Dietary Supplement of Feeding Stimulants on Performance and Digestive Function of Yellowtail, *Seriola quinqueradiata*

Kenji Takii,[*1] Sadao Shimeno,[*2] Masaharu Akutsu,[*3] and Masahiko Takeda[*2]

Synopsis

The supplementary effect of feeding stimulants, a mixture of L-alanine, L-proline and inosine-5'-monophosphate, to a brown fish meal diet was investigated on yellowtail, having mean body weight of 60 g, regarding growth performance and digestive function.

Growth performances such as weight gain, survival, feed conversion efficiency, protein and fat retention rates of the fish fed a test diet supplemented with the feeding stimulants were superior to the fish fed an unsupplemented control diet. Moreover, the test diet supported better digestive function such as protein and carbohydrate digestibilities, pepsin-like and trypsin-like enzyme activities in gastrointestinal digesta, blood glucose and plasma triglyceride levels than the control diet at 3 and 6 h after feeding.

These results indicate that preferential chemical stimuli, feeding stimulant-based, promotes feeding activity as well as digestion, absorption and nutrient retention in young yellowtail.

I Introduction

Improving the palatability of an artificial diet for fish has been expected to increase feed intake and growth rate as well as to decrease stresses attributed to the intensive fish-culture to some extents. In the previous reports,[1-3] the Japanese eel *Anguilla japonica* fed a moist fish meal diet supplemented with a feeding stimulant mixture showed the increases of feed efficiency, pepsin-like enzyme activity in gastric digesta, apparent digestibility of dietary protein and carbohydrate and activities of some hepatic enzyme relating to amino acid and carbohydrate catabolisms; or the decreases of blood glucose and plasma free amino acid nitrogen levels after feeding as compared with the fish fed an unflavored diet. These results indicated that the promotive digestion and absorption result from the much secretion of both digestive fluid and gastrointestinal hormones by favorite chemical stimuli via central nervous system, as documented well in mammals.[4-6]

The objective of the present study was therefore stressed on making certain the hypothesis relating the effect of feeding stimulant in yellowtail, as one of other fishes, following eel and tiger puffer *Fugu rubripes*.[1-3, 13]

[*1]Fisheries Laboratory, Kinki University, Uragami, Wakayama 649-51, Japan.
(滝井健二：浦神実験場)

[*2]Faculty of Agriculture, Kochi University, Nankoku, Kochi 783, Japan.
(示野貞夫・竹田正彦：高知大学農学部)

[*3]Trechnical Research Center, Marubeni Shiryo Co. Ltd., Ono, Hyogo 675-13, Japan.
(阿久津正治：丸紅飼料技術センター)

II Materials and Methods

Fish and Aquarium

Young yellowtail were obtained from a fish-farmer in Tosa city, Kochi and transported to Usa Marine Biological Institute, Kochi Univ. Prior to the start of feeding trial, the fish were fed on sliced sand lance *Ammodytes personatus* and acclimated to experimental conditions for 2 weeks. Two groups of 30 fish, having mean body weight of 60 g, were maintained in each aquarium (150x90x60 cm deep) with a running sea water system at 6 *l*/min and vigorous aeration. Water temperature during the feeding trial was 28.7±0.5°C (mean±SD, n=19).

Diets

The composition of a basal diet and a feeding stimulant mixture used in the present study is shown in Table 1. All ingredients were thoroughly mixed with TH-4 vitamin mixture and T-4 major and O-1 trace mineral mixtures (Table 2). A test diet, a moist pellet with 3 mm in diameter, was prepared by the addition of 40 m*l* tap water to 100 g of the ingredient mixture with 1 g of feeding stimulants, and a control diet, without the feeding stimulants, was done by the same manner. The diets were prepared every 10 days by a experimental pellet machine and were stored at -20°C until use.

Table 1. Composition of basal diet and feeding stimulant mixture

Ingredient	g
Brown fish meal	65
Wheat gluten	10
White dextrin	6.5
Vitamin mixture*1	3
Mineral mixture*1	2.5
CM cellulose	2
Ethoxyquin	0.03
Urso-20*2	0.37
Cellulose	1.6
Pollack liver oil	10
Total	110
Metabolizable energy (kcal/100 g diet)	392
Feeding stimulant mixture	%
L-Alanine	23.2
L-Proline	35.4
Inosine-5'-monophosphate•2Na	41.4
Total	100

*1 Show in Table 2.
*2 Commercially prepared by Tokyo Tanabe Pharmaceutical.

