A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Study on Dietary Protein Requirement for Juvenile Tiger Puffer (*Takifugu rubripes*)

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GRADUATE SCHOOL

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This thesis has been examined and approved.

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CONTENTS

국문초록

단백질은 생명체를 구성하는 필수적인 고분자의 복잡한 유기물로서 근육조직 내에 가장 많이 함유되어 있는 영양소이다. 탄수화물이나 지방과 같이 에너지를 발생할 수 있지만, 질소를 함유하고 있어서 다른 영양소로 대체할 수 없으며, 동물조직을 구성하는 성분이며, 항체, 호르몬 및 각종 효소의 구성성분이다. 단백질 분자는 아미노산이 펩티드 결합(peptide linkage; -C(=O)-N(H)-)으로 연결되어 있는 중합체로서 아미노산 잔기를 약 350~5,000개로 결합되어 그 종류에 따라 다르나 분자량이 35,000에서 500,000 dalton이며, 자연계에는 들어 있지 않는 200여 종 이상의 아미노산들이 있으나 평균 20여 종의 아미노산들로 구성되어 있다.

단백질은 어류의 성장에 가장 큰 영향을 미치고, 양식어류의 사료에 있어서 영양소 중 가장 큰 비중을 차지하며, 그 가격 또한 가장 값비싼 영양소이다. 따라서 최근에는 아미노산의 조성이 좋아 가장 많이 활용되고 있는 동물성 단백질원인 어분(fishmeal)을 대체하고자 하는 연구들이 활발하게 이루어 지고 있는 실정이다. 하지만 영양학적으로 대상동물이 요구하는 단백질의 함량이 우선적으로 수행되어야 한다. 따라서 양식어종을 대상으로 단백질 요구량에 대한 많은 실험이 수행되었다. 하지만 단백질 요구량은 어종, 어체의 사이즈, 성장단계 등에 따라 그 요구량이 다르며, 수온, 염분, pH 등 환경요인에 의해 영향을 받기도 하고, 사료원 및 사료의 조성에 의해 영향을 받기도 한다. 따라서 단백질 요구량에 관한 실험은 많은 연구자들에 의해 대부분의 양식어종을 대상으로 수행되었다.

자주복은 아주 뛰어난 맛과 높은 시장가격 때문에 지난 10년 동안 우리나라에서 유망하게 양식 되고 있는 어종 중에 하나이다. 특히 제주도에서 새로운 양식어종으로 가장 중요한 양식어종 중에 하나인 자주복은 영양학적 요구량에 관한 정보가 전무한 실정이다. 자주복은 위를 가지고 있지 않기 때문에 25g 미만의 어류에게는 하루에 4~6회 사료공급을 하여야 하며, 상품사이즈인 1kg까지 키우는데 약 18개월이 걸린다. 따라서 양식기간을 단축하고 사료단가를 절감시키기 위해서 자주복의 사료 내 영양학적 요구량을 밝히는 것은 성장률을 증가시키기 위해 아주 중요하다고 할 수 있다. 하지만 자주복에 관한 단백질 요구량은 아직까지 밝혀지지 않았다. 따라서 이 실험은 자주복 (*Takkifugu rubrifes*) 치어의 성장에 있어서 사료 내 최적의 단백질 요구량을 결정짓기 위해서 수행되었다.

총 5개의 실험사료는 단백질 함량이 35, 40, 45, 50 및 55%가 포함되도록 casein으로 조절하여 조성되었다. 각 실험사료는 8주 동안 유수식 시스템에서 3반복으로 수행되었다. 8주 동안의 성장실험 종료 후, 단백질 함량이 40%인 실험구에서 단백질 함량이 35%인 실험구보다 유의적으로 높은 성장률을 보였으며, 단백질 함량이 45, 50 및 55%인 실험구와는 유의적인 차이를 보이지 않았다. 또한 단백질 함량이 35%인 실험구에서 가장 낮은 먹이효율을 보였다. 단백질전환효율은 단백질함량이 45, 50 및 55%인 실험구가 단백질 함량이 35와 40%인 실험구보다 유의적으로 낮은 값을 보였다. 먹이섭취율과 생존율에서는 모든 실험구에서 유의적인 차이가 관찰되지 않았다. 헤마토크릿과 헤모글로빈의 수치는 사료 내 단백질 함량이 증가됨에 따라 증가하는 경향을 보였으나 유의적인 차이는 없었다. 혈청의 aspartate aminotransferase의 활성은 단백질 함량이 45%인 실험구에서 35% 보다 유의적으로 낮은 값을

보였으며, 단백질 함량이 40, 50, 55%인 실험구와는 유의적인 차이를 보이지 않았다. 전어체의 일반성분 분석에서는 단백질 함량이 증가함에 따라 전어체의 단백질 함량이 증가하는 경향을 보였으며, 단백질 함량이 50와 55%인 실험구가 35% 실험구 보다 유의적으로 높은 단백질 함량을 보였으며, 단백질 함량이 40과 45%인 실험구와는 유의적인 차이를 보이지 않았다. 따라서 이 연구의 결과를 요약하면 치어 자주복의 성장에 있어서 사료 내 최적의 단백질 함량은 약 41%로 사료된다.

ABSTRACT

This study was conducted to determine optimum dietary protein requirement for the growth of juvenile (initial weight, 17.05 ± 0.17g) tiger puffer, *Takifugu rubripes*. Five Semi-purified diets were formulated with casein to contain graded levels of protein levels of 35, 40, 45, 50 and 55%. Each diet was fed to triplicate groups of the fish in a flow-through system for 8 weeks. After the 8-week feeding trial, growth rate of fish fed 40% diet was not significantly different from that of fish fed 45, 50 and 55% diet, but significantly higher than that of fish fed 35% diet. The lowest feed efficiency was found in fish groups fed 35% diet. Protein efficiency ratio of fish fed 45, 50 and 55% diets was significantly lower than that of fish fed 35 and 40% diets. No significant differences were observed in feed intake and survival among all the fish groups. Hematocrit and hemoglobin values were increased with the increment of dietary protein level, even though it was not significant. Serum aspartate aminotransferase activity of fish fed 45% diet was significantly lower than that of fish fed the 35% diet, but this was not significantly different from that of fish fed 40, 50 and 55% diets. Whole body composition of fish was affected by dietary protein levels. Whole body protein content of fish fed 50 and 55% diet was significantly higher than that of fish fed 35% diet, but was not significantly different from that of fish fed 40 and 45% diet. Condition factor and hepato somatic index did not differ in all fish groups fed the experimental diets.

Our results indicate that juvenile tiger puffer require approximately 41% protein in their diet to obtain optimum growth of juvenile tiger puffer.

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I. INTRODUCTION

1.1 Tiger puffer in aquaculture

Tiger puffer (*Takifugu rubripes*) has been one of the promising cultured fish species in South Korea for the last decade, because of the desirable taste and high market price. Its market price has been a more than 1.5 times that of Japanese flounder and three times that of yellowtail or red sea bream in Japan (Kikuchi et al., 2006). The puffer fish is considered as one of the high value seafood in Asian countries.

