Suitable Sugar Level in Brown Fish Meal Diet for Tiger Puffer

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Tiger puffer *Takifugu rubripes*, weighing about 10 g, were reared for 28 days on brown fish meal diets containing 10, 13, 16, 19, and 22% white dextrin.

Except for a low survival (75%) obtained in the fish reared on the lowest white dextrin diet, the fish reared on other diets and sandeel, as a reference, showed high survival (>95%). Under a similar daily ration size, the best weight gain, feed efficiency, and protein efficiency ratio were commonly noted in the fish fed the 16% dextrin diet. No marked differences among the dietary treatments were found in the proximate compositions of whole body and the hepatopancreas. Apparent digestibilities of dietary carbohydrate and protein remained high in the fish given 13–16% and 10–19% dextrin diets, respectively, using the intestinal digesta dissected at 5 h after feeding.

These results indicate that a suitable dietary dextrin level for the puffer is estimated around 16% and dietary carbohydrate content about 20%.

Key words: tiger puffer, growth performance, digestibility, protein sparing effect, dietary sugar

Physiologically, tiger puffer *Takifugu rubripes* do not have a stomach but an expansion sac, a portion between the esophagus and the intestine, differentiating from an intestinal organ at their early life stages.¹⁾ Moreover, somatic growth and digestion were improved by feeding a preferable diet supplemented with scallop *Patinopecten yessoensis* extract.¹⁾ Kanazawa *et al.*²⁾ had already shown that dietary levels of casein, pollack liver oil, mineral mixture, and vitamin mixture for the puffer were estimated at 50, under 6, at 4, and at 3%, respectively. Otherwise, Kakuta *et al.*³⁾ also showed that suitable dietary levels of white fish meal, pollack liver oil and dextrin were about 50, 8 and 34%, respectively. The estimation of a suitable dietary sugar level in brown fish meal diet is necessary to conduct and progress the development of practical diet and the culture technique for the puffer.

Materials and Methods

Test Diet

Test diets 1–5 were composed of graded white dextrin levels of 10, 13, 16, 19, and 22% at the expense of cellulose, as appeared in Table 1. All diets were commonly composed of 49% brown fish meal, 10% corn gluten meal, 5% pollack liver oil, 3% vitamin mixture,⁴⁾ 8% mineral mixture,⁴⁾ and 3% carboxymethylcellulose. Moreover, feeding stimulants,⁵⁾ mixture of L-aspartic acid, L-serine, glycine, L-alanine, and betaine, were supplemented to test diets for activating feeding of the puffer.

Test diets, a moist type pellet, were prepared using a laboratory pelleting machine after thoroughly mixing 100 parts of both the dry ingredients and pollack liver oil with 30 parts of cold tap water. Pellets, 1.9 mm in diameter and 2–3 mm in length, were stored at –20°C until use.

Proximate compositions of test diets are shown in Table

Table 1. Formula and proximate composition of test diets (%)

Ingredient	Diet					Sandeel
	1	2	3	4	5	
Brown fish meal	49	49	49	49	49	—
Wheat gluten	10	10	10	10	10	—
Pollack liver oil	5	5	5	5	5	—
White dextrin	10	13	16	19	22	—
Cellulose	12	9	6	3	—	—
Vitamin mixture* ¹	3	3	3	3	3	—
Mineral mixture* ¹	8	8	8	8	8	—
CM-cellulose	3	3	3	3	3	—
FS (g)* ²	1.183	1.183	1.183	1.183	1.183	—
Proximate composition						
Crude protein	46.9	46.0	43.3	45.6	47.2	71.3
Crude fat	8.7	9.0	8.9	9.4	9.1	14.4
Crude ash	11.6	12.0	11.9	12.1	12.0	14.4
Crude sugar	14.0	17.7	20.5	23.6	25.1	—
Gross energy* ³ (kcal/100 g diet)	405	418	413	443	456	539

*¹ Halver mixture.⁴⁾

*² Feeding stimulants (mg): L-aspartic acid; 21, L-serine; 24, glycine; 329, L-alanine; 130, and betaine; 679.

*³ Gross energy (kcal/g): protein; 5.65, fat; 9.45, and sugar; 4.10.

1. Dietary crude sugar were 14.0, 17.7, 20.5, 23.6, and 25.1% in diets 1–5, in that order. No marked differences were found in dietary crude protein, crude fat, and crude ash contents among test diets. Cutted sandeel *Ammodytes personatus*, as a reference, was given to compare growth performance with that of test diet groups.