Table 2. Composition of vitamin and mireral mixtures

Vitamin[*1] (mg)		Mineral[*2] (mg)	
Thiamine•HNO_3	2.4	Major component	
Riboflavin	4.4	KH_2PO_4	412
Pyridoxine•HCl	2.4	$Ca(H_2PO_4)_2•H_2O$	618
Nicotinic acid	7.2	Ca•lactate	282
Ca•panthotenate	14	Peptide•Fe[*3]	166
Inositol	169	Cellulose	522
Biotin	0.14	Total	2000
Folic acid	2.4	Trace element	
Choline cloride	584	$ZnSO_4$	11.1
Ascorbic acid•Ca	178	$MnSO_4$	6.25
Cyanocobalamin	0.032	$CuSO_4•5H_2O$	2.0
DL-αTocopherol acetate	88	$CoCl_2•6H_2O$	0.05
Menadione•$NaHSO_3$	7.7	KIO_3	0.15
Vitamin AD_3 750	1.2	Cellulose	30.45
Vitamin A 750	1.5	Dextrin	450
Cellulose	1937.6	Total	500
Total	3000		

[*1] TH-4 vitamin mixture.
[*2] T-4 major and 0-1 trace mineral mixtures.
[*3] Commercially prepared by Eisai Pharmaceutical.

Feeding and Test Items

The fish were given the test and control diet twice a day for 29 days. Daily ration size in dry weight basis was adjusted about 3.8% of live body weight. To evaluate the feeding activity of the fish for each diet, the following 6 point method was adopted based on the consuming time in every feeding.

Point 0: consuming time taken above 5 min and diet still remained.
1: consuming time taken from 4 min to under 5 min.
2: consuming time taken from 3 min to under 4 min.
3: consuming time taken from 2 min to under 3 min.
4: consuming time taken from 1 min to under 2 min.
5: consuming time taken under 1 min.

Significant difference of the feeding activity between the groups was tested at the 5% level using Student's *t*-test.

At the end of the experiment, eight fish, having near mean body weight, were taken from each group and stored at -20°C for subsequent proximate analysis of whole body and the liver after decapitation. Moreover, apparent digestibility of dietary protein and carbohydrate, free amino acid nitrogen (FA) and carbohydrate (FC) levels in gastrointestinal digesta, protease and amylase activities in gastrointestinal tissues and digesta, and some plasma constituent levels were also measured at 3 and 6 h after feeding on the final experimental day.

Assays

Gastral and intestinal digesta were obtained by squeezing the digestive organ dissected carefully at 6 h after feeding. The digestibility was measured by the indirect method[7] using Cr_2O_3 as indicator in intestinal digesta. FA and FC were assayed by DNFB method[8] and phenol sulfuric acid method,[9] respectively, using the supernatant fluid after the centrifugation of the digesta at 3000 rpm for 20 min.

Protease and amylase activities were assayed as follows: digestive organs, such as the stomach and intestine including the pyloric caeca, and those digesta were carefully dissected and homogenized with nine volumes of deionized water by using a Potter-Elvehjem glass homogenizer. After the centrifugation of the resultant homogenate at 10,000 x *g* for 20 min, the supernatant fluid was used as enzyme solution. These were conducted under 4°C. Pepsin-like (EC 3.4.4.1) enzyme and trypsin-like (EC 3.4.4.4) enzyme and amylase (EC 3.2.1.1) activities were assayed by Casein-Folin method[10] and Tauber-Kleiner method[11] modified by Kawai and Ikeda,[12] respectively. These activities were expressed as units which were defined as μ mols of L-tyrosine or glucose liberated from each substrate per min per g of tissue or digesta.

Blood was obtained from the aortic bulb with a heparinized syringe. Hematocrit value (Ht) was measured by the centrifugation at 10,000 rpm for 5 min. Plasma constituents such as blood glucose (BG) and plasma free amino acid nitrogen (PAA) were measured by the same methods described previously.[3] Plasma triglyceride (PTG) was assayed by the enzyme method using Triglyceride C Test Wako (Wako Purechemicals, Osaka).

III Results

Performance

Growth performance of the fish fed on the test and control diets for 29 days are shown in Table 3. Weight gain of the test diet group was superior to those in the control diet group. Moreover, feed efficiency, protein efficiency ratio, energy efficiency and apparent nutrient retention, all were entirely improved in the test group, in spite of the similar ration size conducted. No significant difference was statistically found in the feeding activity between the test and control group ($p>0.05$), but fairly higher mean value and smaller deviation were detected in the test group than the control group.