There are approximately 100 different species of puffers in the world. In Korea, 18 species of puffers have been found along the coastline. Little is known about their nutritional requirements, especially the species *Takifugu rubripes*, which is one of the most important and newest aquaculture fish species in Jeju-Island, South Korea. Dry and moist pellets are fed by hand four to six times a day for those fish weighing less than 25 g size (Kumamoto Prefectural Fisheries Research Center 2001), because puffer fish has no stomach. The tiger puffer grew up to 1 kg, which is the minimum market size, within 18 months. Determination of dietary nutritional requirements is very important to increase growth rate of the species, to reduce its culture period and to decrease the cost of feeds.

1.2 Aquaculture of South Korea

The flounder, together with black rockfish, has been a key marine finfish species cultured in Korea since late 1980s. The highest production of cultured fish was from olive flounder (56.1%), followed by black rockfish (31.6%), mullet (4.8%), sea bream (3.4%), and sea bass (3.0%). The overproduction of the above fish species, especially flounder and rockfish, has resulted in a lower market price of them in South Korea. Therefore, many aquaculturists have been trying to find new fish species, such as tiger puffer, parrot fish and groupers.

1.3 Protein and amino acids

Proteins are composed of up to 20 α-amino acids linked into chains by peptide bonds. The amino acid compositions of proteins, particularly feed proteins, are markedly different. Several feed proteins such as gelatin or casein, are largely or even entirely deficient in one or more specific amino acids. However, fishmeal has a well balanced amino acids composition efficient for many fish species.

Protein source is predominated in feed ingredients, because it is a major important factor affecting growth performance of fish and the most expensive component in the fish feeds. Dietary protein provides the energy for maintenance and growth of fish, and plays the most important role as the components for the antibody, enzymes and hormones. Therefore, the continuous supply of protein with well-balanced amino acids is required for the health of the fish.

1.4 Protein requirements in fish

The protein requirement, meaning the minimum amount needed to meet requirements for amino acids and to achieve optimum growth of fish, is the first key factor to establish a formulated feed for a specific fish. The protein allowances in fish diets are appreciably higher than those in the diets of terrestrial warm-blooded animals.

Dietary Protein requirement usually become lower as fish grow. For instance, 35% protein produced faster growth rate than did 25% protein in 14 – 100g fish, but 25% dietary protein was enough to produce an optimum growth in channel catfish of 114 – 500g (Page and Andrews, 1973). Little evidence has exists to show that protein requirement is affected by water temperature. However, protein requirement for rainbow trout was unchanged from 35% at water temperatures ranging from 9° to 18°C (National Research Council, 1981).

Therefore, many studies have been done concerning dietary protein requirement of fish according to fish species, fish size, dietary protein ingredient and environmental conditions. However, the optimum dietary protein level for growth of tiger puffer is not available. In this study, we aimed to investigate the effect of the dietary protein levels on growth, hematological parameters and whole body compositions of juvenile tiger puffer fed semi-purified diets.

II. MATERIALS AND METHODS

2.1 Experimental diets

A feeding trial was conducted using a completely randomized design. The experimental diet formulation and proximate compositions are presented in Table 1, and 2. Five isocaloric experimental diets (gross energy, 17.4 MJ/kg) were formulated to contain graded level of protein (35, 40, 45, 50 and 55 %). The energy value of each diet was determined by using values of 16.7 KJ/g protein or carbohydrate and 37.6 KJ/g fat (Garling and Wilson, 1976). White fish meal, casein and soybean meal were used as protein sources, starch and wheat meal as carbohydrate sources and squid liver oil as a lipid source. The procedure of making experimental diets was shown in Figure 1. All dry ingredients were thoroughly mixed with distilled water. Pellets were extruded through the meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size and freeze dried at –40°C for 24 h. The pellets were crushed into desirable particle sizes (0.4 – 2.0 mm) and stored at –20°C until use.

Table 1. Formulation of the experimental diets (% dry matter)

	Dietary protein levels (%)				
Ingredients	**35**	**40**	**45**	**50**	**55**
White fish meal[a]	32.00	32.00	32.00	32.00	32.00
Casein (vitamin-free)[b]	5.50	11.00	16.50	22.00	27.50
Soybean meal[a]	6.50	6.50	6.50	6.50	6.50
Wheat flour[c]	18.00	18.00	18.00	18.00	18.00
Yeast[a]	2.00	2.00	2.00	2.00	2.00
Mineral mix[d]	1.00	1.00	1.00	1.00	1.00
Vitamin mix[e]	1.00	1.00	1.00	1.00	1.00
Squid liver oil[f]	8.00	8.00	8.00	8.00	8.00
Starch[a]	26.00	20.50	15.00	9.50	4.00

[a] Provided by Suhyup Feed Co. Ltd., Uiryeong, Korea.

[b] Casein was purchased from USB Co. Ltd., Cleveland, OH, USA.

[c] Wheat flour was purchased in the market.

[d] Mineral premix (g/kg of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; $CuCl_2$, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[e] Vitamin premix (g/kg of mixture): L-ascorbic acid monophosphate, 100.0; DL-α tocopheryl acetate, 20.0; thiamin hydrochloride, 4.0; riboflavin, 4.4; pyridoxine hydrochloride, 4.0; niacin, 30.0; D-pantothenic acid hemicalcium salt, 14.5; myo-inositol, 40.0; D-biotin, 0.2; folic acid, 0.48; menadione, 0.2; retinyl acetate, 1.0; cholecalficerol, 0.05; cyanocobalamin, 0.01.

[f] Squid liver oil was purchased from E-Wha oil Co. Ltd., Busan, Korea.

Table 2. Proximate composition of the experimental diets (% dry matter)

	Dietary protein levels (%)				
Proximate composition	**35**	**40**	**45**	**50**	**55**
Dry matter, %[a]	12.5	10.9	13.2	12.6	10.7
Protein, % DM[b]	34.6	41.1	45.9	50.9	56.5
Lipid, % DM	10.7	10.6	10.6	10.7	10.6
Ash, % DM[c]	5.3	5.3	5.4	5.6	5.4
NFE, %[d]	36.9	32.1	24.9	20.2	16.8
Gross energy, MJ/kg[e]	17.4	17.4	17.4	17.4	17.4
P:E ratio (mg kJ^{-1})[f]	19.9	23.6	26.4	29.3	32.6

[a] Dry matter was analyzed according to AOAC (1995).

[b] Protein was analyzed using Kjeltec 2003 Analyzer Unit (Foss Tecator, Sweden).

[c] Ash was analyzed according to AOAC (1995).

[d] Nitrogen Free Extracts = 100 - (%Moisture + %CP + %Lipid + %Ash).

[e] Estimated energy (Garling and Wilson 1976).

[f] Protein to energy ratio in mg protein kJ^{-1} of gross energy.

