水研センター研報，第40号，39－49，平成27年
Bull. Fish. Res. Agen. No. 40, 39－49, 2015

Development of a New Type of Fish Diet, Non-Fish Meal Extruded-Pellet

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Abstract: We developed a non-fish meal diet using plant and/or animal protein materials. Three kinds of non-fish meal diets and a control diet containing 50% fish meal were processed. In the non-fish meal diets, the fish meal was replaced with commercially available plant or animal materials and supplemented with taurine and materials for maintaining palatability. These diets were fed to one year old yellowtail (BW 753±96g) in net cages. There was no difference in growth, daily weight gain, daily feeding rate, feed conversion ratio and protein efficiency ratio among the diets. Non-fish meal diets were processed in a factory and dietary properties were studied such as uptake, stomach evacuation rate, and comparative disease resistance in fish fed the experimental diets. In addition, palatability of each substitute protein material for fish was examined and materials to enhance palatability of the non-fish meal diets were clarified. Non-fish meal diets have the potential to support the growth of one year old yellowtail.

Key word: Fish diet, Non-fish meal, Extruded-pellet, Yellowtail, Palatability, Substitute protein material

In Japan, fish meal is the main material in fish diets used in aquaculture and is contained at a level of over 50%. Over 90% of the fish meal used in fish diets for aquaculture is imported. Globally, the price of fish meal has increased dramatically in recent years due to reduced production caused by fishery regulation in South America and the increased worldwide demand for aquacultured fish. The increasing fish meal price threatens of the viability of production for Japanese aquaculture farmers. Therefore, a new type of diet that is independent of fish meal is strongly required. Low or non-fish meal extruded-pellets have been developed. In our preliminary study, we prepared diets containing 10% fish meal, and fed them to cultured yellowtail to compare the fish growth performances to a diet containing 50% fish meal. It was observed that fish growth was similar in both diet groups. Three experimental non-fish meal extruded-pellets were designed and fed to one year old yellowtail which were at an immature and rapidly growing size. We also studied dietary, uptake, stomach evacuate rate, palatability, and comparative disease resistance of fish. Then the meat quality fed the experimental diets was evaluated using a panel sensory test.

Materials and Methods

Experimental diets and processing: The control diet and three kinds of non-fish meal diets were prepared using a variety of ingredients (Table 1) (Tsuzaki *et al*., 2013 and 2014). The control diet (FM) contained 50% fish meal to provide a suitable balance of amino acids. Fish meal was replaced with commercially available plant (PP) and animal (AP) materials in the non-fish meal diets. Soy protein concentrate, defatted soy bean meal, and corn gluten meal were used for plant materials and pork meal, feather

2015年１月30日受理（Received on January 30, 2015）
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Table 1. Formulation of the experimental diets (%)

	Diet group			
Ingredient	FM	PP-SP	PP-FS	AP-SP
Fish meal [1]	50.0	0	0	0
Soy bean concentrate [2]	0	30.0	30.0	0
Defatted soy bean meal [3]	3.5	11.0	11.0	8.0
Corn gluten meal [4]	0	12.0	12.0	9.0
Krill meal	0	3.0	3.0	3.0
Pork meal [5]	0	0	0	30.0
Feather meal [5]	0	0	0	3.0
Blood meal [6]	0	0	0	3.0
Fish oil [7]	15.4	17.0	17.0	14.0
Wheat	13.0	10.8	10.8	12.0
Defatted rice brain [8]	8.5	0	0	1.8
Tapioca starch [9]	6.6	8.0	8.0	8.0
Vitamin mixture [10]	2.0	2.0	2.0	2.0
Mineral mixture [10]	1.0	1.0	1.0	1.0
Taurine	0	1.0	1.0	1.0
Amino acid mixture [11]	0	2.2	2.2	2.2
Calcium phosphate [12]	0	2.0	2.0	2.0
Enzyme mixture [13] (external addition)	0	(0.2)	(0.2)	(0.2)
Skipjack peptide [14] (external addition)	0	(0.5)	0	(0.5)
Fish sauce [15] (external addition)	0	0	(0.5)	0