Relative Organ Weight to Body weight and Proximate Composition of Whole body and The Liver

The relative organ weights of the liver, stomach and intestine including the pyloric caeca to body weight at the end of the experiment are shown in Table 4. The relative liver and stomach weights were similar between the groups, but the relative intestinal weight had a trend of increasing in the control group.

Proximate compositions of the whole body and liver are shown in Table 5. Fat content of both whole body and the liver increased in the test group as compared with those in the control group. No marked differences were found in the other constituents between the dietary trestments.

Table 3. Performance and feeding ativity[*1] of yellowtail fed on test and control diets for 29 days

Group:	Test	Control
Initial No. of fish	30	30
Mean body wt (g)		
initial	60.2	59.5
final	111.6	93.5
Mean wt gain (%)	51.4	34.0
Survival (%)	100	50
Feed intake (g)	2475.7	1478.5
Daily feeding rate (%)	3.82	3.42
Feed conversion efficiency (%)	53.5	29.2
Protein efficiency ratio	1.02	0.572
Energy efficiency (%)	13.6	7.63
Protein retention (%)	19.6	11.3
Fat retention (%)	51.7	25.0
Energy retention (%)	23.1	12.5
Feeding activity	3.34±1.38[*2]	3.00±2.06

[*1] Detailed explanation was described in the text.
[*2] Mean ±SD (n=58).

Table 4. Relative organ weight to body weight of yellowtail fed test and control diets on the final experimental day

Group:	Test	Control
Liver	1.65±0.25[*1]	1.78±0.31
Stomach	0.99±0.07	1.06±0.07
Intestine[*2]	1.79±0.26	1.92±0.19

[*1] Mean±SD (n=8).
[*2] Including pyloric caeca.

Digesta Weight and Apparent Digestibility

Postplandial changes in digesta weight expressed as relative weight to body weight on wet weight basis are shown in Fig. 1. Although the expression of postplandial digesta changes in wet weight basis had some problems, the gastral digesta weight of the test group linearly decreased until 6 h after feeding, in contrast to the control group showing a abrupt decrease until 3 h after feeding. Therefore, the intestinal digesta of the test group was less maintained at near constant level than the control group, having much amounts of digesta after feeding.

Table 5. Proximate composition (%) of whole body and liver of yellowtail fed test and control diets for 29 days

	Moisture	Crude protein	Crude fat	Ash	Glycogen
Whole body					
Initial	74.0	19.2	2.23	3.74	
Final					
Test group	69.2	19.2	7.55	3.50	
Control group	73.0	19.3	4.33	3.61	
Liver					
Initial	60.4	15.8	18.7	1.03	0.37
Final					
Test group	55.7	12.6	22.7	0.91	4.68
Control group	63.3	14.2	15.0	0.94	5.45