A) Grinding of ingredients
B) Weighing of ingredients
C) Mixing of ingredients with oil and distilled water
D) Pelletting of mixed dough
E) Freeze dryer
F) Sieving of size

Figure 1. The procedure of making experimental diet

2.2 Fish and feeding trial

Juvenile tiger puffer fish were transported from a private hatchery (Sa-Jo Fisheries Co.) in Jeju-Island to Marine and Environmental Research Institute, Cheju National University. All the transported fish were fed a commercial diet for 2 weeks to be acclimated in the experimental facilities and conditions, and to be recovered from the stress of transportation. One hundred eighty fish (initial body weight 17.05 ± 0.2 g) were randomly distributed into fifteen 100L-tanks (12 fish/tank) in a flow through system supplied with sand filtered seawater at water flow rate of 3 L/min. The daily water replacement rate was about 60% of the total volume after feeding. Then one of the experimental diets was fed to three groups of fish at a feeding rate of 4% to 2% total body weight/day for 8 weeks. Fish were fed six times a day at 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00, seven days a week. The growth of fish was measured every two weeks and feeding rate was adjusted accordingly. Water quality parameters are presented in Table 3. Water temperature varied between 22℃ and 17.5℃ due to natural fluctuation in sea water temperature. Water temperature, dissolved oxygen and salinity were measured with electronic oxygen meter (Oxi 315i, WTW GmbH, Germany) and NH_4^+ (RQflex 10, Merck KGaA, Germany) and pH (SevenEasy, Mettler-Toledo GmbH, Switzerland) was measured every 2 weeks, sampling each tank. The diurnal cycle was 12-h light/12-h dark. Salinity during the experimental period was approximately 33 ‰.

Table 3. Water quality parameters (mean ± SD) of the fish rearing system

Temperature (°C)	pH	O_2 (mg L^{-1})	NH_4^+ (mg L^{-1})	Salinity (‰)
22-17.5	7.7-8.4	6.3-6.7	5.8-6.8	32.5-34.0

2.4 Sample collection

At the beginning and the end of feeding trial, all fish were weighed. Weight gain, feed conversion ratio, protein efficiency ratio and specific growth rate were calculated. Blood samples were obtained from the caudal vein of six fish from each tank (18 fish per treatment) using heparinized syringed with syringes after they were starved with tricaine methanesulfonate (MS-222) at a concentration of 100 mg/L. Immediately, hematocrit was measured using microhematocrit technique. After of measure hematocrit, blood plasma was collected after centrifugation (300 × g for 5 min) and stored at -70°C as separate aliquots for analysis of protein, cholesterol, hemoglobin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Six fish from each tank were randomly sampled at the end of the feeding trial and stored at -70°C subsequent proximate analysis of the whole body.

2.5 Proximate analyses

Proximate compositions of all ingredient, experimental diets and whole body were analyzed by AOAC methods. The fish samples were then rigorously blended into a homogeneous crumble/meal and used for determination of whole body lipid, protein and ash contents. Lipid content in 2 g samples was determined using the Soxhlet Method with extraction in ether at 120 °C (Soxhlet Extraction System C-SH6, Korea). Protein content ($N \times 6.25$) was determined in the fish crumble using the automated Kjeldahl method (Kjeltec Analyzer Unit 2300, FOSS, Sweden). Ash contents were determined after heating of the fish crumble at 550°C for 4 h. Nitrogen-free extract (NFE) was computed by taking the sum values for crude protein, lipid, ash, and moisture and subtracting this from 100.

2.6 Hematological parameters

Haematocrit (PVC) was determined by the microhaematocrit method (Brown 1980) and hemoglobin, ALT, AST, and cholesterol were measured using a Photometer CH100 Plus (Calenzano, Firenze, Italy). The hemoglobin and cholesterol were determined by end point method and ALT and AST activity were determined by kinetic method. Protein content in the serum was determined by the method of Bradford (1976).

2.7 Morphological parameters

The total length, body weight, liver weight and gonad weight of 9 fish/diet (3 fish/tank) were individually measured. Condition factor (CF; 100 x [fish weight (g)/fish length $(cm)^3$], and hepato somatic index (HSI; 100 x liver weight/body weight) were calculated.

2.8 Calculation and statistical analysis

The following variables were calculated:

Weight gain (%) = 100 × (final mean body weight – initial mean body weight)/initial mean body weight.

Specific growth rate (%) = [($\log_e$ final body weight – $\log_e$ initial body weight)/days] × 100.

Protein efficiency ratio = wet weight gain/total protein given.

Feed conversion ratio = dry feed fed/wet weight gain.

Feed intake (dry matter, g/fish) = total feed fed (g)/fish.

Data were subjected to one-way ANOVA in SPSS version 11.0. The significant differences between group means were compared using Duncan's multiple test. Data were presented as means ± standard deviations (SD). The percentage data were arcsine transformed before the ANOVA analysis. Differences were considered significantly at $P<0.05$.

III. RESULTS

3.1 Growth performance

Results of growth performance and feed utilization of fish fed the experimental diets were influenced by dietary protein level (Fig 2, 3, 4 and 5). After 8 weeks of the feeding trial, growth rate of fish fed 40% diet was not significantly different from that of fish fed 45, 50 and 55% diets, but significantly higher than that of fish fed 35% diet ($P < 0.05$). The lowest feed efficiency was found in fish groups fed 35% diet. Protein efficiency ratio of fish fed 45, 50 and 55% diets was significantly lower than that of fish fed 35 and 40% diets ($P < 0.05$). No significant differences were observed in feed intake and survival among all the fish groups(Fig 6 and 7).

