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Short communication

Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*)

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ABSTRACT

This study was conducted to determine the optimum dietary protein requirement for the growth of juvenile (initial weight, 17.05±0.17 g) tiger puffer. 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 of fish fed the 40% diet was not significantly different from that of fish fed 45, 50 or 55% dietary protein, but significantly higher than that of fish fed the 35% diet. The lowest feed efficiency was found in fish groups fed 35% diet. Protein efficiency ratio of fish fed the 45, 50 and 55% diets was significantly lower than that of fish fed the 35 and 40% diets. No significant differences were observed in feed intake and survival among all the fish groups. Serum aspartate aminotransferase activity of fish fed the 45% diet was significantly lower than that of fish fed the 35% diet, but was not significantly different from that of fish fed the 40, 50 and 55% diets. Whole body protein content of fish fed the 50 and 55% diets was significantly higher than that of fish fed the 35% diet, but was not significantly different from that of fish fed the 40 and 45% diets. This result indicates that juvenile tiger puffer requires approximately 41% dietary protein for optimum growth and physiological performances.

1. Introduction

Tiger puffers have been one of the promising cultured fish species in Japan and South Korea for the last decade, because of their desirable taste and high market price. The puffer fish is considered as a high value seafood specie 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 specie *Takifugu rubripes*, which is one of the most important and the newest cultured fish species in South Korea. Dry or moist pellets are fed to this specie at four to six times a day (Kumamoto Prefectural Fisheries Research Center, 2001), because puffer fish do not have a stomach. The puffer fish grow very slowly and reach 1 kg, body weight by 17–18 months. Determination of nutritional requirements is very important to increase growth of the species, to reduce its culture period and to decrease feed cost.

The protein requirement, meaning the minimum amount needed to meet the amino acid requirements and achieve optimum growth, is the first nutritional parameter to be determined for formulated feed production for a newly established cultured fish specie.

Many studies have been conducted concerning the dietary protein requirement of fish which many vary with fish species, fish size, dietary protein source and environmental conditions. Nutritional studies are available on dietary lipid (Takii et al., 1995a) and carbohydrates (Takii et al., 1995b) for tiger puffer. However, the optimum dietary protein level for optimum growth and/or physiological performances of juvenile tiger puffer is limited (Kanazawa et al., 1980). In this study, therefore, we aimed to investigate the optimum dietary protein level for this fish specie with semi-purified diets.

2. Material and methods

2.1. Experimental diets and design

A feeding trial was conducted using a completely randomized design. Five isocaloric experimental diets (gross energy, 17.4 MJ/kg) were formulated to contain graded levels of 35, 40, 45, 50 and 55% crude protein (Table 1). The gross energy value of each diet was determined by using values of 16.7 kJ/g protein or carbohydrate and 37.6 kJ/g lipid (Garling and Wilson, 1976). White fish meal, casein and soybean meal were used as protein sources. All dry ingredients were thoroughly mixed with distilled water. The mixed dough were extruded through the meat chopper machine (SMC-12, Korea) into 3.0 mm diameter size and freeze-dried at −40 °C for 24 h. The pellets were crushed into desirable particle sizes and stored at −20 °C until used.

2.2. Experimental fish and feeding trial

Juvenile tiger puffers (*Takifugu rubripes*) were transported from a private hatchery (Sa-Jo Fisheries Co., Jeju-Island, Korea) to the Marine and Environmental Research Institute, Cheju National University, South Korea. The fish were fed a commercial diet for 2 weeks to

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Table 1
Formulation and proximate composition of experimental diets (% dry matter)

Ingredients	Dietary protein levels (%)				
	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
Potato starch[a]	26.00	20.50	15.00	9.50	4.00
Proximate composition					
Dry matter, % DM	12.5	10.9	13.2	12.6	10.7
Crude protein, % DM	34.6	41.1	45.9	50.9	56.5
Crude lipid, % DM	10.7	10.6	10.6	10.7	10.6
Ash, % DM	5.3	5.3	5.4	5.6	5.4
NFE, % DM[g]	36.9	32.1	24.9	20.2	16.8
Gross energy, MJ/kg DM[h]	17.4	17.4	17.4	17.4	17.4
P:E ratio (mg kJ^{-1})[i]	19.9	23.6	26.4	29.3	32.6

[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): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; $CuCl_2$, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 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.
[g] Nitrogen Free Extracts = 100 − (%moisture + %protein + %lipid + %ash).
[h] Estimated energy (Garling and Wilson, 1976).
[i] Protein to energy ratio in mg protein kJ^{-1} of gross energy.

acclimate them to the experimental facilities and conditions and to recover from the stress of transportation. One hundred eighty fishes (initial body weight 17.05 ± 0.2 g) were randomly distributed into 15–100 L tanks (12 fishes/tank) in a flow-through system supplied with sand filtered seawater at flow-rate of 3 L/min. Each of the experimental diets was fed to three replicate groups of fish at a feeding rate of 2 to 4% biomass per day (actual amount the fish fed satiation) for 8 weeks. Fishes were fed six times a day at 08:00, 10:30, 12:30, 14:30, 16:30 and 18:00 h, 7 days a week. Growth was measured every 2 weeks and the feeding rate was adjusted accordingly. Water temperature was between 22 and 17.5 °C by natural fluctuation in seawater temperature. The diurnal cycle was 12-h light/12-h dark. Salinity during the experimental period was approximately 33‰.