The gross energy for dietary protein, fat, and sugar were based on values 5.65, 9.45, and 4.10 kcal/g, respectively.⁶⁾

Fish and Feeding Trial

The tiger puffer, having a mean body weight about 10 g, which were reared in Fisheries Laboratory, Kinki University after artificial fertilization, were used for the present study. After domesticating the fish to rearing conditions on an artificial diet (Marubeni Shiryō Co., Ltd.) for 7 days, 12 groups of 20 fish each were accommodated into 300 l indoor rectangular tanks (60 × 100 × 50 cm deep) under a running water system at 4 l filtered sea water per min. Water temperature during the feeding trial was 24.5 ± 1.2°C (Mean ± SD; n=28). The puffer were fed on test diets 3 times a day, 6 days a week for 28 days. A daily ration size was adjusted about 2.7% of body weight through the feeding trial. A 12 h light:dark cycle was carried under fluorescent tubes. The feeding trial was conducted under duplicated conditions.

On the initial and final day of feeding trial, the fish were weighed and then several fish were collected and decapitated to assay the proximate composition of whole body and the hepatopancreas. Growth performance of the dietary groups was estimated by the following expressions:

$$\text{feed efficiency} = \frac{\text{weight gain (wet)}}{\text{feed intake (dry)}} / 100;$$

$$\text{protein efficiency ratio} = \frac{\text{weight gain (wet)}}{\text{protein intake}};$$

$$\text{energy efficiency} = \frac{\text{weight gain (wet)}}{\text{energy intake (kcal)}} / 100;$$

and

$$\text{apparent nutrient retention} = \frac{\text{body nutrient deposited}}{\text{nutrient intake}} / 100.$$

For measuring apparent protein and sugar digestibilities, the fish fasted for 2 days and were fed on the test diets with 0.5% Cr₂O₃ after the end of feeding trial. We dissected the abdominal cavity and took out the intestines from 5 or 6 individuals in each group at 5 h after feeding, and then squeezed and pooled the intestinal digesta. The digesta was dried in an oven at 70°C for a day and stored in a desiccator at room temperature until assay.

Assays

The proximate compositions of the diets, whole body and the hepatopancreas were assayed by AOAC method.⁷⁾ Glycogen contents of the hepatopancreas and acid-digesti-

ble carbohydrate (crude sugar) contents of the diets were respectively measured by the anthron method⁸⁾ and phenol-sulfuric acid method.⁹⁾ The digestibilities of dietary protein and sugar were assayed by the indirect method cited by Furukawa and Tsukahara.¹⁰⁾

Statistical Analysis

The data was analyzed by one-way ANOVA to detect an experimental treatment effect ($p < 0.05$), if this was significant, differences between dietary treatments were identified by Duncan's multiple range test ($p < 0.05$).¹¹⁾ The standard deviations obtained from duplicate lots were divided by 0.798 for estimating population.¹²⁾

Results

Growth Performance

Survival and growth performance of the tiger puffer which were fed the test diets are shown in Table 2. No infectious diseases were superficially noted on the fish throughout the feeding trial. Mortality was mainly attributed to serious injuries, loss of caudal fin and peduncle, by biting each other. Despite the hypersensitive characteristic of the puffer, relatively good growth was obtained in all dietary groups.

High survivals of 95.0–97.5% were maintained in the dietary groups, except 77.5% in the group fed the diet 1. Weight gain, feed efficiency, protein efficiency ratio, and energy efficiency improved significantly by increasing the dietary crude sugar up to 20.5% in diet 3 group, but fell above the content. The highest apparent protein retention was noted in the group fed the diet 4 containing 23.6% crude sugar, followed by the diet 3, 5, 2, and 1 groups. Significant differences of the feed efficiency were not found among the reference feed group and diet 2–4 groups, but the weight gain, protein efficiency ratio, and energy efficiency of the latter groups were higher than the former group.

Proximate Composition

Tables 3 and 4 show the proximate composition of whole body and the hepatopancreas, respectively.

On the final day of the feeding trial, the crude protein content of whole body rose slightly by increasing the dietary crude sugar, and crude fat content in the diet 3 group was only higher than those in other groups. There were no meaningful differences in moisture and crude ash of the whole body among the dietary treatments.