[1] Fish meal was made from anchovy meal as the main raw material, imported from Peru.
[2] Soycomil; Archer Daniels Midland Japan Ltd.
[3] Nisshin Oillio Co., Ltd.
[4] Japan Corn Starch Co., Ltd.
[5] Kagoshima Pure Foods Co., Ltd.
[6] APC Japan Co., Ltd.
[7] Tsuji Oil Mills Co., Ltd.
[8] Suzuki Oil Mills Co., Ltd.
[9] Sanguan Wongse Industries Co., Ltd.
[10] See Takagi *et al.* (2005).
[11] See Aoki *et al.* (2000b).
[12] Onoda Chemical Industry Co., Ltd.
[13] Alltech.
[14] Marubeni Nisshin Feed Co., Ltd.
[15] Sakaiovex Co., Ltd.

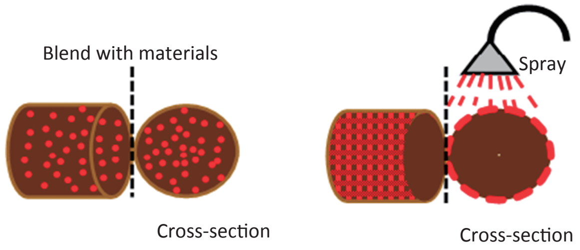

Fig. 1. Method to add palatability materials and enzyme solution to fish pellets. Right is sprayed after being shaped to reduce the material volume. Left is shaped after blending all the materials.

meal, and blood meal were used for animal materials in this study. Taurine was added to the non-fish meal diets at a 1% composition. Experimental diets were carefully shaped using an extruder to have suitable physical properties in the sea and sink at an appropriate rate rather than float.

It is known that yellowtail become less responsive to a diet as the amount of fish meal included is reduced (Aoki *et al.*, 2000a and 2000b). Therefore, two commercially available materials, a skipjack peptide (SP) and a fish sauce (FS) were supplemented to maintain the palatability of the non-fish meal diet. The skipjack peptide is concentrated from the cooking water of skipjack. The fish sauce is made from mackerel gut using a patent-pending method for rapid production. An enzyme solution and amino acid chelated trace metals were added to the non-fish meal diets to improve digestibility and absorption. These palatability materials and enzyme solution were added by a spray method after the extruder processing to minimize the volume (Fig. 1).

Therefore, the three kinds of non-fish meal diets were as follows:

PP-SP was a plant protein based diet with the skipjack peptide as a material to enhance palatability.

PP-FS was a plant protein based diet with the fish sauce as a material to enhance palatability.

AP-SP was an animal protein based diet with the skipjack peptide as a material to enhance palatability.

Two sizes of experimental diets, 13mm and 15mm grain diameter, were prepared at the Kagoshima plant of Marubeni Nisshin Feed Co., Ltd. There were minimal differences in the general composition among the four experimental diets. Small differences were seen in the fatty acid composition. AP-SP had 16% of n-3 PUFA while the other diets ranged from 22–27%. AP-SP also had 41% of total monoenes, higher than the 30–35% found in the other diets (Table 2).

Feeding experiment: One year old yellowtails which originated from seedling production in Goto Island, Nagasaki Prefecture were used in the experiments (Tsuzaki *et al.*, 2013 and 2014). The average body weight of 220 fish was 753±96g. Four groups of 55 fish fed different experimental diets to satiation three times a week for six months from July to January were reared in four 4 m x 4 m replicate net cages on the sea surface. After January fish continued to be fed with the leftover experiment diet until March.

The feeding experiment was divided into two periods, summer season (July to September) and autumn season (October to December). Sampling and analysis were performed for each period.

Body weight and fork length were monthly measured for 20–25 live fish at random from every group. At the end of the experiment, all the fish were sampled and weighed and the following data were measured or calculated.