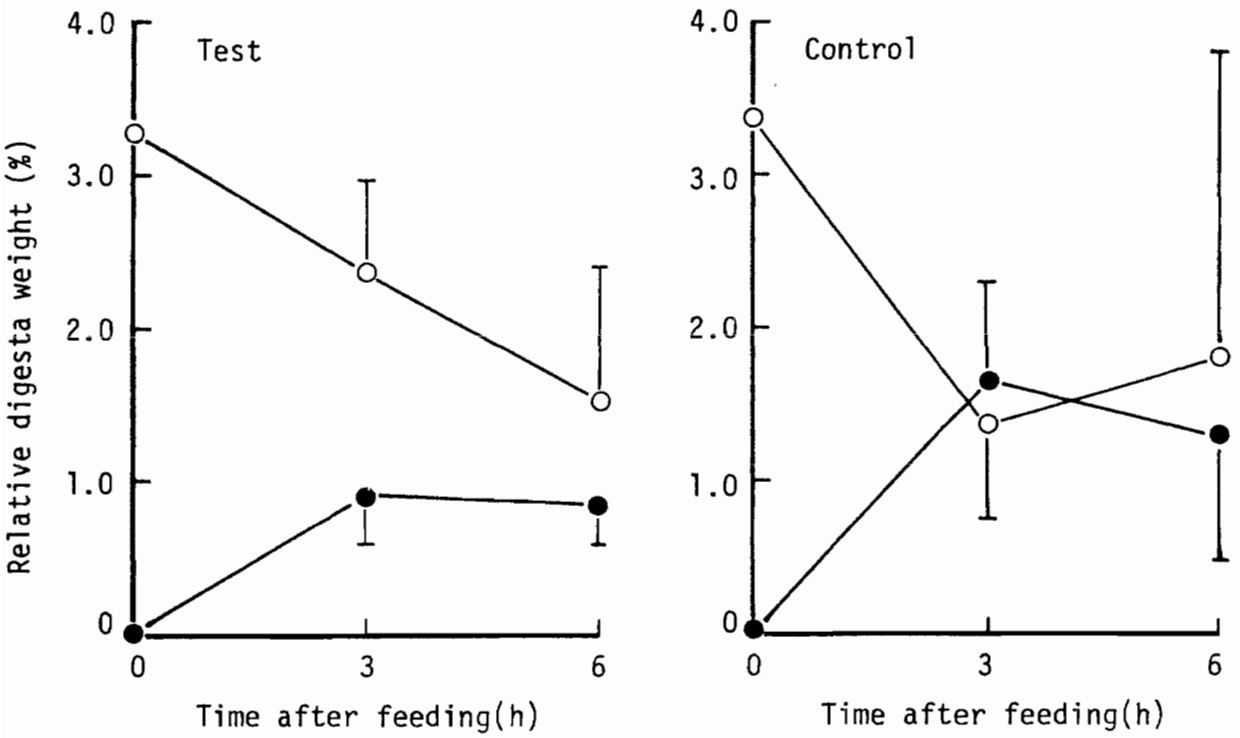

Fig. 1. Postplandial changes in relative digesta weight to body weight of test and control groups.
○ Gastric digesta, ● Intestinal digesta.

The protein and carbohydrate digestibilities measured at 6 h after feeding on the final day of the experiment are shown in Table 6. The digestibilities of the test group were better than those of the control group. The carbohydrate digestibility of the test group was especially superior to the control group.

Table 6. Protein and carbohydrate digestibility (%) of yellowtail at 6 h after feeding test and control diets

Group:	Test	Control
Protein	77.2	72.0
Carbohydrate	78.8	50.9

FA and FC Levels, Digestive Enzyme Activity of Gastrointestinal Tissues and Digesta, and Plasma Constituents

As shown in Table 7, FA and FC levels of gastral digesta were relatively higher in the test group than the control group. In the test group, FA level of intestinal digesta was also higher, whereas FC level was reversely decreased at 3 and 6 h after feeding.

Table 7. Free amino acid nitrogen (FAA) and glucose levels (mg/g digesta) in gastrointestinal digesta of yellowtail at 3 and 6 h after feeding test and control diets

	Test group		Control group	
	3 h	6 h	3 h	6 h
Gastral digesta				
FAA	1.40	1.50	1.28	0.78
Glucose	11.6	4.81	10.2	2.86
Intestinal digesta				
FAA	4.35	5.21	3.67	2.27
Glucose	11.9	6.36	15.3	6.51

Fig. 2 shows postplandial changes in protease and amylase activities of gastrointestinal tissues and digesta. Although, the pepsin-like enzyme activities of gastral tissue were maintained a relatively constant level in the control group after feeding, the activity in the test group decreased immediately after feeding, reaching a minimum level at 3 h, thereafter increasing until 6 h. Or pepsin-like enzyme activity of gastral digesta in the test group was

maintained at higher level than the control group at 3 and 6 h after feeding. In the intestinal tissue including the pyloric caeca, trypsin-like enzyme and amylase activities were lower in the test group than the control group, whereas these activities in intestinal digesta were reversely higher in the test group. These results indicate that the promotive secretion of both gastric and pancreatic fluid are supported by the fish fed the test diet.