2.3. Sample collection and analytical methods

At the beginning and end of feeding trial, all the fishes 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 6 fishes from each tank (18 fishes per dietary treatment) using heparinized syringe after anesthetization of the fish with tricaine methanesulfonate (MS-222) at a concentration of 100 mg/L. Immediately, hematocrit and hemoglobin were measured using microhematocrit technique (Brown, 1980) and CH 100 plus blood biochemical auto analyzer (SEAC, Italy), respectively. After the measurement with whole blood, blood plasma were collected after centrifugation at 300 ×g for 5 min and stored at −70 °C as separate aliquots for analyses of protein, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by blood biochemical auto analyzer. The hemoglobin and cholesterol were determined by an end point method and alanine aminotransferase and aspartate aminotransferase activity were determined by a kinetic method. Protein content in the serum was determined by the method of Bradford (1976). The remaining 6 fishes from each tank were sampled at the end of the feeding trial and stored at −70 °C for the subsequent proximate analysis of whole body by standard methods (AOAC International, 1997).

Table 2
Growth performance and feed utilization of juvenile tiger puffer fed the semi-purified diets with increasing levels of dietary protein for 8 weeks[a]

Parameters	Protein level (%)					Pooled SEM[g]
	35	40	45	50	55	
WG (%)[b]	322[a]	389[b]	385[b]	390[b]	371[b]	8.98
SGR (%)[c]	2.57[a]	2.83[b]	2.82[b]	2.83[b]	2.76[b]	0.03
PER[d]	2.00[a]	1.93[a]	1.74[b]	1.53[c]	1.33[d]	0.07
FCR[e]	1.43[a]	1.30[b]	1.28[b]	1.31[b]	1.37[a,b]	0.02
FI, (DM, g/fish)[f]	77.8	86.7	84.3	87.5	85.5	1.44
Survival (%)	83.3	83.3	83.3	80.6	88.9	2.36

[a] Data are means of three replicate groups; values in the same row with different superscripts are significantly different ($P<0.05$).
[b] Weight gain (%) = 100 × (final mean body weight − initial mean body weight)/initial mean body weight.
[c] Specific growth rate (%) = [($\log_e$ final body weight − $\log_e$ initial body weight)/days] × 100.
[d] Protein efficiency ratio = wet weight gain/total protein given.
[e] Feed conversion ratio = dry feed fed/wet weight gain.
[f] FI (dry matter, g/fish) = total feed fed (g)/fish.
[g] Pooled standard error of mean: SD/√n.

2.4. Statistical analysis

All data were subjected to one-way ANOVA in SPSS version 11.0 (Chicago, IL, USA). Significant differences among the group means were compared using Duncan's multiple test. Data are presented as mean ± standard error.

3. Results

After 8 weeks of the feeding trial, growth of fish fed the 40% diet was not significantly different from that of fish fed the 45, 50 and 55% diets, but significantly higher than that of fish fed the 35% diet (Table 2). The lowest feed conversion ratio was found in fish groups fed the 35% diet. Protein efficiency ratio of fish fed the 45, 50 and 55% diets was significantly lower than that of fish fed the 35 and 40% diets. No significant differences were observed in feed intake or survival among all fish groups.

There were no significant differences in hematocrit, hemoglobin, ALT activity or serum protein level of fish fed all the experimental diets (Table 3). However, serum AST activity of fish fed the 45% diet was significantly lower than that of fish fed the 35% diet, but this was not significantly different compared to that of fish fed the 40, 50 and 55%

Table 3
Hematological parameters of juvenile tiger puffer (initial weight, 17.05 ± 0.17 g) fed the semi-purified diets with increasing levels of dietary protein for 8 weeks[a]

Parameters	Protein level (%)					Pooled SEM[d]
	35	40	45	50	55	
Hematocrit (%)	23.0	23.8	25.4	24.00	22.6	0.46
Hemoglobin (%)	5.22	5.31	6.18	5.74	5.89	0.16
ALT (U/L)[b]	32.7	25.2	27.1	28.8	21.1	1.85
AST (U/L)[c]	48.6[a]	40.5[a,b]	31.8[b]	34.4[a,b]	35.2[a,b]	2.39
Cholesterol (mg/dL)	159.9[a]	139.9[b]	137.7[b]	127.4[b]	133.6[b]	3.66
Serum protein (μg/μL)	2.99	3.00	2.97	2.99	2.92	0.04

[a] Data are means of three replicate groups; values in the same row with different superscripts are significantly different ($P<0.05$).
[b] ALT = alanine aminotransferase, unit per liter (U/L) is the amount of enzyme which oxidizes 1 μmol/L of NADH per minute.
[c] AST = aspartate aminotransferase.
[d] Pooled standard error of mean: SD/√n.