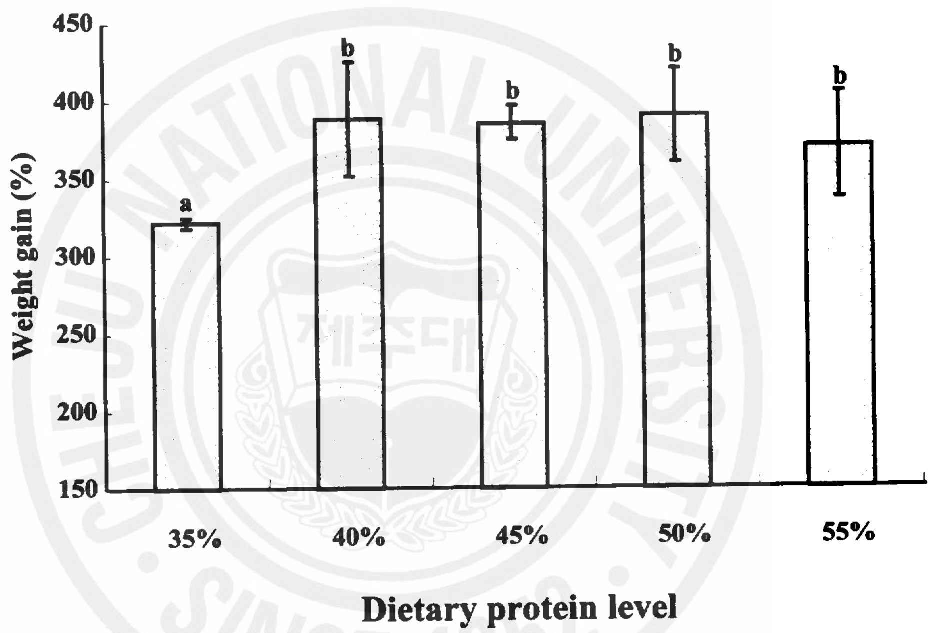

Figure 2. Weight gain (%) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

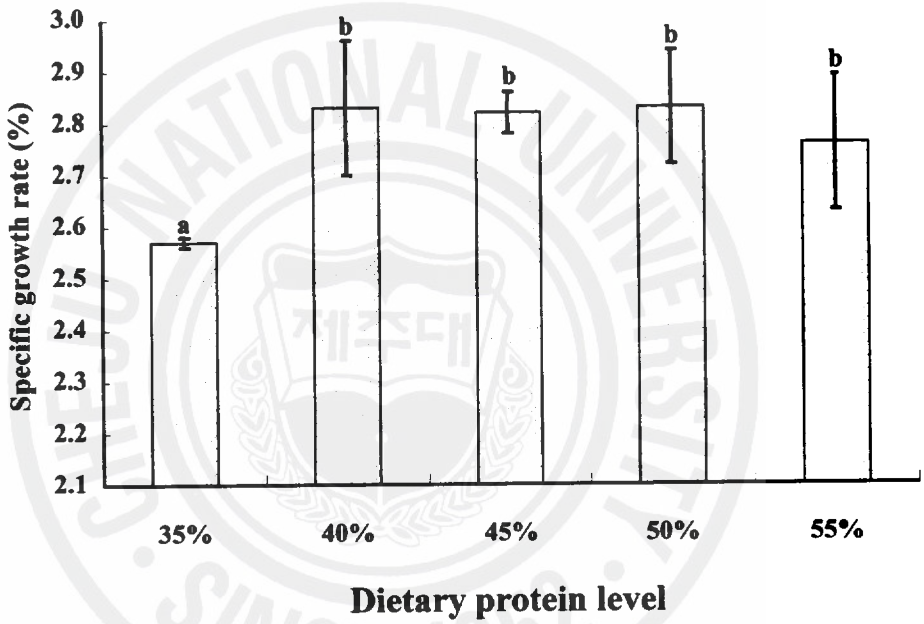

Figure 3. Specific growth rate (%) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

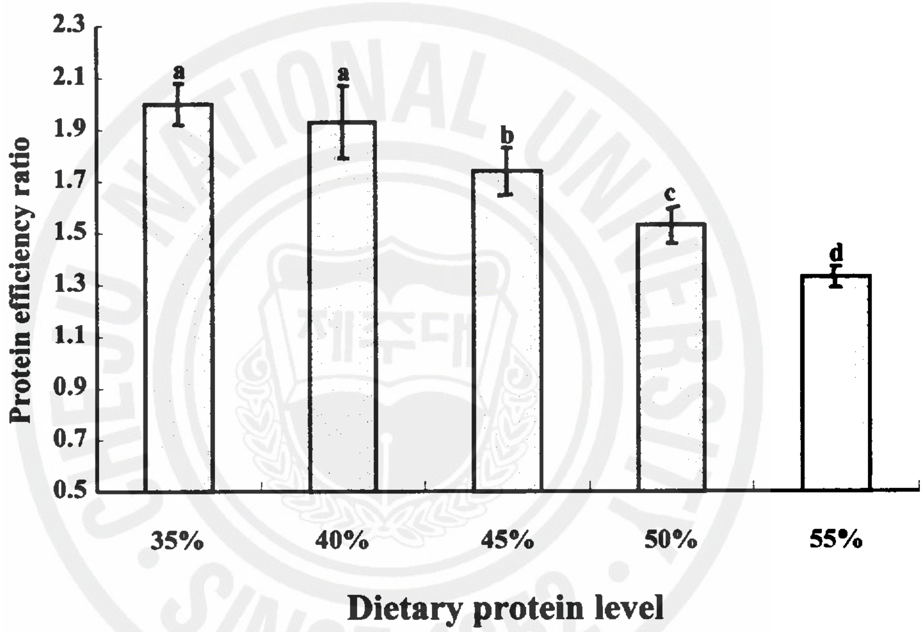

Figure 4. Protein efficiency ratio of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

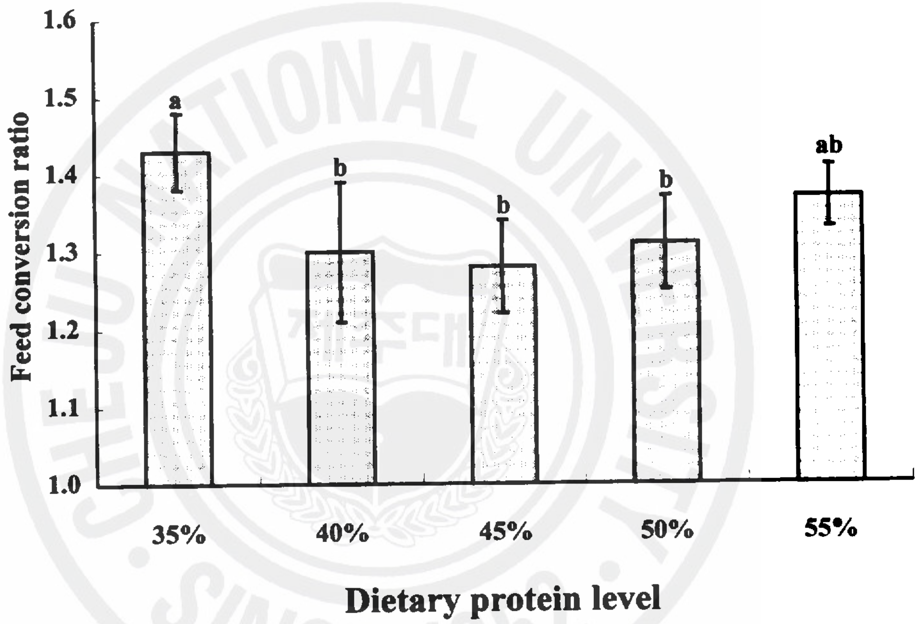

Figure 5. Feed conversion ratio of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