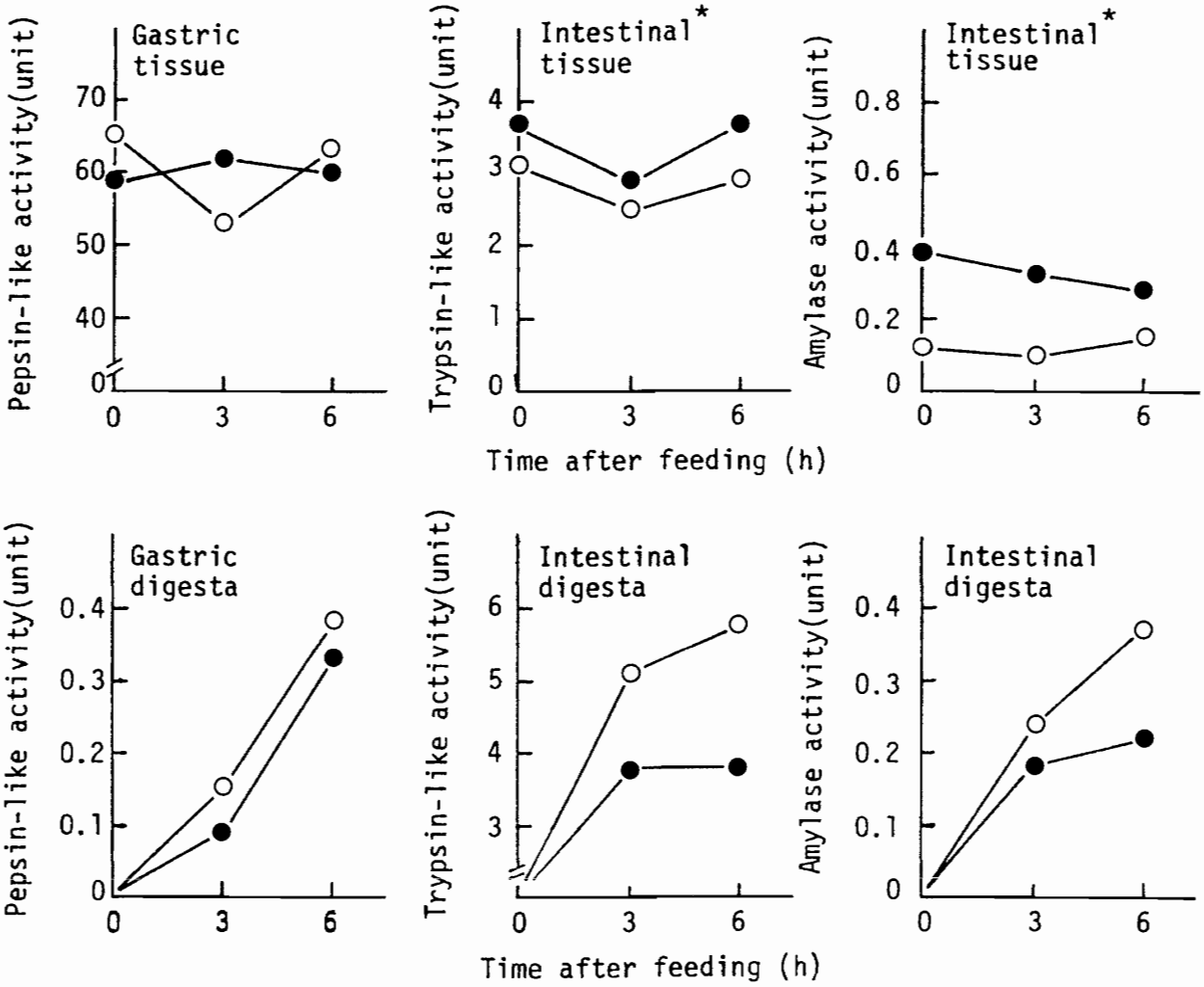

Fig. 2. Postplandial changes in digestive enzyme activities per g of tissue and digesta in gastrointestinal tissue and digesta of test and control groups.
❍ Test group, ● Control group.
* Intestinal tissue included pyrolic caeca.

Changes in Ht, BG, PAA and PTG after feeding are shown in Fig. 3. PAA level showed a similar postplandial change, increasing slightly with time after feeding, in both diet groups. The test group supported higher Ht, BG and PTG levels than the control group after feeding.

