Dietary Protein Requirement of Juvenile Turbot (*Scophthalmus maximus* Linnaeus)

LIU Xingwang[1, 2], MAI Kangsen[1], LIUFU Zhiguo[1], and AI Qinghui[1, *]

1) *Key Laboratory of Nutrition and Feed of Department of Agriculture of China, Ocean University of China, Qingdao 266003, P. R. China*
2) *Guangdong Evergreen Feed Industrial Co., Ltd., Zhanjiang 524094, P. R. China*

Abstract   The dietary protein requirement of juvenile turbot (initial average weight, $38.2\,g \pm 0.1\,g$) reared indoor in aerated aquaria was determined in this study. Five energy equal experimental diets were formulated with fish meal as protein source, which contained different concentrations of protein (47.2%, 51.0%, 54.6%, 59.3% and 63.6% of dry diet). Three groups of fish with 18 individuals in each, were cultured in 300-L tanks and fed twice a day for 8 weeks. During culture, temperature was controlled between 15.0 and 18.0℃, salinity was controlled between 28.5 and 32.0, acidity was controlled between pH7.8 and pH8.5, and ammonia nitrogen was maintained below $0.03\,mg\,L^{-1}$ and dissolved oxygen was maintained about $7\,mg\,L^{-1}$. Results showed that the growth of fish was significantly affected by dietary protein content ($P < 0.05$). Specific growth rate (*SGR*) of turbot increased when dietary protein content varied between 47.2% and 51.0% ($P < 0.05$), and then kept stable when dietary protein content was higher than 51.0%. Fish which were fed the diet containing 63.6% protein showed the highest *SGR* while those fed the diet containing 59.3% protein showed the highest feed efficiency rate. No significant difference of feed intake and protein efficiency ratio was found among experimental diets ($P > 0.05$). Broken-line regression analysis of *SGR* showed that the optimal dietary protein requirement of turbot was about 57.0%.

Key words   turbot; *Scophthalmus maximus*; protein requirement; feed nutrition

1 Introduction

An optimal dietary protein content is important for fish growth and farming environment maintenance (Guo *et al*., 2012). Accurate information on the protein requirement of fish is critical for initiating a new aquaculture species as dietary protein usually represents the highest proportion of a commercial feed cost (Ng *et al*., 2008). It is important to determine the optimum dietary protein content for meeting success in fish culture.

Due to its fast growth and high consuming demand, turbot, *Scophthalmus maximus*, has been widely cultured indoor intensively Northern China. A series of studies on protein nutrition of turbot have been carried out. The documented protein requirement ranged from 35% to 69.8% (Adron *et al*., 1976; Caceres-Martinez *et al*., 1984; Lee *et al*., 2003). The optimum dietary protein content for the most cost-effective turbot culture is not fixed. The variation of the optimum reported may be due to the difference in fish size and age, dietary protein quality and energy richness, and protein energy balance (Wilson, 2002). Most in use commercial diets of turbot in China contained 11%–12% of crude lipid, which is the maximum that can be achieved with extruders in China currently. The objective of this study was to optimize the protein requirement of turbot with practical ingredients containing 11.5% lipid. The results may provide more practical value to Chinese turbot feed industry.

2 Materials and Methods

2.1 Diets

Five energy equal (about $20.5\,MJ\,kg^{-1}$) testing diets were formulated to contain different concentrations of protein (47.2%, 51.0%, 54.6%, 59.3% and 63.6% of dry diet) (Table 1). Fish meal was used as the dietary protein source. Wheat flour and wheat-middlings were used to replace dietary protein. Ingredients were ground into a fine powder through a 320 μm mesh. All the ingredients were thoroughly mixed in a V-type blender (Shanghai Tianxiang & Chentai Pharmaceutical Machinery Co., Ltd., China) and 30% water was added to produce stiff dough. The dough was then extruded through a 3-mm die using an extruder (260, Shandong Weihai Youyi Machinery Co., Ltd., China). The moist pellets were dried in an oven set at 60℃ for about 10 h, and stored at −20℃.