Condition factor $= (W / FL^3) \times 10^3$

Weight gain ratio (%) $= (TWt + SDW - TW0)/TW0 \times 100$

Daily weight gain ratio (%/day) $= (TWt + SDW - TW0)/[(TWt + SDW + TW0)/2 \times t] \times 100$

Daily feeding rate (%/day) $= F/[(TWt + SDW + TW0)/2 \times t] \times 100$

Feed conversion ratio (FCR) $= F/(TWt + SDW - TW0)$

Protein efficiency ratio (PER) $= (TWt + SDW - TW0)/P$

W: Body weight (g)
FL: Fork length (cm)
TW0: Initial total body weight (g)
TWt: Final total body weight (g)
SDW: Total body weight of sampled fish and dead fish (g)
Nt: Number of final fish
F: Total feed intake (g)
t: Rearing days

Table 2. Proximate composition, phosphorus content, and fatty acid composition of the experimental diets

	Diet group			
Analysis	FM	PP-SP	PP-FS	AP-SP
Proximate composition (%)				
Moisture	7.6	7.7	8.8	4.9
Crude ash	9.1	5.6	5.6	7.4
Crude protein	39.8	38.1	37.4	42.0
Crude lipid	18.9	16.1	17.4	17.6
Phosphorus content				
P (mg/g)	15.4	9.7	9.7	13.4
Fatty acid composition (%)				
14: 0*1	4.8	4.2	4.3	5.4
16: 0	18.3	16.5	18.3	15.8
18: 0	3.8	3.5	4.1	4.2
16: 1n-7	5.8	5.2	5.5	5.5
18: 1n-9	16.0	16.8	19.0	16.4
20: 1n-9	2.6	2.7	4.5	7.5
20: 1n-11	2.2	2.3	1.5	1.5
22: 1n-11	4.0	4.1	4.5	9.8
18: 2n-6	4.5	7.0	7.1	5.6
20: 4n-6	1.0	0.9	0.9	0.4
18: 3n-3*2	1.0	1.2	1.1	0.9
18:4n-3	1.8	1.5	1.3	2.0
20: 4n-3	0.6	0.6	0.6	0.6
20: 5n-3 (EPA)	8.0	6.8	6.0	5.2
22: 5n-3 (DPA)	2.0	1.9	1.9	1.0
22: 6n-3 (DHA)	13.5	13.6	11.3	6.2
Others	10.2	11.1	8.0	12.2
n-3 HUFA	25.9	24.3	21.2	14.9

*1 Left figure represents the number of carbonatoms and right figure after colon represents the number of carbon unsaturated bonds.

*2 "n-3"represents the omega-3 fatty acid.

n-3 HUFA=18: 4n-3+20: 4n-3+EPA+DPA+DHA.

Experimental diets are shown in Table 1.

P: Total protein intake (g)

Blood, muscle, and liver samples were collected from 5 fish in every group at the start (initial), late summer (intermediate), and late autumn (end). The ratio of liver weight per body weight was calculated. Hematocrit value and plasma component levels were analyzed. General content, phosphorus content, fatty acid composition, and amino acid composition of muscle were analyzed.

The statistical differences between trial diets were determined by Tukey's multiple range comparison test ($p<0.05$).

Experimental diet uptake, stomach evacuate rate, and comparative disease resistance of fish fed experimental diets: Nutritional absorption was analyzed from fish feces collected from fish reared in a conical tank (Fig. 2) using special diets containing chrome oxide as an internal standard material (Satoh *et al.*, 2013).

Fish were anesthetized and administered the diet at 2% of body weight directly into the stomach. Stomach residue was analyzed over time by the Kjeldahl nitrogen method and stomach evacuation rate was estimated (Tohata *et al.*, 2012).

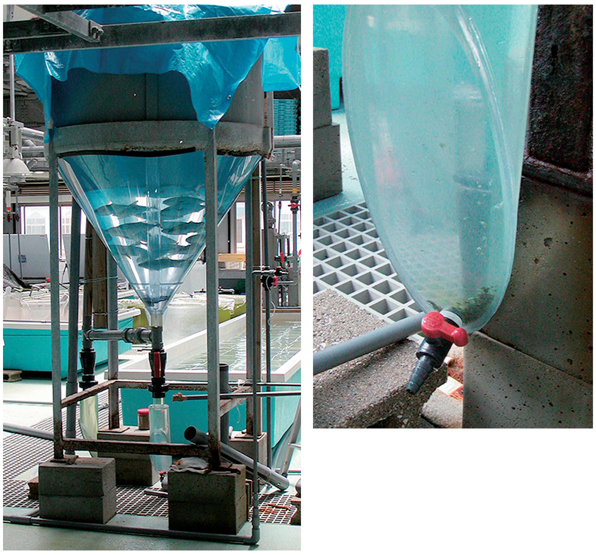

Fig. 2. The conical tank used to collect feces of fish fed the experimental diets to analyze nutritional absorption.
Right: Feces at the bottom of the tank

Disease resistance was carried out using *Streptococcus dysgalactiae*, an infectious streptococcal bacterium. Fish were reared on each diet in indoor tanks for 3 months and fed the bacterium-containing feed for 5 days. Fish mortality was observed over a 40 day period (Ishida *et al.*, 2013).