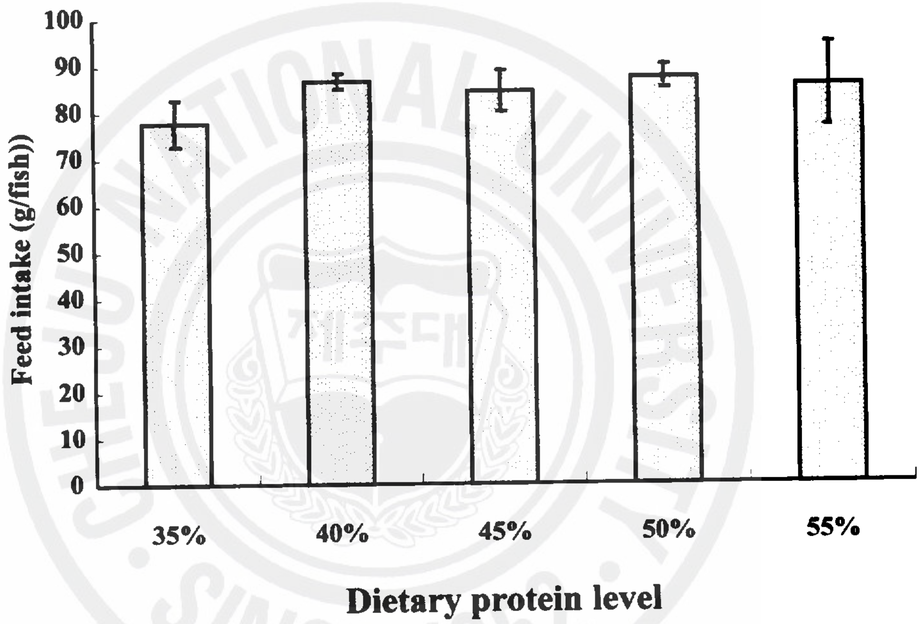

Figure 6. Feed intake (g/fish) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

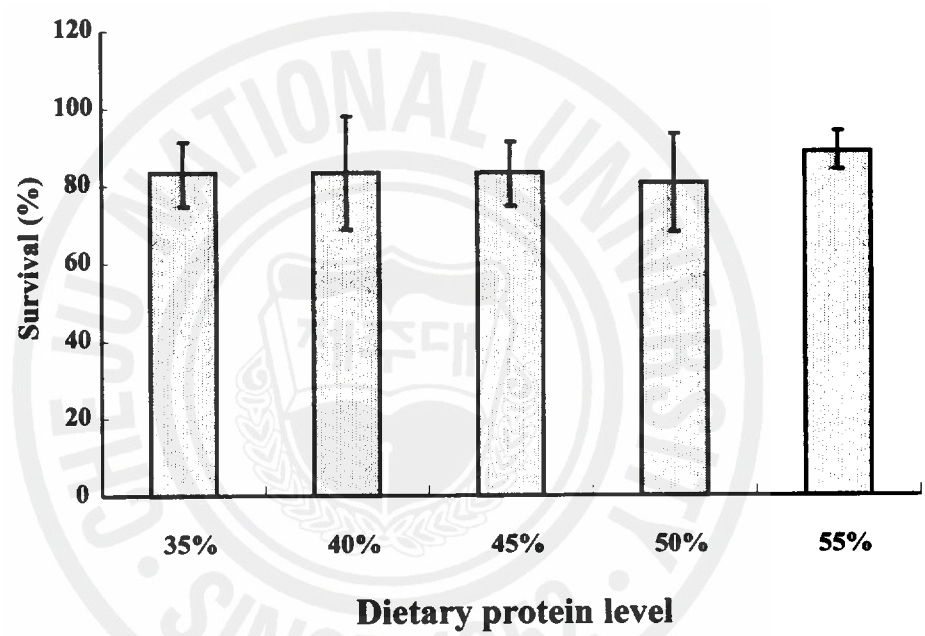

Figure 7. Survival (%) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

3.2 Whole body composition

Whole body composition of tiger puffer after 8 weeks feeding trial was affected by dietary protein levels (Table 4). Whole body protein content of fish fed 50 and 55% diet was significantly higher than that of fish fed 35% diet, but was not significantly different from that of fish fed 40 and 45% diet. Moisture and ash contents of whole body showed a similar trend of protein content affected by dietary protein level, the highest value of moisture and ash contents were found in 50% diet. Whole body lipid content was not significantly affected by dietary protein level.

Table 4. Whole body composition of juvenile olive flounder fed the experimental diets with increasing levels of dietary protein for 8 weeks*

Parameters	Initial	Protein level (%)					Pooled SEM[1]
		35	40	45	50	55	
Moisture (%)	79.6±0.6	75.5	75.2	75.7	76.8	75.6	0.29
Protein (%, DM)	69.9±2.1	56.3[a]	57.9[ab]	60.3[ab]	61.5[b]	61.2[b]	0.74
Lipid (%, DM)	10.2±0.5	12.8	15.6	15.0	13.4	16.1	0.81
Ash (%, DM)	14.6±0.5	9.1[a]	9.4[ab]	9.8[ab]	10.2[b]	9.6[ab]	0.21

* Values are presented as mean ± SD. Values in the same row having different letters are significantly different ($P<0.05$).

[1] Pooled standard error of mean: SD/√n.

3.3 Hematological parameters

Hematological parameter of tiger puffer after 8 weeks feeding trial was influenced by dietary protein level. There were no significant differences in hematocrit, hemoglobin, alanine aminotransferase and protein content in serum of fish fed all the experimental diets. However, serum alanine aminotransferase and aspartate aminotransferase activity were lower by dietary protein level. Serum aspartate aminotransferase activity of fish fed the 45% diet was significantly lower than that of fish fed the 35% diet, but this was not significantly different from that of fish fed 40, 50 and 55% diets (Fig 12). Cholesterol content in serum of fish fed the 35% diet was significantly higher than the other groups (Fig 13).

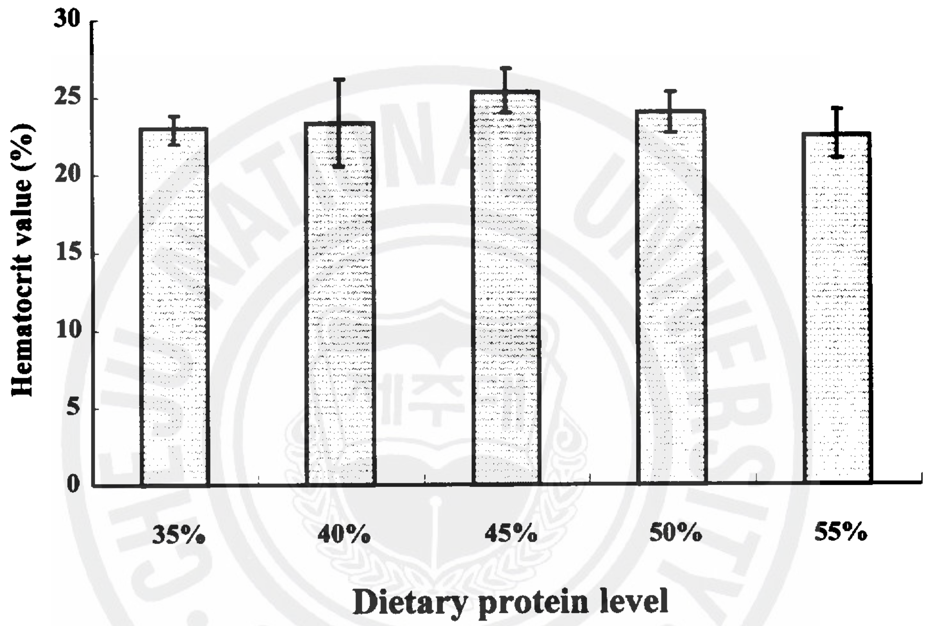

Figure 8. Hematocrit value (%) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

