride, total protein and glucose were not significantly (P>0.05) affected by either dietary protein

or dietary lipid level. However, significant (P<0.05) interaction of dietary protein and energy levels on plasma cholesterol and total protein was observed.

Fatty acid compositions of dorsal muscle in starry flounder at the end of the feeding trial are given in Table 8. Fatty acids of 16:0, 18:1n-9, 20:5n-3 and 22:6n-3 were the primary saturated, monoenic and n-3 highly unsaturated fatty acids (HUFA), respectively in fish. The highest percent of n-3 HUFA were found in starry flounder fed the 47% protein diet with 17% lipid. And n-3 HUFA content of starry flounder increased with dietary lipid level in the 47% protein diets.

The results of this study suggest that the diets containing 47% protein with 17% lipid or 52% protein with 12-17% lipid are optimal for growth and feed utilization of adult starry flounder under these ex-

Table 6. Proximate composition of liver from starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Glycogen (%)
Initial	70.7	12.0	14.2	0.7
P <sub>47-7</sub>	68.7±6.96 <sup>ns</sup>	11.7±1.01 <sup>ns</sup>	11.4±8.55 <sup>ns</sup>	5.0±2.52 <sup>ns</sup>
P <sub>47</sub> L <sub>12</sub>	58.9±5.03	10.5±0.88	27.0±7.05	4.8±1.15
P <sub>47</sub> L <sub>17</sub>	64.0±4.98	10.3±1.48	19.0±1.95	2.2±0.19
P <sub>52-7</sub>	55.0±1.74	8.0±0.41	27.1±5.42	6.3±0.61
$P_{52}L_{12}$	60.1±7.03	9.1±1.08	21.6±5.88	7.8±1.13
P <sub>52</sub> L <sub>17</sub>	64.8±6.33	10.4±1.68	16.9±6.78	4.6±1.99
Two-way ANOVA				
Dietary protein (DP)	P<0.4	P<0.1	P<0.06	P<0.09
Dietary lipid (DL)	P<0.7	P<0.9	P<0.06	P<0.2
DP×DL	P<0.4	P<0.3	P<0.3	P<0.9

<sup>&</sup>lt;sup>1</sup>Values are mean±SE of three replications. <sup>ns</sup>Not significant (P>0.05).

Table 7. Plasma chemistry of starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

Diets	Cholesterol (mg/100 mL)	Triglyceride (mg/100 mL)	Teotal protein (mg/100 mL)	Glucose (mg/100 mL)
P <sub>47</sub> L <sub>7</sub>	110±11.3°	28.3±2.03 <sup>ns</sup>	2.8±0.15 <sup>a</sup>	30.4±3.03 <sup>ns</sup>
$P_{47}L_{12}$	146±53.7 <sup>a</sup>	26.0±7.09	4.3±0.68 <sup>ab</sup>	27.8±5.26
$P_{47}L_{17}$	304±20.7 <sup>b</sup>	35.3±6.12	4.0±0.39 <sup>ab</sup>	28.2±4.50
P <sub>52</sub> L <sub>7</sub>	285±17.7 <sup>b</sup>	43.0±13.75	4.9±0.15 <sup>b</sup>	31.5±3.04
P <sub>52</sub> L <sub>12</sub>	276±33.2 <sup>b</sup>	41.3±19.84	4.2±0.53 <sup>ab</sup>	21.2±4.13
P <sub>52</sub> L <sub>17</sub>	193±51.4 <sup>ab</sup>	30.3±8.45	3.8±0.55 <sup>ab</sup>	21.4±0.55
Two-way ANOVA				
Dietary protein (DP)	P<0.01	P<0.4	P<0.2	P<0.2
Dietary lipid (DL)	P<0.01	P<0.9	P<0.7	P<0.2
DP×DL	P<0.01	P<0.6	P<0.05	P<0.5

<sup>&</sup>lt;sup>1</sup>Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05). <sup>ns</sup>Not significant (P>0.05).

Table 8. Major fatty acid composition (% of total fatty acids) of dorsal muscle in starry flounder fed the experimental diets containing various protein and lipid levels<sup>1</sup>

<u> </u>	$\mathcal{C}$							
	Diets							OEM <sup>2</sup>
	Initial	P <sub>47</sub> L <sub>7</sub>	P <sub>47</sub> L <sub>12</sub>	P <sub>47</sub> L <sub>17</sub>	P <sub>52</sub> L <sub>7</sub>	P <sub>52</sub> L <sub>12</sub>	P <sub>52</sub> L <sub>17</sub>	SEM
Fatty acids								
16:0	21.6	23.3	22.5	23.4	22.5	20.8	21.5	0.83
16:1n-7	4.6	3.2	3.1	1.9	2.9	3.2	2.8	0.48
18:0	4.2	3.2	3.2	3.6	2.0	3.0	3.1	0.41
18:1n-9	16.3	12.1	12.9	12.6	15.3	14.6	12.9	1.19
18:2n-6	6.9	5.0	3.3	2.5	3.8	2.3	3.8	0.89
20:4n-6	3.1	3.9	3.2	2.9	3.2	3.1	3.0	0.38
20:5n-3	12.1	12.8	14.7	14.0	14.1	14.4	14.4	0.74
22:5n-3	2.1	1.7 <sup>a</sup>	2.5 <sup>b</sup>	2.1 <sup>ab</sup>	2.0 <sup>ab</sup>	2.5 <sup>b</sup>	2.2 <sup>ab</sup>	0.20
22:6n-3	23.4	28.8	28.6	30.8	27.3	29.4	29.2	0.85
Saturated	28.3	28.3	27.4	28.6	26.3	25.0	26.8	1.02
Monoenes	22.0	17.2	18.6	17.6	21.0	21.4	18.3	1.84
n-3 HUFA	38.3	44.7 <sup>a</sup>	47.1 <sup>ab</sup>	48.1⁵	44.5 <sup>a</sup>	46.8 <sup>ab</sup>	45.4 <sup>ab</sup>	0.96

<sup>1</sup>Values (mean of three replications) in the same row not sharing a row superscript are significantly different (P<0.05). <sup>2</sup>Standard error of the treatment mean calculated from the residual mean square in the analysis of variance.

perimental conditions.

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