Diet palatability: Fish behavior was divided into five responses after placing the diets into a fish tank described as recognition, ingestion, orientation within the mouth, swallowing, or spitting out (Fig. 3). The actions were analyzed by using a camera to film red sea bream, yellowtail, and coho salmon using single dry pellets made from only one material (Fig. 4) (Niinuma *et al.*, 2012, Yamauchi *et al.*, 2012).

Meat quality of raw sashimi: A panel test using the order method, color-difference meter analysis, and electronic nose system analysis were performed for each experimental fish fed PP-SP, PP-FS, AP-SP, and control diet (Tohata *et al.*, 2013).

RESULTS

Processing: All the experimental diets were processed using a double-screw extruder. At the beginning in the autumn season, plant protein based diets did not sink at a suitable rate and flowed out from the net cage compared to the other diets. Therefore these diets were re-made. Palatability ingredients and an enzyme solution were sprayed onto diets to minimize materials used to 0.05% from the original 2% required in the blending method (Fig. 1).

Growth performances and biological indices in yellowtail: The growth performances in both the summer and autumn period are shown in Table 3.

In the summer period when the water temperature was 22.8 – 29.2 ℃ all diet groups

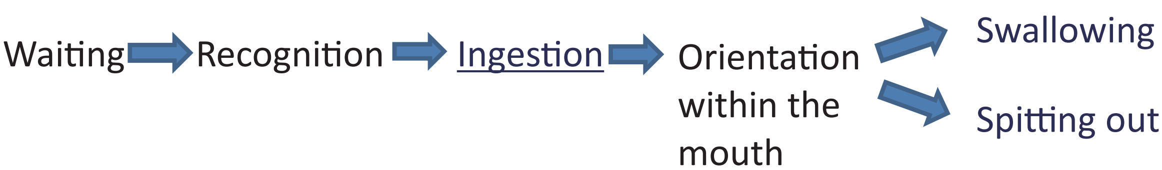

Fig. 3. Fish behaviors for the diet.

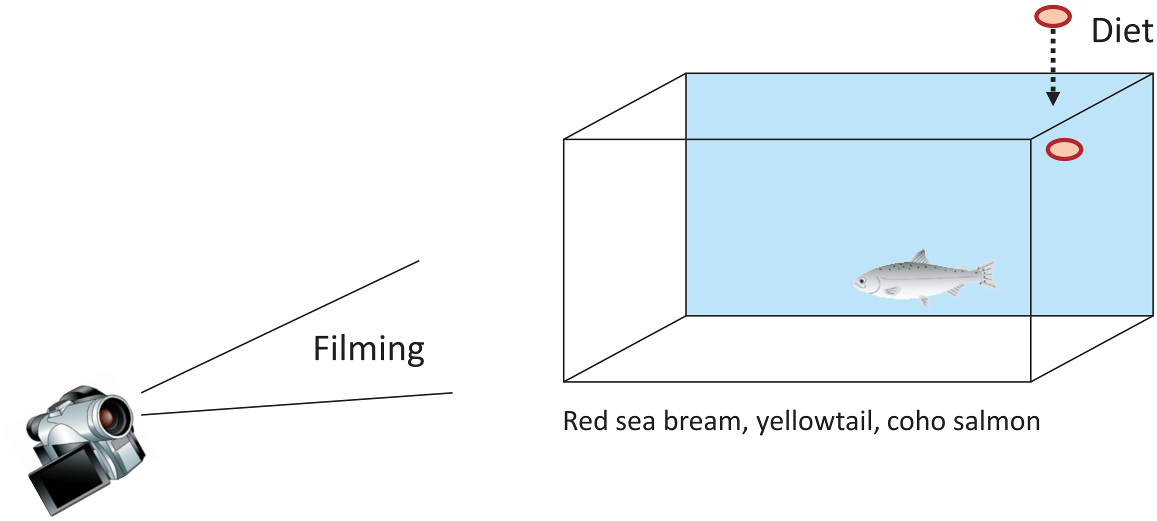

Fig. 4. Behavioral analysis of fish after feeding the diets.