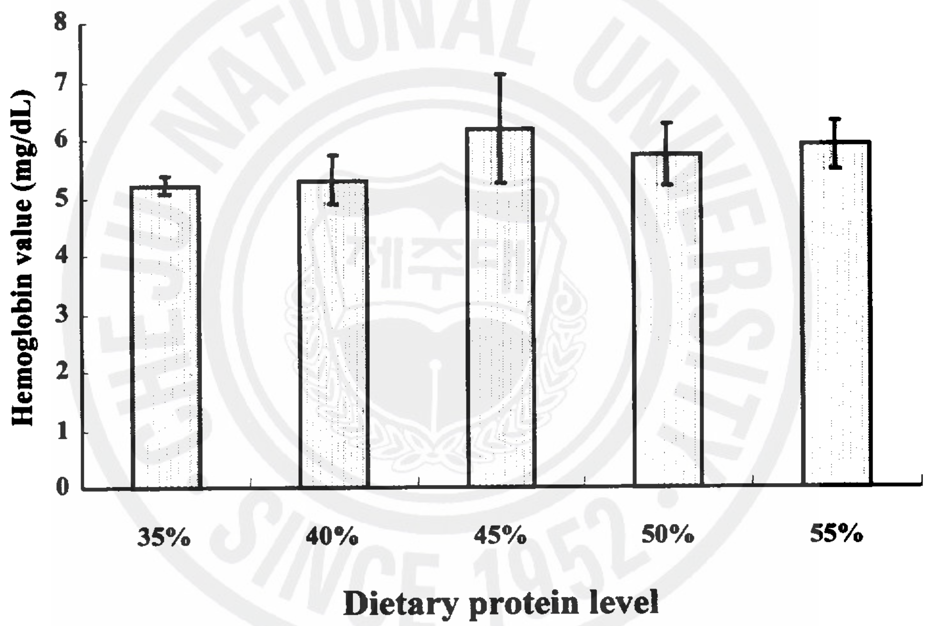

Figure 9. Hemoglobin value (mg/dL) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

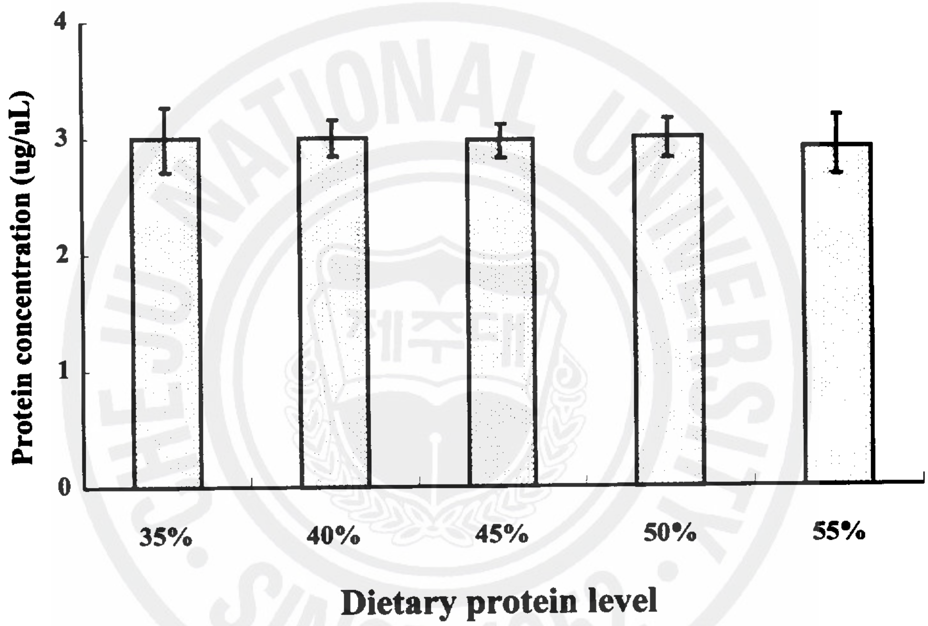

Figure 10. Protein concentration in serum (ug/uL) of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

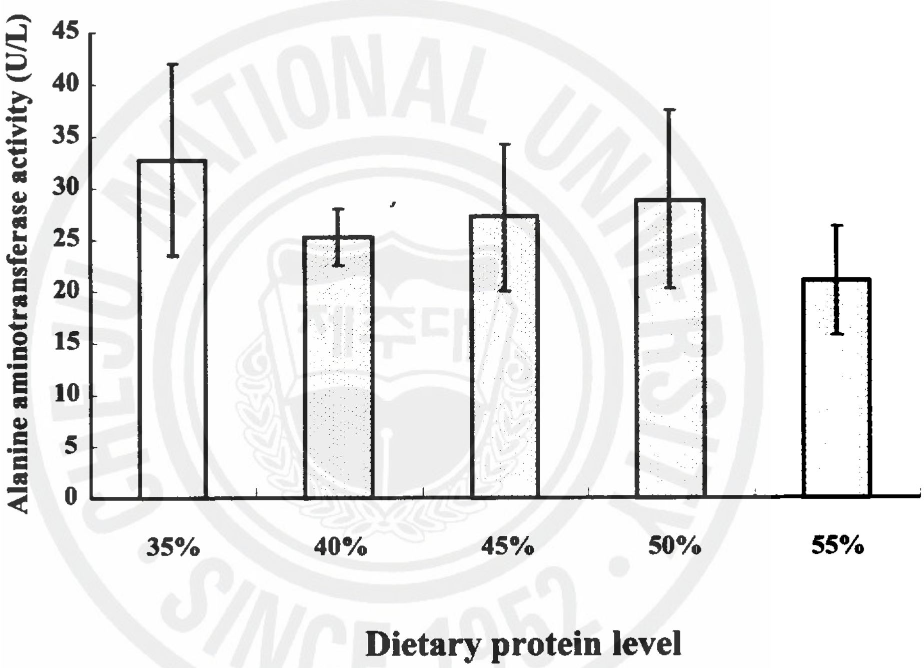

Figure 11. Alaine aminotransferase activity of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different ($P<0.05$).

