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Effects of Protein and Lipid Levels in Practical Diets on Growth and Body Composition of Tongue Sole, *Cynoglossus semilaevis* Gunther

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Abstract

A 9-wk feeding experiment was conducted to estimate the optimal dietary protein and lipid levels for tongue sole, *Cynoglossus semilaevis* Gunther (initial average weight of 43.8 ± 0.18 g). Six practical test diets were formulated to contain three protein levels (45, 50, and 55%, respectively) at two lipid levels (12 and 16%, respectively) with *P/E* ratios ranging from 87.1 to 110.5 mg protein/kcal. Each diet was randomly fed to triplicate groups of 20 fish per tank (1000 L). The results showed that fish fed the diet with 55% protein and 12% lipid (*P/E* ratio of 110.5 mg protein/kcal) had the highest thermal-unit growth coefficient (TGC), feed efficiency ratio, protein productive value, and energy retention. TGC was significantly increased with increasing dietary protein levels irrespective of dietary lipid levels ($P < 0.05$). However, fish fed the diet with 16% lipid showed significant lower growth than fish fed the diet with 12% lipid. These results suggest that the diet containing 55% protein and 12% lipid with *P/E* of 110.5 mg protein/kcal is optimal for tongue sole and the increase of dietary lipid level has no effective protein-sparing effect.

Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish. Accurate information on the protein requirement of fish is critical for any new aquaculture initiative because of the high cost of protein ingredients (Ng et al. 2008). The utilization of dietary protein is related to both protein levels and availability of non-protein energy sources (Ai et al. 2004). Ideally, dietary lipid or carbohydrates should be maximized in fish feeds to spare dietary protein from being used for energy (Deng et al. 2011). Reducing the dietary protein content by increasing fat and/or digestible carbohydrate content without any negative effect on the growth performance is known as "the protein-sparing effect" (Yamamoto et al. 2005).

Tongue sole, *Cynoglossus semilaevis* Gunther, is an endemic carnivorous flatfish species in Northern China. Owing to its rarity and delicious meat, the price of the tongue sole is the highest among flatfish in China (Ma et al. 2006). To our knowledge, about 5000 tons tongue soles have been produced every year, and the culture of this sole in indoor intensive system or earthen ponds along China's coast became popular since the success of artificial hatchery (Fang et al. 2010). However, only few nutritional studies on tongue sole have been reported. These researches were performed with larvae and live food, and the results showed that fish protein hydrolysate was a potential protein source and sodium alginate is one of suitable binders in microdiet of postlarval tongue sole (Chang et al. 2006; Liu et al. 2008). To our knowledge, no information is available about the study of dietary protein and energy requirements of this species. This study was conducted to evaluate the effects of protein and lipid levels in practical feeds on growth, feed utilization, and body composition of tongue sole.

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TABLE 1. *Formulation and proximate composition of experimental diets (% dry matter).*

Ingredients	Diet no. (protein/lipid)					
	Diet 1 (45/12)	Diet 2 (50/12)	Diet 3 (55/12)	Diet 4 (45/16)	Diet 5 (50/16)	Diet 6 (55/16)
Fish meal[a]	51.0	60.0	69.0	52.5	62.0	70.5
Wheat flour	15.0	15.0	15.0	15.0	15.0	15.0
Wheat middlings	23.7	15.1	6.6	18.1	9.2	1.1
Oils[b]	6.8	6.4	5.9	10.9	10.3	9.9
Soybean lechithin	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mix[c]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[d]	1.5	1.5	1.5	1.5	1.5	1.5
Proximate analysis ($n = 3$)						
Crude protein (% dry matter)	45.2	50.2	55.1	45.0	51.1	55.3
Crude lipid (% dry matter)	11.9	12.2	11.9	16.4	16.4	16.4
Ash (% dry matter)	11.9	12.9	14.7	12.0	13.4	14.6
Gross energy (kcal/g)	4.95	4.97	4.99	5.18	5.21	5.18
P/*E* (mg/kcal)	91.3	101.3	110.5	87.1	97.9	106.7

[a]Fish meal, obtained from Qihao Bio-tech Company (Qingdao, China), crude protein, 743 g/kg (dry matter basis), and crude lipid 73 g/kg (dry matter basis).