Table 2. Growth performance of the puffer fed test diets for 28 days

Diet group	Dietary sugar (%)	Mean body weight(g)		Daily feeding rate (%)	Survival (%)	Growth rate (%)	Feed efficiency (%)	Protein efficiency ratio	Energy efficiency (%)	Apparent protein retention (%)
		Initial	Final							
1	14.0	10.3	25.7	2.60	77.5 ^{b*2}	133.2 ^b	102.0 ^b	2.18 ^b	25.2 ^c	24.2 ^{bc}
2	17.7	9.94	26.1	2.76	95.0 ^a	157.1 ^{ab}	112.7 ^a	2.51 ^a	27.0 ^{ab}	33.6 ^{ab}
3	20.5	10.4	27.3	2.77	97.5 ^a	163.3 ^a	115.0 ^a	2.65 ^a	27.9 ^a	35.2 ^a
4	23.6	10.0	25.8	2.68	95.0 ^a	155.5 ^{ab}	114.9 ^a	2.52 ^a	26.0 ^{bc}	37.5 ^a
5	25.1	10.3	25.8	2.80	95.0 ^a	144.4 ^{ab}	107.5 ^{ab}	2.28 ^b	23.6 ^d	33.2 ^{ab}
Sandeel	—	10.9	25.9	2.53	95.0 ^a	134.2 ^b	114.5 ^a	1.61 ^c	21.3 ^e	19.7 ^c
SE ^{*1}					3.13	6.91	2.62	0.06	0.33	2.79

*1 Standard error of mean.

*2 Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3. Proximate composition (%) of whole body of the puffer fed test diets for 28 days

Diet group	Moisture	Crude protein	Crude fat	Crude ash
Initial	77.6	15.7	3.1	2.3
Final				
1	78.6	13.3	3.5	2.5
2	77.6	14.5	3.5	2.3
3	79.6	14.2	3.7	2.2
4	78.0	15.2	3.4	2.4
5	78.2	15.1	3.2	2.6
Sandeel	79.5	13.7	3.0	2.7

Table 4. Hepatosomatic index and proximate composition (%) of the puffer fed test diets for 28 days

Diet group	Hepatosomatic index	Moisture	Crude protein	Crude fat	Crude ash	Glycogen
Initial	9.56 ± 0.77 ^{*1}	38.1	7.9	49.7	0.60	—
Final						
1	8.06 ± 0.86 ^{a*2}	36.4	6.9	52.0	0.81	5.4
2	8.16 ± 0.75 ^a	36.2	6.8	50.4	0.61	5.2
3	8.46 ± 1.34 ^a	35.7	6.7	51.7	0.60	8.5
4	8.02 ± 0.39 ^a	34.3	6.3	54.9	0.70	6.0
5	8.07 ± 0.53 ^a	36.6	6.5	52.1	0.60	8.8
Sandeel	7.13 ± 0.65 ^b	36.4	7.0	50.5	0.74	4.0

^{*1} Mean ± SD (n = 10).

^{*2} Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 5. Apparent sugar and protein digestibilities using intestinal digesta dissected at 5 h after feeding

Digestability	Diet group				
	1	2	3	4	5
Sugar	38.2	43.0	45.7	39.2	38.3
Protein	46.8	50.1	47.1	45.2	26.7

No significant differences were detected in the hepatosomatic indices among test diet groups, but that of the group fed sandeel was significantly lower than those of test diet groups on the final day of the feeding trial. Marked differences in moisture, crude protein, crude fat, and crude ash contents of the hepatopancreas were also not found among the dietary groups on the final day. The highest glycogen was accumulated in the diet 5 group, followed by diet 3, 4, 1, and 2 groups. The lowest glycogen content was detected in the reference group.

Apparent Digestibility of Sugar and Protein

Digestibilities assayed using intestinal digesta are shown in Table 5. Sugar digestibilities of diets 2 and 3 were about 44%, which were slightly higher than those of diets 1, 4, and 5 by about 38%. Meanwhile, protein digestibilities of diets 1–4, up to 23.6% dietary crude sugar, were commonly about 47%, being higher than that of diet 5, having the highest white dextrin level.

Discussion

We adopted a 4-week feeding trial in the present study. The short feeding period was obligated to the hypersensitive character of tiger puffer, such as serious injuries caused by biting each other, as cited above.

The digestible energy for dietary nutrients had not been estimated in the puffer. Then, we used gross energy values for dietary protein, fat, and sugar as 5.65, 9.45, and 4.10 kcal/g,⁶ respectively. There are problems to use such convenient values for determining dietary energy content, but these values were necessary as an indicator for formulating test diet compositions.