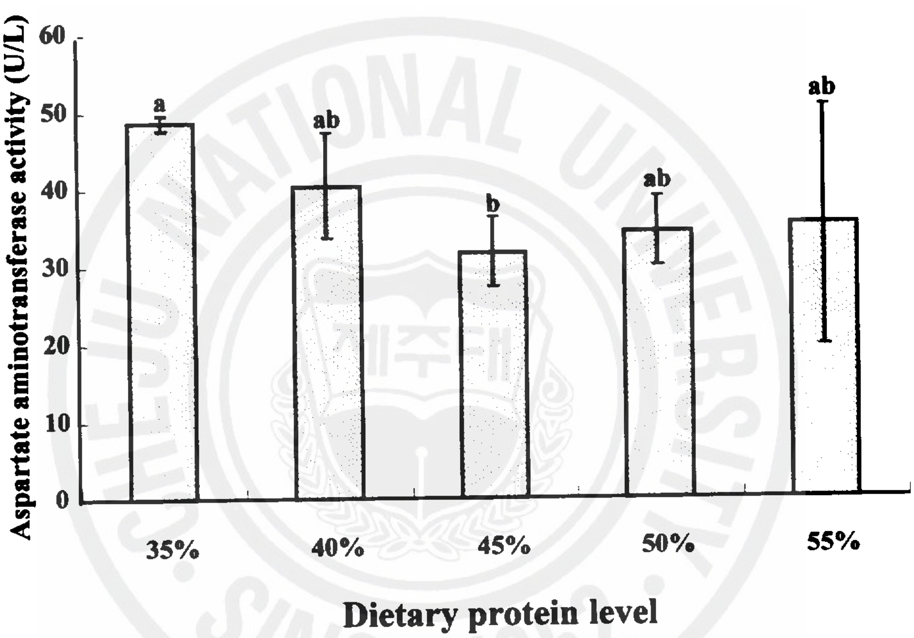

Figure 12. Aspartate aminotransferase activity of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

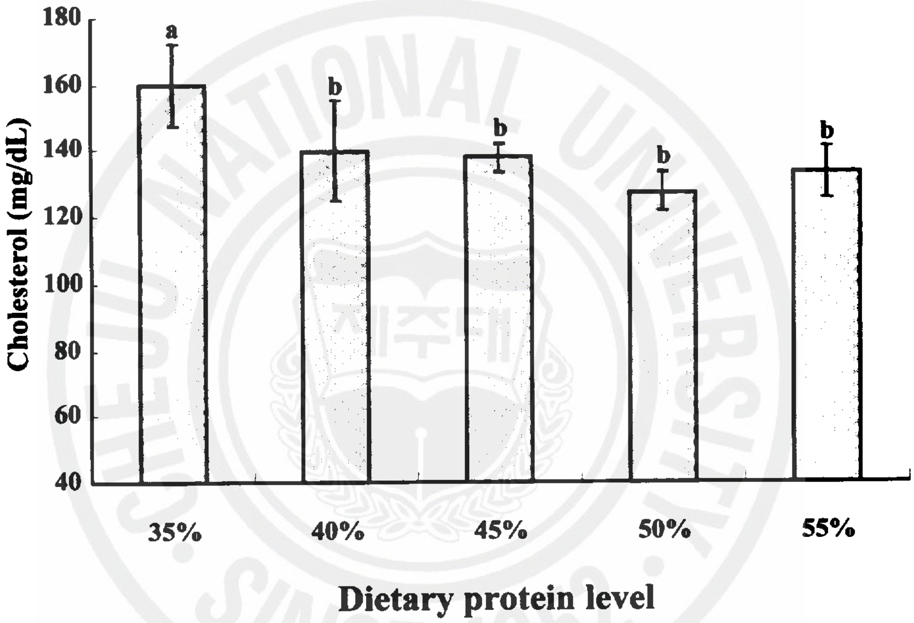

Figure 13. Serum cholesterol concentration of juvenile tiger puffer fed the experimental diets for 8 weeks. Values are mean of three replicates per treatment. Bars with different letters are significantly different (P<0.05).

3.4 Morphological parameter

Condition factor (CF) and hepato somatic index (HSI) did not differ in all fish groups fed the experimental diets (Table 5). However, HIS was extremely high compared to the result of other species, such as olive flounder and parrot fish.

Table 5. Morphological parameter of juvenile tiger puffer fed the experimental diets with increasing levels of dietary protein for 8 weeks*

Parameters	Initial	Protein level (%)					Pooled SEM[1]
		35	40	45	50	55	
Hepato somatic index	7.1±1.3	12.25	12.01	12.34	11.99	12.00	0.23
Condition factor	2.4±0.3	2.66	2.75	2.82	2.73	2.63	0.05

* Values are presented as mean ± SD. Values in the same row having different letters are significantly different (P<0.05).

[1] Pooled standard error of mean : SD/√n.

III. DISCUSSION

In the present study, dietary protein level had a significant effect on the growth of the fish. Dietary protein level of 40% showed significantly better growth performance than the 35% protein level, but not significantly different from that of fish fed 45, 50 and 55% diets. No improved growth performances of the tiger puffer by higher protein levels (45-55 g kg^{-1}) were clearly found in this study. There have been many studies showing that the excess protein in the diet can result in additional energy costs in many other fish for deamination and consequent reduction in energy for growth (Cho et al., 1985; De Silva and Perera, 1985; Tacon and Cowey, 1985; Shiau and Huang, 1989; Vergara et al., 1996). In the case of excessive dietary protein, the growth rate of fish remains constant or decreases because dietary protein is used to metabolize the excess amino acids absorbed (Jauncey, 1982).

Tiger puffer did not seem to have enough protein for their optimum growth when fed with 35% dietary protein level. Weight gain of juvenile tiger puffer increased with the increment of dietary protein levels up to 40% and then no more increment at higher protein levels. Similar trends were also observed in many other fish species (Yang et al. 2002; Martinez-Palacios et al. 2007). However, weight gain in this study was higher compared to the result of Kanazawa et al. (1980) who used casein-based purified diets in the puffer fish (*Fugu rubripes*). The present study suggests that semi-purified diet could be utilized better than the purified diet.

Juvenile tiger puffer in this study seemed to have an estimated optimum protein requirement of 41%, similar to that reported for juvenile fish of many species (NRC 1993) such as chinook salmon (DeLong et al. 1958), coho salmon (Zeitoun et al. 1974), gilthead sea bream (Sabaut and Luquet. 1973), largemouth bass (Anderson et al. 1981), milk fish (Lim et al. 1979), and mossambique tilapia (Jauncey, 1982). However, the protein level was lower than that recommended for carnivorous fish, such as Atlantic salmon, Japanese ell, smallmouth bass, red sea bream, snakehead, and yellowtail (Lall and Bishop. 1977; Nose and Arai. 1972; Anderson et al. 1981; Yone 1976; Wee and Tacon. 1982; Takeda et al. 1975), which have maximal growth when fed diets with protein levels of 45 to 55%. The dietary protein level that resulted in maximum weight gain of juvenile tiger puffer in this study was much different to the value of 55% reported by Kanazawa et al. (1980). The difference between the two studies can be explained by different experimental conditions (feeding methods, feeding frequency, age, stocking density, and water temperature) used. The biggest difference the studies, which made a big gap in dietary protein requirement (41% and 55%) was the protein sources used (white fish meal versus casein) and/or different fish sizes.