[b]Fish oil : soybean oil = 1:1.

[c]Vitamin premix (mg or IU/kg of diet): thiamin, 24.5 mg; riboflavin, 36 mg; pyridoxine HCl, 19.8 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 5.1 mg; inositol, 784 mg; pantothenic acid, 58.8 mg; niacin acid, 198 mg; folic acid, 19.6 mg; biotin, 1.20 mg; retinol acetate, 16,000 IU; cholecalciferol, 2500 IU; alpha-tocopherol, 200 mg; choline chloride (50%), 2500 mg; ascorbic acid (35%), 1000 mg; mold inhibitor, 1000 mg; and ethoxyquin, 500 mg.

[d]Mineral premix (mg/kg of diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; $ZnSO_4 \cdot H_2O$, 50; $FeSO_4 \cdot H_2O$, 80; $MnSO_4 \cdot H_2O$, 45; CoCl (1%), 50; Na_2SeO_3 (1%), 20; $Ca(IO_3)_2$ (1%), 60; zeolite, 8485; and $Ca(H_2PO_3)_2 \cdot H_2O$, 5000.

Materials and Methods

Experimental Diets

Using fish meal as the protein source, fish oil and soybean oil as lipid sources, and wheat meal as the carbohydrate source, six practical test diets were formulated to contain three protein levels (45, 50, and 55%, respectively) at two lipid levels (12 and 16%, respectively) with *P*/*E* ratios ranging from 87.1 to 110.5 mg protein/kcal (Table 1).

Ingredients were ground into fine powder in order to pass through a 320-μm mesh. All the ingredients were thoroughly mixed with oils and water was added to produce a stiff dough. The dough was then pelleted with an experimental feed mill (SPHS 22, Xiamen Huiyi Mould Factory, Xiamen, China) and dried for about 12 h in a ventilated oven at 60 C. The size of pellets was 5.0 × 5.0 mm. All diets were stored at −20 C until used.

Experimental Procedure

Juvenile tongue soles were obtained from Haiyang Yellow Sea Aquatic Product Co., Ltd, Yantai, China. Prior to the start of the experiment, the juveniles of tongue sole were reared in circular fiberglass tanks for 2 wk in order to acclimate to the experimental conditions. The fish were maintained in a recirculation system with a flow rate of approximately 3 L/min. Continuous aeration was maintained in each tank. During this period, fish were fed twice a day on a commercial diet (Qihao Bio-tech Co., Ltd., Qingdao, China) to satiation. At the start of the experiment, fish were fasted for 24 h before weighing. Fish of similar sizes (initial weight 43.8 ± 0.18 g, mean ± SEM) were distributed into eighteen 1000-L fiberglass tanks with 20 juveniles per tank. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice (0830 and 1730 h) daily. The feeding trial lasted for 9 wk. During the experimental period, the temperature ranged from 24.0 to 28.0 C, the salinity from 28.5 to 32.0 g/L, pH from 7.8 to 8.5, and dissolved oxygen content was approximately 7 mg/L. Photoperiod was 14 h light : 10 h dark during the period of experiment.

Measurement and Analysis

At the start of the experiment, six stock fish were killed, weighed, and kept frozen for subsequent initial whole-body composition analysis. At the end of the experiment, the fish were starved for 24 h before harvest. Total number and mean body weight of fish in each tank were measured. Five fish from each tank were killed, individually weighed, and their total length measured. Liver and viscera were excised and weighed for the determination of hepatosomatic index (HSI) and viscerosomatic index (VSI), respectively. Livers from five other fish per tank were collected, frozen in liquid nitrogen, and stored at −80 C prior to total lipid, glycogen, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) analysis. The remaining fish from each tank were pooled and stored frozen at −20 C for subsequent whole-body composition analysis. Fish carcasses were then blended, dried, and ground into fine powder.