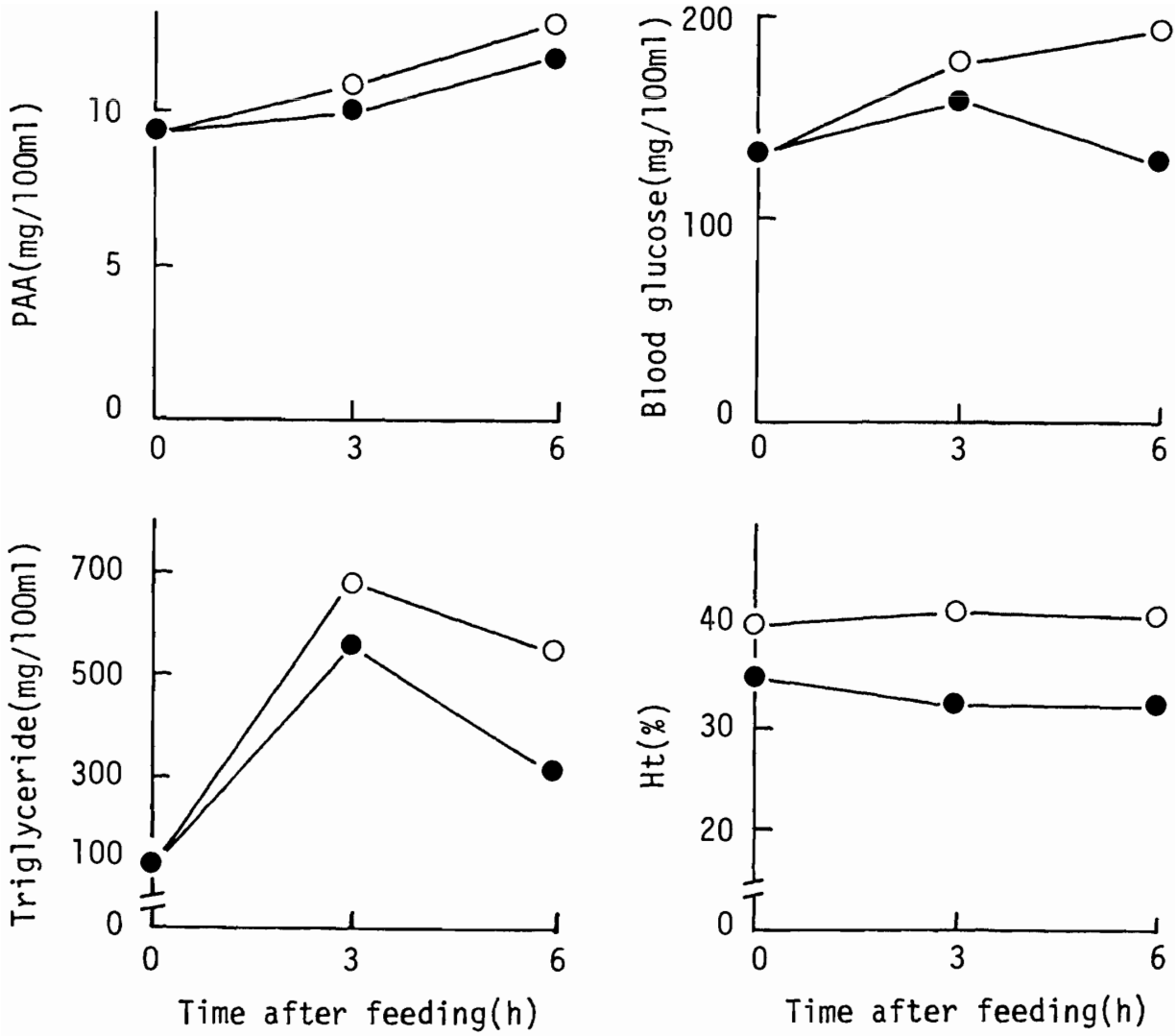

Fig. 3. Postplandial changes in plasma constituents and Ht in test and control groups. ○ Test group, ● Control group.

IV Discussion

In the present study, weight gain, feeding activity, feed conversion efficiency and nutrient retentions of which the fish fed the diet supplemented with feeding stimulants, were almost superior to those of the fish fed the unflavored diet, in spite of similar ration size and same dietary composition practiced. Furthermore, digestive enzyme activities, protein and carbohydrate digestibilities and some plasma constituents of the test diet group also showed higher levels than the control diet group at 3 and 6 h after feeding. Otherwise, the low survival of the control group was detected and introduced by a temporal stop of sea water-supply, resulting in troubles of pumps, but the test diet supposed high survival under the same trouble. From these results, we intensely suggest that favorite chemical stimuli relating feeding have very important roles, not only improving feeding activity and some kinds of stress but also promoting nutrient assimilation and retention, in yellowtail, as eel and tiger puffer described previously.[1-3, 13] In mammals, favorite taste and odor stimuli induce the establishment of feeding anticipation, which arrange the digestive and absorptive organ functions until feed swallow and promote the metabolism of assimilated

nutrients, via a cephalic nervous system.[4] The similar mechanisms might function in fishes as mammals.