Hematological parameters, such as, hematocrit, hemoglobin, protein concentration, alanine aminotransferase (ALT), aspartate amonotransferase (AST) and cholesterol in tiger puffer fed the five diets were shown in Fig 8, 9, 10, 11, 12 and 13. There were no significant differences in hematocrit, hemoglobin, ALT and protein content in serum of fish fed all the experimental diets. The AST activity and cholesterol content of serum were significantly lower by dietary protein level (Fig 12 and 13). It is well known that ALT and AST activities are usually used as general indicators for the functioning of vertebrate liver. High AST and ALT generally, but not definitively, indicate a weakening or damage of normal liver function. For finfish, AST and ALT have been used extensively in studies that evaluate finfish response to toxins, stress, disease, and malnutrition (Chien et al., 2003). The present results indicated that an increase in ALT and AST values was responded to the poor growth performance, especially in the 35% groups.

The importance of dietary protein in the regulation of cholesterol metabolism has been well established in various species including humans and rats (Huang et al. 1993). Saeki and Kiriyama (1990) reported that the difference in the amino acid composition of casein is the main factor affecting plasma cholesterol level. Also, Madani et al (1998) reported that dietary protein affect enzyme activities involved in cholesterol metabolism and fatty acid desaturation. Non-protein components (such as fiber, phytic acid, minerals and isoflavones) also seemed to have an effect on cholesterol metabolism (Potter, 1995). Gaye-Siessegger et al. (2006) reported that a low-protein/high-carbohydrate diet led to significantly higher lipid gain and apparent lipid conversion values. The finding in the present study was well demonstrated by the previous studies (Saeki and Kiriyama, 1990; Madani et al. 1998; Potter, 1995; Gaye-Siessegger et al. 2006). However, the mechanism for the decreased serum cholesterol in fish fed higher protein levels can not be explained through the present study.

Whole body composition of tiger puffer after 8 weeks feeding trial was affected by dietary protein levels (Table 4). Generally, whole body protein contents were positively correlated with the dietary protein levels. In this study, whole body protein contents of fish fed 50 and 55% diets were significantly higher than that of fish fed 35% diet, but not significantly different from 40 and 45% diets. A similar result has been reported for many fish species (Bai et al. 1999; Yang et al. 2002; Kim et al. 2001; Lee et al. 2001). Ash content of whole body showed a similar trend of protein content, whereas moisture and lipid contents of fish were not significantly different among all the fish groups. Body moisture was not correlated to the dietary protein levels, which was consistent with reports for other fishes. Results from our study showed body lipid and moisture were not positively correlated to dietary protein levels. However, body protein and ash of whole body were significantly increased with increasing dietary protein levels.

Some studies have reported that the Condition factor (CF) and hepatosomatic index (HSI) were significantly affected by the dietary nutritional factors, especially main nutrients, such as carbohydrate, lipid and protein (Jover et al. 1999; Kim and Lall 2001). No significant differences were observed in CF and HSI among all the fish groups in this study (Table 5). However, HSI value of this study was extremely high compared to the result of the other fish species such as olive flounder, parrot fish, rockfish and silver perch (Lim et al. 2006; Pham and Lee. 2007; Lee et al. 2002; Yang et al. 2002). Further studies are needed to determine parameters related to HSI in juvenile tiger puffer.

Many studies have been done concerning protein to energy ratio. Protein to energy ratio is very important to establish a formulated feed for a specific fish species and usually affects its protein requirement (Kim et al. 2004; Kim et al. 2005; Somsueb and Boonyaratpalin. 2001; Ramseyer and Garling. 1998). In this study, the protein/energy ratio of the experimental diets was ranged from 19.9 to 32.6 mg/kJ and thus might have affected the growth performance of juvenile tiger puffer. Further studies are needed to determine the optimum potein/energy ratio for juvenile tiger puffer.

We concluded that the optimum dietary protein level is approximately 41% for optimum growth rate of juvenile tiger puffer.

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VI. SUMMARY

In nutrition study of fish, determining the optimum dietary protein level for growth is generally a primary consideration. Protein requirements change with respect to changes in biotic factors (e.g., species, physiological state, size) and dietary characteristics (e.g., protein quality, protein:energy ratio). Therefore, many studies have been done concerning dietary protein requirement of fish according to fish species, fish size, dietary protein ingredient and environmental conditions. However, the optimum dietary protein level for growth of tiger puffer is not available.

Tiger puffer did not seem to have enough protein for their optimum growth when fed with 35% dietary protein level. In this study, weight gain of juvenile tiger puffer increased with increment in dietary protein levels up to 40% and then no more increment at higher protein levels. In this study, the protein/energy ratio of the experimental diets was ranged from 19.9 to 32.6 mg/kJ and thus may affect the growth performance of juvenile tiger puffer.

Our results indicate that juvenile tiger puffer require approximately 41% protein in their diet to obtain optimum growth of juvenile tiger puffer. Further studies are needed to determine the optimum protein/energy ratio and amino acid requirements on growth performance of juvenile tiger puffer.

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그리고 연구하는 동안 정성껏 많은 지원과 격려를 해 주신 양어사료영양학 실험실의 가장 큰 형님이며, 든든한 후원자이신 하나동물약품의 장계환형님과 남원에서 양식장을 경영하고 있는 박영준 형님, 실험실 선배로서 많은 가르침과 격려를 해준 임세진선배님, 지금은 멀리 미국에 있지만 항상 많은 가르침과 조언을 해준 이봉주선배님, 얼마 전에 공부한다고 미국으로 떠난 차지훈선배님 고맙습니다. 또한 매 실험마다 헌신적으로 도와준 우리 양어사료영양학실험실 식구 후배님들, 장지웅, 고경용, 어진이, 오대한, 송진우 정말 고맙습니다. 멀리 칠레에서 한국까지

유학와서 고생하는 헤르만 페드로 부에노 갈라즈 삼춘과 베트남에서 와서 정말 열심히 공부하고 있는 팜민안형에게도 감사의 말씀을 드립니다. 또한 사육실험에 물심양면으로 많은 도움을 주신 해양과환경연구소 행정실 직원들과 발생학실험실원, 무척추동물양식실험실원, 면역학실험실원 모두에게도 감사의 마음을 전합니다.

항상 멀리 고향에서 학문에 전념할 수 있도록 노심초사 뒷바라지를 해주신 어머니, 아버지, 그리고 언제나 지지해 주고 큰 버팀목이 되어준 큰형님, 작은형님의 가족들에게도 가슴속 깊이 감사를 드립니다.

끝으로 지금의 제가 있기까지 헌신적인 믿음과 사랑을 주고 내조를 해준 사랑하는 아내 곽명수와 이 기쁨과 영광을 함께하고 싶습니다.

정말 많은 분들의 도움으로 지금의 제가 여기까지 오게 되었습니다. 제 인생의 좌우명처럼 항상 감사하며 살겠습니다. 정말 많은 도움과 격려를 주신 모든 분들께 다시 한번 감사드리며, 앞으로 더욱 더 인내와 끈기를 가지고 성실하게 학문에 매진하고자 합니다. 열심히 하겠습니다. 감사합니다.