Analyses of proximate composition of feed ingredients, experimental diets, and fish body were performed based on standard methods described by AOAC (1995). To determine moisture content, samples of diets and fish were dried to a constant weight at 105 C. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; lipid by ether extraction using Soxhlet (BUCHI Company, Flawil, Switzerland); ash by combustion at 550 C; and energy contents were measured by PARR1281 Calorimeter (PARR Instrument Company, Moline, IL, USA). Total lipid of liver samples was determined according to Folch et al. (1957), whereas liver glycogen was determined by a colorimetric enzymatic method using commercial kits (supplied by Nanjing Jiancheng Bio-Tech Co., Nanjing, China). Crude extracts of liver for assaying protein-metabolism enzyme activities were obtained by homogenization of frozen tissue in ice-cold 0.7% saltwater. Following centrifugation (3200 *g* for 20 min at 4 C), activities of ALT and AST of liver supernatants were measured using specific analytical procedures and commercially available kits (supplied by Nanjing Jiancheng Bio-Tech Co., Najing, China).

Calculations and Statistical Analysis

The following variables were calculated:

$$\text{Thermal-unit growth coefficient (TGC)} = \left(W_t^{1/3} - W_0^{1/3}\right) \times 100 / \sum [T \times D]$$

$$\text{Feed intake (FI)} = \text{feed consumption (g)} / \left[((W_t + W_0)/2) \times t\right]$$

$$\text{Feed efficiency ratio (FER)} = \text{Wet weight gain (g)/dry feed fed (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Wet weight gain (g)/protein intake (g)}$$

$$\text{Protein productive value (PPV)} = (W_t \times P_1 - W_0 \times P_2)/(I_d \times P)$$

$$\text{Energy retention} = (W_t \times E_1 - W_0 \times E_2)/(I_d \times E)$$

$$\text{Condition factor } (K) = \left[\text{final body weight}/(\text{total length})^3\right] \times 100$$

$$\text{Hepatosomatic index (HSI)} = [\text{liver weight (g)/body weight (g)}] \times 100$$

$$\text{Viscerosomatic index (VSI)} = [\text{visceral weight (g)/body weight (g)}] \times 100$$

where W_t is final body weight, W_0 is initial body weight, T is water temperature (C), D is number of days, t is experimental duration

in days, and I_d is feed intake of dry matter. P, P_1, and P_2 represent protein contents in diet, final fish body, and initial fish body, respectively. E, E_1, and E_2 represent energy content in diet, final fish body, and initial fish body, respectively.

The effects of dietary protein and lipid levels and their interactions on growth and whole-body proximate composition were analyzed by factorial (two-way) analysis of variance (ANOVA). Tukey multiple range tests were used for determining significant differences among means. Differences were regarded as significant when $P < 0.05$. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) for Windows.

Results

No mortality was recorded within treatments during the trial. The growth response of tongue sole fed the experimental diets is given in Table 2. Fish fed the feed containing 55% protein and 12% lipid exhibited the highest TGC and fish fed the diets with 50 or 55% protein and 12% lipid exhibited the highest feed efficiency ratio (FER) among the fish fed the experimental diets. Two-way ANOVA revealed that dietary protein levels significantly affected final weight, TGC, FER, and PER for fish performance after 9 wk of feeding ($P < 0.05$). Final weight was significantly increased with increasing dietary protein levels from 45 to 55% for both lipid levels ($P < 0.05$), whereas TGC and FER were significantly increased