From the present study, we could indicate that the suitable dextrin level of the diet for the puffer is 16%, dietary crude sugar being about 20%. The difference in the suitable dietary dextrin level between the present study and the previous report³) might result from the differences of experimental conditions, such as body weights of the puffer used, or other dietary ingredients, and rearing conditions. The formula of diet 3 in the present study is more profitable for the puffer-culture because brown fish meal has been most vigorously and currently used as a dietary protein source for fish-cultures in Japan.

The low sugar utility of fish was ordinarily estimated as compared with terrestrial mammals.^{13,14} Shimeno,¹⁵ Nagai and Ikeda,^{16,17} and Furuichi¹⁸ studied on the carbohydrate metabolism of yellowtail *Seriola quiqueradiata*, carp *Cyprinus carpio*, and red sea bream *Pagrus major*, respectively. These reports suggested that low amylase and hepatic glycolytic enzyme activities, shortage of insulin synthesis and secretion, and high activities of hepatic enzymes relating gluconeogenesis resulted in the low sugar utilization of fishes. Amino acids and fatty acids were preferably used in fishes for energy source.¹⁹ The suitable dietary sugar level for yellowtail, carp, and red sea bream was estimated about 10, 35, and 15%, respectively.^{15,18} Ikeda¹⁹ suggested that the differences of sugar utility among fishes were related to their feeding habits and migration. Juveniles, young, and adult tiger puffers that preferentially ate crustaceans and juvenile fin fishes are carnivorous fish.²⁰ Judging from the relatively high sugar utility, the puffer is suspected to be classified with the omnivorous fish rather than the absolute carnivorous fish such as the yellowtail and the red sea bream. The ecological character of the puffer, feeding selection, migration, mating, and others are now being estimated. Much understanding of their character will surely make progress in improving the culture technique of the tiger puffer.

The puffers fed diet 3, having 20% dietary crude sugar, had the best protein efficiency ratio and relatively high apparent protein retention in the diet groups. This indicates the protein sparing effect by dietary sugar in the puffer. Based on the protein efficiency ratio of the dietary groups, the quantitative requirements of protein for 100 g weight gain were calculated as 45.9, 40.9, 37.7, 39.7, and 43.9 g in the diet 1, 2, 3, 4, and 5 groups, respectively. In comparison with the diet 1 group, therefore, the sparing effect by dietary sugar for dietary protein was estimated to be 17.9 and 13.5% in the diet 3 and 4 groups, respectively. It has already been shown in terrestrial animals that glucose

depresses many degradative reactions of protein in tissues.²¹⁾ This mechanism might support the protein sparing effect of dietary sugar in the puffer. Shimeno²²⁾ suggested that sugar is not always necessary to maintain fish growth and health, but should be consciously used to spare dietary proteins as well as to lower feed costs economically. Otherwise, Takeda²³⁾ and Cowey et al.²⁴⁾ showed the protein sparing effect was more preferable to supplement the proper ratio of both fat and sugar than only fat in yellowtail and plaice *Pleuronectes platessa*. We intend to clarify the optimum fat level in BFM diet and the additional effect of fat to sugar supplement on the protein sparing in the puffer.

Although low digestibilities were ordinarily detected by using intestinal digesta squeezed at 5 h after feeding, relatively high sugar and protein digestibilities were detected in the diet 2 and 3 groups and the diet 1–4 groups, respectively. These supported the good growth performance of the diet 3 group. The fish fed diet 5, having 25% dietary crude sugar, showed low sugar and protein digestibilities which were comparable to half of those in the diet 3 group. Kitamikado et al.²⁵⁾ and Shimeno¹⁵⁾ also reported that protein and sugar digestibilities fell with relatively large increases of dietary sugar content in yellowtail. It is of great interest to infer what control the synthesis, secretion, and activation of digestive enzymes in fish; it is possible that complex mechanisms, such as nervous system via chemical input,^{1,26)} gastrointestinal hormones, the balance of dietary nutrients, correlation between nutrient assimilation and endocrine hormone secretion, are involved in this process. The puffer does not have a stomach. Many differences in digestive process of dietary nutrients are expected between the puffer and fish that have stomachs, such as rainbow trout, yellowtail, and red sea bream.

In conclusion, around 20% dietary crude sugar was suitable for the tiger puffer, when 49% brown fish meal, 10% wheat gluten, 16% white dextrin, and 5% pollack liver oil were used as protein, sugar, and lipid sources, respectively. Relatively high content of dietary sugar profits to prepare an expanding and paste type diet and to lower feed cost for tiger puffer-culture. The paste type diet may be more reasonable for the tiger puffer, considering the shape of their teeth and feeding behavior.

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