Table 1 Formulation and proximate composition (% dry weight) of five experimental diets fed to juvenile turbot

Ingredients	Diet no.				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	54.0	61.0	69.0	77.0	84.0
Wheat flour	15.0	15.0	15.0	12.6	6.1
Wheat-middlings	19.4	12.8	5.2	0.0	0.0
Fish oil	6.7	6.3	5.9	5.5	5.0
Soybean lechithin	1.0	1.0	1.0	1.0	1.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0
Mineral mix	2.0	2.0	2.0	2.0	2.0
Ascorbic acid (35%)	0.1	0.1	0.1	0.1	0.1
Choline chloride (50%)	0.3	0.3	0.3	0.3	0.3
$Ca(H_2PO_3)_2$	0.5	0.5	0.5	0.5	0.5
Proximate composition					
Crude protein (%)	47.2	51.0	54.6	59.3	63.6
Crude lipid (%)	11.5	11.7	11.4	11.6	11.7
Ash (%)	12.6	14.3	15.3	16.6	17.9
Gross energy ($MJ\,kg^{-1}$)	20.7	20.5	20.5	20.7	20.5

Notes: Fish meal, obtained from Qihao Bio-tech Company (Shandong, China), produced by Alimar Co. Ltd. (Chile), crude protein, 74.3% dry matter, crude lipid 7.3% dry matter. Vitamin premix (mg or $IU\,kg^{-1}$ diet): thiamin 24.5 mg; riboflavin, 36 mg; pyridoxine HCl, 19.8 mg; vitamin B12, 0.1 mg; vitamin K3, 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, 16000 IU; cholecalciferol, 2500 IU; alpha-tocopherol, 200 mg; Mold inhibitor 1000 mg; Ethoxyquin 500 mg. Mineral premix ($mg\,kg^{-1}$ 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.

2.2 Growth Evaluation

Juvenile turbot were obtained from Haiyang Yellow Sea Aquatic Product Co., Ltd., Yantai, China. Prior to experiment, the fish were acclimated to a commercial diet (Qihao Bio-tech Co., Ltd., China) for 2 weeks and were fed twice a day to apparent satiation. At the beginning of experiment, fish were starved for 24 h, and those in similar size (initial weight 38.2 g±0.1 g) were distributed into 15 300-L fiberglass tanks, 18 each. Each testing diet was randomly assigned to triplicate tanks. Juvenile turbot were fed to visual satiety twice a day for 8 weeks. Each tank was provided a continuous flow of sand-filtered seawater (3 L min^{-1}) with continuous aeration. Water quality was monitored daily. During experiment, temperature was controlled at 15.0–18.0℃, salinity was controlled at 28.5–32.0, acidity was controlled between pH7.8 and pH8.5, ammonia nitrogen was maintained lower than 0.03 mg L^{-1} and dissolved oxygen was maintained about 7 mg L^{-1}.

2.3 Sampling and Chemical Analysis

Fish were fasted for 24 h before harvesting. The total number and mean body weight of fish each tank were measured to calculate specific growth rate, feed efficiency ratio, protein efficiency ratio and survival. Six fish at beginning and six at the end each tank were randomly collected and frozen (−70℃) for determining proximate analysis. Analysis of dry matter (105℃, 24 h), crude protein (Kjeldahl nitrogen × 6.25), crude lipid (ether extraction by Soxhlet method) and ash (550℃, 18 h) in diets and whole-body were performed following standard laboratory procedures (AOAC, 1990). Gross energy in diet was determined by an adiabatic bomb calorimeter (Parr 1281, USA).

2.4 Calculations and Statistical Analysis

The following variables were calculated:

$$\text{Specific growth rate } (SGR, \%) = \frac{\ln W_t - \ln W_0}{t} \times 100,$$

$$\text{Feed intake } (FI) = \frac{\text{Feed consumption (g)} \times 2}{(W_t + W_0) \cdot t},$$

$$\text{Feed efficiency ratio } (FER) = \frac{\text{Wet weight gain (g)}}{\text{Dry feed fed (g)}},$$

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

where W_t is final body weight, W_0 is initial body weight, t is experimental duration in days.