TABLE 2. *Growth response of tongue sole fed the test diets for 9 wk.*

Lipid	12%			16%			
Protein	45%	50%	55%	45%	50%	55%	Pooled SEM
Initial weight (g)	43.8	43.8	43.8	43.8	43.8	43.8	0.18
Final weight (g)	80.7	85.3	92.8	70.3	79.0	89.7	1.94
FI[a] (g/100 g BW/d)	1.2	1.15	1.28	1.42	1.46	1.41	0.03
TGC[b] (%/d)	0.00155	0.00174	0.00199	0.00122	0.0015	0.00186	0.00003
FER[c]	0.77	0.89	0.9	0.53	0.61	0.76	0.04
PER[d]	3.22	2.41	2.25	4.66	3.59	2.64	0.21
PPV[e] (%)	26.4	25.9	25.3	16.9	16.2	20.9	1.09
ER[f] (%)	22.9	24.2	25.6	13.1	16.8	19.6	1.24

	Variation source[g]			Tukey HSD for protein level[h]		
Two way ANOVA	Protein	Lipid	Interaction	45%	50%	55%
Initial weight (g)	ns	ns	ns			
Final weight (g)	***	**	ns	a	a	b
FI (g/100 g BW/d)	ns	***	ns			
TGC (%/d)	***	**	ns	a	ab	b
FER	*	***	ns	a	a	b
PER	***	***	ns	a	b	b
PPV (%)	ns	***	ns			
ER (%)	ns	***	ns			

SEM = standard error of means; BW = body weight; ns = nonsignificant.

[a]FI: feed intake = Feed consumption (g)/$[((W_t + W_0)/2) \times t]$.

[b]TGC: thermal-unit growth coefficient = $(W_t^{1/3} - W_0^{1/3}) \times 100/\sum [T \times D]$.

[c]FER: feed efficiency ratio = Wet weight gain (g)/dry feed fed (g).

[d]PER: protein efficiency ratio = Wet weight gain (g)/protein intake (g).

[e]PPV: protein productive value = $(W_t \times P_1 - W_0 \times P_2)/(I_d \times P)$.

[f]ER: energy retention = $(W_t \times E_1 - W_0 \times E_2)/(I_d \times E)$.

[g]$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[h]Significant differences are indicated by different letters ($P < 0.05$).

TABLE 3. *Body composition of tongue sole fed the test diets for 9 wk.*

Lipid		12%			16%			
Protein	Initial body composition	45%	50%	55%	45%	50%	55%	Pooled SEM
Moisture (%)	76.3	75.5	76.7	76	77.1	76	76.1	0.165
Crude protein (% ww)	15.5	16.3	15.8	16.4	15.7	16	16.2	0.078
Crude lipid (% ww)	5.12	5.55	5.07	5.16	4.97	5.75	5.36	0.111
Crude ash (% ww)	1.93	2.09	1.95	2.01	2.19	2.01	2.13	0.035
Energy (kcal/g ww)	1.24	1.42	1.35	1.42	1.31	1.43	1.36	0.071

	Variation source[a]			Tukey HSD for protein level[b]		
Two way ANOVA	Protein	Lipid	Interaction	45%	50%	55%
Moisture (%)	ns	ns	**			
Crude protein (% ww)	*	ns	**	a	ab	b
Crude lipid (% ww)	ns	ns	ns			
Crude ash (% ww)	ns	ns	ns			
Energy (kcal/g ww)	ns	ns	ns			

SEM = standard error of means; ww = wet weight; ns = nonsignificant.

[a] $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[b] Significant differences are indicated by different letters ($P < 0.05$).

with increasing dietary protein levels for 16% lipid only. Correspondingly, PER was significantly decreased with the increase of protein levels for 16% lipid only. Two-way ANOVA showed a significant effect due to the lipid level with the fish fed the 12% lipid diets having a significantly higher final weight, TGC, FER, PPV, and ER compared with the 16% lipid diets ($P < 0.05$). On the contrary, FI and PER of fish fed the 12% lipid diets showed significantly lower values compared with the 16% lipid diets ($P < 0.001$). FI, PPV, and ER were only affected by the dietary lipid level; specifically, the increase of dietary lipid level resulted in increased FI and decreased PPV or ER at all protein levels.