The test diet group maintained the smaller intestinal digesta than the control group. This suggested the skillful regulation of digesta transportation from gastral lumen to intestinal lumen was occurred in the test group, in relation to the high protein and carbohydrate digestibilities detected. Passing digesta from the stomach to intestine in mammals have not fully defined, but some free chemicals and pH of gastral digesta, gastrointestinal hormones like gastric inhibitory polypeptide, secretin and cholecystokinin, and nervous system concern to the transport function.[14] The high levels of FA and FC in gastral digesta might well regulate the transportation of digesta in the test group. Trypsin-like enzyme and amylase activities of intestinal digesta, blood glucose and plasma triglyceride in the test group surpassed those in the control group after feeding. We had recognized the reversed results in the previous report using eel.[3] The reasons for getting the reversed results may be attributed to the difference of ecological character between fish species; yellowtail being a migratory fish vs. eel being a nocturnal fish. Yellowtail require more energy for swimming, metabolisms and maintenance than eel even after feeding.

The higher Ht recognized in the test group than the control group was partially attributed to the promoting absorption of vitamin B_{12}. The vitamin is necessary to bind the intrinsic factor, mucoprotein secreted with gastric juice from the parietal cells, for being absorbed from the intestinal epithelium in mammals.[15] There is few information of the intrinsic factor in fishes. The pepsin-like enzyme secretion enhanced by the feeding stimulants might also relate to the promotive secretion of intrinsic factor in yellowtail.

We reaffirm from the present results that favorite taste and/or odor stimuli in diet are important factor to improve feeding activity, digestion, absorption and retention of nutrients in fish like yellowtail, following eel and tiger puffer.[1-3,13] These results will throw a new standpoint relating to the applications of feeding stimulants to chemical physiology and practical fish-culture.

Acknowledgment

The present investigation was conducted at Usa Marine Biological Institute, Kochi Univ. We are indebted to associate professor H. Hosokawa, Kochi Univ., for his valuable suggestion through this work. We also thank the stuff of Fish Nutrition Lab. and Usa Marine Biological Institute, Kochi Univ., for technical facilitation. One of the authors (K. Takii) was sincerely indebted to Director of Fish. Lab., Kinki Univ., professor H. Kumai, for his critical reading.

References

1) K. Takii, M. Takeda and Y. Nakao: *Nippon Suisan Gakkaishi*, **50**, 1039-1043 (1984).
2) K. Takii, S. Shimeno, M. Takeda and S. Kamekawa: *Nippon Suisan Gakkaishi*, **52**, 1449-1454 (1986).
3) K. Takii, S. Shimeno and M. Takeda: *Nippon Suisan Gakkaishi*, **52**, 2131-2134

(1986).
4) K. Yumikari and K. Torii: in "Mikaku no Kagaku" (ed. by M. Satou), Asakura Shoten, Tokyo, 1981, pp. 244-266.
5) S.A. Giduck, R.M. Threatte and M.R. Kare: *J. Nutr.*, **117**, 1191-1196 (1987).
6) K. Iwai: *Nippon Nogeikagaku Kaishi*, **61**, 1453 (1987).
7) A. Furukawa and H. Tsukahara: *Nippon Suisan Gakkaishi*, **32**, 502-506 (1966).
8) J.F. Goodwin: *Clin. Chem.*, **14**, 1080-1090 (1968).
9) J.E. Hodge and B.T. Hofreiter: in "Methods in Carbohydrate Chemistry" (ed. by R.L. Whistler and M.L. Wolfrom), Vol. 1, Academic Press, New York, 1962, pp. 388-389.
10) B. Ogihara: in "Kouso Kenkyu Hou" (ed. by S. Akabori), Vol. 1, Asakura Shoten, Tokyo, 1955, pp. 237-246.
11) H. Tauber and I.S. Kleiner: *J. Biol. Chem.*, **99**, 249-255 (1932).
12) S. Kawai and S. Ikeda: *Nippon Suisan Gakkaishi*, **37**, 333-337 (1971).
13) H. Kumai, I. Kimura, M. Nakamura, K. Takii and H. Ishida: *Nippon Suisan Gakkaishi*, **55**, 1035-1043 (1989).
14) Z. Itoh: in "Shoukakan Hormone" (ed. by Kodansha Scientific), Kodansha, Tokyo, 1980, pp. 212-258.