All data obtained for the test diets were subjected to one-way ANOVA to determine if there was significant effect due to dietary crude protein. When significant ($P < 0.05$) differences were detected, Duncan's multiple range was used to compare mean values among testing diets. Optimal dietary protein level for juvenile turbot was determined by the broken-line model using *SGR* data. All analyses were conducted using SPSS (version 11.0, Chicago, IL, USA).

3 Results

In 8 weeks feeding trial, mean survival ranged from 90.7% to 100%, which was not related to dietary protein content (Table 2). *FI* and *PER* were not significantly different among testing diets ($P > 0.05$). However, *SGR* and *FER* were significantly affected by dietary protein content ($P < 0.05$). *SGR* and *FER* of turbot increased when dietary protein was increased from 47.2% to 51.0% ($P < 0.05$). When the protein concentration was higher than 51.0%, no significant difference was found. Fish fed the diet with 63.6% protein had the highest *SGR* while those fed the diet with 59.3% protein obtained the highest *FER*. Based on *SGR* response using the broken-line model (Fig.1), optimum dietary protein content for the growth of juvenile turbot was estimated to be 57.0% ($Y = 1.54 - 0.05(57.00 - X)$ ($R^2 = 0.69$)).

Carcass moisture, protein, lipid and ash contents were not significantly affected by protein content of diets ($P > 0.05$) (Table 3). Carcass moisture was fairly consistent as it ranged from 76.7% to 77.5%. Carcass protein ranged

from 15.2% to 15.9%. Carcass lipid ranged from 3.8% to   4.1% and carcass ash ranged from 3.2% to 3.5%.

Table 2 Growth performances of turbot fed diets containing various protein levels

Diet no.	Initial weight (g)	Final weight (g)	FI (g (100 B.W.)$^{-1}$ d^{-1})	SGR (% d^{-1})	FER	PER	Survival (%)
Diet 1	38.2	70.5[b]	0.98	1.10[b]	1.00[c]	2.12	90.7
Diet 2	38.2	75.2[ab]	0.93	1.20[ab]	1.17[bc]	2.29	98.2
Diet 3	38.2	86.4[ab]	0.94	1.46[ab]	1.44[ab]	2.64	98.2
Diet 4	38.2	89.9[a]	0.92	1.53[a]	1.58[a]	2.66	100.0
Diet 5	38.2	91.8[a]	0.94	1.56[a]	1.52[ab]	2.39	94.4
Pooled S.E.M.	0.1	2.74	0.02	0.06	0.07	0.08	1.60
ANOVA							
F		5.179	0.433	6.238	8.148	2.313	1.110
P		0.016	0.782	0.009	0.003	0.129	0.404

Notes: Values are presented as means of triplication. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$). S.E.M., standard error of means; ANOVA, one-way analysis of variance; *FI*, feed intake; *SGR*, specific growth rate; *FER*, feed efficiency ratio; *PER*, protein efficiency ratio.
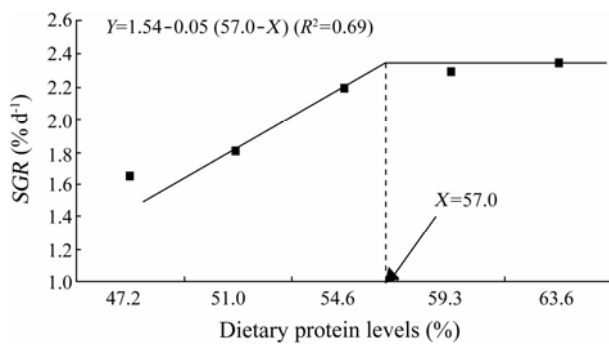

Fig.1 Optimal dietary protein requirement of turbot juveniles, based on the broken-line regression model of *SGR* versus dietary protein level.