Analysis of fish body composition showed that dietary protein and lipid levels did not significantly affect the carcass lipid, ash, and energy (Table 3). Two-way ANOVA identified that the interaction between dietary protein and lipid level had significant effect on the carcass moisture and protein content ($P < 0.05$). The protein content was significantly increased with increasing dietary protein levels from 45 to 55% at 16% dietary lipid levels, whereas the moisture content was significantly increased with increasing dietary lipid level for 45% protein only.

Condition factor (K), HSI, VSI, liver lipid content, hepatic glycogen concentrations, and hepatic enzymes related to amino acids metabolism of tongue sole fed the different protein/lipid diets are presented in Table 4. Dietary protein and lipid concentrations had no significant effect on K, VSI, and liver lipid content ($P > 0.05$). Two-way ANOVA revealed that HSI and hepatic glycogen concentrations were significantly decreased with the increase of lipid from 12 to 16% ($P < 0.05$), whereas AST and ALT in fish liver were significantly increased with increasing dietary lipid level ($P < 0.001$). Multiple comparison testing showed that the 16% lipid diets had significantly higher AST compared with 12% lipid for all protein levels, whereas 12% lipid diets showed a significantly higher HSI for the 45 and 55% protein diets only.

Discussion

In this study, filtered natural seawater has been used and the temperature ranged from 24 to 28 C during the experimental period. However, the results of Fang et al. (2010)

TABLE 4. *Biometric indices, liver composition, and hepatic AST, ALT of tongue sole.*

Lipid	12%			16%			
Protein	45%	50%	55%	45%	50%	55%	Pooled SEM
Condition factor	0.66	0.69	0.71	0.62	0.65	0.65	0.01
VSI	3.61	3.41	3.53	3.39	3.45	3.31	0.05
HSI	0.92	0.8	1.02	0.69	0.7	0.61	0.04
Hepatic glycogen (mg/g)	38.6	30.7	48.3	19.1	24.5	25	3.09
Lipid of liver (mg/g wet liver)	16.1	11.5	10.9	15.4	11	11.8	1.09
AST of liver (U/mg protein)	6.65	6.73	4.95	26.1	22.7	24.9	2.39
ALT of liver (U/mg protein)	7.95	9.08	7.55	23.3	33.8	34.9	3.42

	Variation source[a]			Tukey HSD for protein level[b]		
Two way ANOVA	Protein	Lipid	Interaction	45%	50%	55%
Condition factor	ns	ns	ns			
VSI	ns	ns	ns			
HSI	ns	**	ns			
Hepatic glycogen (mg/g)	ns	**	ns			
Lipid of liver (mg/g wet liver)	ns	ns	ns			
AST of liver (U/mg protein)	ns	***	ns			
ALT of liver (U/mg protein)	ns	***	ns			

SEM = standard error of means; VSI = viscerosomatic index; HSI = hepatosomatic index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ns = nonsignificant.

[a] $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[b] Significant differences are indicated by different letters ($P < 0.05$).

showed that the proportion of food energy allocated to growth decreased with increasing temperature. They suggested that commercial farmers could feed juvenile tongue sole to satiation at 22 C to obtain higher growth rate. Being a eurythermal and euryhaline species, tongue sole is distributed widely in the Bohai Sea, the Yellow Sea, and the East China Sea, China (Ma et al. 2006). This sole can be cultivated in both indoor intensive systems and earthen ponds along China's coast and the fertilized eggs can be hatched at temperatures from 18 to 28 C (Zhang et al. 2006). Also, the purpose of this study was to examine the effects of dietary protein and lipid level on growth and body composition cultured under practical conditions.

In this study, the tongue sole grew best when fed the diets containing 12 and 16% lipid at 55% protein. Especially, growth and FER of fish with 16% lipid were significantly increased with increasing dietary protein levels. As for 12% lipid, similar trends have also been found although significant differences were not detected. As the optimal dietary protein level of tongue sole has not been determined, these results suggested tongue sole should require dietary protein of above 50% to sustain a quick growth. Some previous studies have indicated that Japanese flounder required dietary crude protein (CP) of 50% (Lee et al. 2000; Alam et al. 2004), turbot of 49.4% CP (Lee et al. 2003), southern flounder of 50.8% CP (Gonzaleza et al. 2005), plaice of 50.0% CP (Cowey et al. 1972), starry flounder of 50% CP (Lee et al. 2006), winter flounder of 50% CP (Hebb et al. 2003), and Senegalese sole of 53% CP (Rema et al. 2008). Dietary protein requirement of tongue sole appears to be similar to those of other flatfish.

Two-way ANOVA showed that the growth performance of tongue sole was significantly affected by dietary lipid level in this study. Fish fed the diets containing 12% lipid showed

higher final weight, TGC, and FER than those of fish fed the diets containing 16% lipid, suggesting that the tongue sole has a relatively poor capacity to utilize dietary lipids as energy sources, and 16% dietary lipid appears to be excessive for this species. Excess lipid not only suppresses *de novo* fatty acid synthesis but also reduces the ability of fish to digest and assimilate it, leading to reduced growth rate (Sargent et al. 1989; Mohanta et al. 2007). The use of high fat diets may lead to fatty fish (Reinitz et al. 1978; Regost et al. 2001), inhibit the utilization of other nutrients (Winfree and Stickney 1981), and affect the oxidative status of the fish (Rueda-Jasso et al. 2004). In this study, fish growth reduction when fed high dietary lipid suggests that dietary lipid did not result in protein sparing on tongue sole. This is in agreement with the results reported for other fish species such as winter flounder, white seabream, greater amberjack, Malaysian mahseer, and Pacific threadfin (Hebb et al. 2003; Ozorio et al. 2006; Takakuwa et al. 2006; Ng et al. 2008; Deng et al. 2011). In contrast, an apparent protein-sparing effect has been reported for several other fish species such as Japanese seabass, bagrid catfish, and cuneate drum (Ai et al. 2004; Kim and Lee 2005; Wang et al. 2006). The lack of protein-sparing effect in this study was probably due to the fact that the 12% dietary lipid level used was already providing sufficient amount of metabolizable energy for growth of tongue sole or that the capacity to utilize dietary lipids for tongue sole is poor.

In addition, HSI, hepatic glycogen concentrations, AST, and ALT in fish liver were also significantly affected by dietary lipid level. AST and ALT in fish liver were significantly increased with increasing dietary lipid level ($P < 0.001$). The activities of AST and ALT are useful to evaluate the feeding status in some fish (Moyano et al. 1991; Melo et al. 2006). High protein level or protein/energy ratio usually causes an increase of liver AST and ALT (Meton et al. 1999). In this study, the increase of hepatic ALT and AST suggests that 16% lipid level in diets of sole may affect protein metabolism of tongue sole. Rueda-Jasso et al. (2004) also reported that the high dietary lipid affected the oxidative status of Senegalese sole and inhibited the growth rate of fish. On the other hand, HSI decreased significantly with lipid level increasing from 12 to 16% for tongue sole. However, Lee et al. (2002) reported that the HSI of rockfish increased with increasing dietary lipid. Therefore, more studies are needed to elucidate the optimum dietary lipid level and the metabolism of tongue sole in the future. In addition, hepatic glycogen of sole fed 16% lipid was significantly lower than that of fish fed 12% lipid. This was confirmed by some previous studies, which indicated that liver glycogen content increased with increasing dietary digestible carbohydrate level in European sea bass (Moreira et al. 2008).

The overall results of this study show that a diet with dietary protein of 55 and 12% lipid with P/E of 110.5 mg protein/kcal is recommended for maximum growth of tongue sole. Increasing dietary lipid level from 12 to 16% did not result in protein-sparing effect.

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