Table 3 Proximate composition (% wet weight) of the whole body of turbot fed diets containing various protein levels

Diet no.	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Diet 1	77.0	15.5	3.9	3.5
Diet 2	77.1	15.5	3.8	3.4
Diet 3	77.5	15.3	4.1	3.2
Diet 4	77.4	15.2	4.0	3.3
Diet 5	76.7	15.9	3.9	3.4
Pooled S.E.M.	0.12	0.10	0.07	0.05
ANOVA				
F	1.595	1.318	0.407	2.005
P	0.250	0.328	0.800	0.170

Notes: Values are presented as means of triplication. Means in the same column with different superscripts are significantly different from each other ($P < 0.05$). S.E.M., standard error of means; ANOVA, One-way analysis of variance.

4 Discussion

Results of the present study showed that *SGR* and *FER* of turbot increased when dietary protein content increased from 47.2% to 51.0%, and then became stable when in the dietary protein content increased higher. The best growth was observed in fish fed 63.6% of protein. A broken-line model equation on basis of regression analysis of *SGR* against dietary protein content indicated that the optimal dietary protein for the maximum growth was 57.0%. This requirement value is higher than that reported in other flatfish including plaice, Japanese flounder, winter flounder, southern flounder and starry flounder (50.0%–50.8% crude protein) (Cowey *et al*., 1972; Lee *et al*., 2000; Alam *et al*., 2004; Hebb *et al*., 2003; Gonzaleza *et al*., 2005; Lee *et al*., 2006). For turbot, a series of studies on protein nutrition have been carried out. Adron *et al*. (1976) reported that the growth of 10 g turbot fed 35% protein containing diet was almost the same as those fed 50% protein containing diet while Caceres-Martinez *et al*. (1984) observed the best growth when the diet contained 69.8% of protein. For 89–155 g turbot, Lee *et al*. (2003) concluded that the optimal dietary protein content was 49.4% with white fishmeal as protein source. The difference among these experiments may be due to different fish sizes, energy levels or ingredients. In this study, practical formulations were designed and high quality fishmeal was used as the only protein source. And the lipid level designed in this research was about 11.5%, which was the maximum of fat that could be achieved by extruder now used in China. So the result obtained from this study would be more valuable for Chinese turbot farming industry.

Normally, protein efficiency ratio (*PER*) tends to decrease with dietary protein content increase (Deng *et al*., 2011; Coutinho *et al*., 2012). It is generally known that fish cannot utilize excess dietary protein for protein synthesis but can utilize it for energy generation (Kim and Lee, 2009). In this study, there was no significant difference of *PER* among different testing diets ($P > 0.05$). The maximum *PER* was observed at 59.3% dietary protein content, which was similar to the optimal obtained by broken-line model. Similar result has also been reported by Lee *et al*. (2003). These results suggested that the protein requirement of turbot was relatively higher than that of other flat fish.

In this study, dietary protein content did not significantly affect final whole-body content of turbot (Table 3). This agreed well with the results obtained from turbot by other researchers (Adron *et al*., 1976; Caceres-Martinez

et al., 1984; Lee *et al.*, 2003). It was reported that the protein and moisture content of whole body of fish generally tended to increase as dietary protein content increased while body lipid content showed an inverse relationship with body protein and moisture (Martinez-Palacios *et al.*, 1996; Allan *et al.*, 2001; Luo *et al.*, 2004; Craig *et al.*, 2006). However, other results showed that proximate composition of the whole body of fish was not significantly affected by dietary protein content (Moore *et al.*, 1988; Ng *et al.*, 2001). Shearer (1994) pointed out that the body proximate composition of fish was influenced by both endogenous factors such as fish size and sex and exogenous factors such as diet composition and culture environment. This may partly explain the different conclusions of the experiments.

The results of the present study indicated that turbot juvenile require about 57.0% of protein in their diets to obtain maximum growth, which was in the range of reported for other carnivorous fish. Further studies will be conducted to investigate the essential amino acid requirements and the apparent digestibility coefficient of protein.

Acknowledgements

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