Table 3. Growth performance and feed utilization by yellowtail fed the experimental diets

Rearing season (days)		Initial	Summer (90 days)				Autumn (91 days)			
			FM	PP-SP	PP-FS	AP-SP	FM	PP-SP	PP-FS	AP-SP
Number of fish		220[*1]								
	Dead		0	0	1	2	1	0	1	1
	Sampled		3	4	3	4	7	7	7	7
	Final		52	51	51	49	44	44	43	41
Total food intake (kg)		–	113.0	113.9	113.6	118.0	98.5	102.9	101.0	90.5
Body weight (g)[*2]		753 ± 96								
	Final		1663 ± 204^{a}	1700 ± 221^{a}	1698 ± 156^{a}	1688 ± 204^{a}	2242 ± 333^{a}	2178 ± 285^{a}	2234 ± 244^{a}	2205 ± 222^{a}
Fork length (cm)[*2]		36.9 ± 1.5								
	Final		44.8 ± 2.3^{a}	44.8 ± 1.8^{a}	45.0 ± 1.2^{a}	44.1 ± 1.2^{a}	48.4 ± 2.2^{a}	48.6 ± 1.8^{a}	49.0 ± 1.7^{a}	48.5 ± 1.5^{a}
Condition factor[*2]		15.0 ± 0.8								
	Final		18.3 ± 1.1^{b}	18.8 ± 0.9^{b}	18.6 ± 0.7^{b}	19.7 ± 1.4^{a}	19.6 ± 1.2^{a}	18.9 ± 1.2^{b}	19.0 ± 1.1^{ab}	19.3 ± 1.0^{ab}
Weight gain ratio (%)[*3]		–	116.4	119.9	119.1	113.6	34.2	30.4	31.0	32.3
Daily weight gain (%/day)[*3]		–	0.82	0.83	0.83	0.80	0.32	0.29	0.30	0.31
Daily feeding rate (%/day)[*3]		–	1.92	1.91	2.02	1.91	1.08	1.15	1.05	1.12
Feed conversion ratio[*3]		–	2.35	2.29	2.30	2.51	3.33	3.91	3.76	3.39
Protein efficiency ratio[*3]		–	1.07	1.14	1.16	0.94	0.75	0.67	0.71	0.70

[*1] Fishes were divided into 4 diet groups, each group contains 55 fishes.
[*2] Data are shown as mean ± standard deviation (n=20–44). Values having different superscript letters are significantly different ($P < 0.05$).
[*3] Shown in the text.
Experimental diets are shown in Table 1.

showed a similar total feed intake ranging from 113 to 118 kg and a similar body weight gain (Fig. 5). In the autumn period when the water temperature was 11.5 ℃ lower than during the summer period total food intake of all groups was less than in the summer ranging from 90 to 103 kg. No seasonal difference in weight gain was seen among diet groups.

Final fork length and body weight were 48.4 cm and 2,242 g in the control (FM), 48.6 cm and 2,178 g in the PP-SP, 49.0 cm and 2,234 g in the PP-SF, and 48.5 cm and 2,205 g in the AP-SP. Differences between control and non-fish meal diets were not observed and the growth was almost the same. There was no seasonal difference between daily weight gain (%/day), daily feeding rate (%/day), and weight gain ratio (%). For example, feed conversion ratio in the autumn season was 3.33 in the control (FM) and 3.91 in PP-SP, 3.76 in PP-FS, and 3.39 in AP-SP.

Table 4 shows the results of the biological indices of cultured fish (n=5). The liver to body weight ratio in every group was 1.2 to 1.3 and no significant difference was seen. The average hematocrit value of 5 fish in every group was 46.2 to 50.6 with no significant difference observed. There were three, two, and one mortalities of fish in AP-SP, PP-FS, and FM respectively. Green liver, which is thought to be a sign of taurine deficiency (Takagi *et al.*, 2005), was not seen in any of the dead fish.

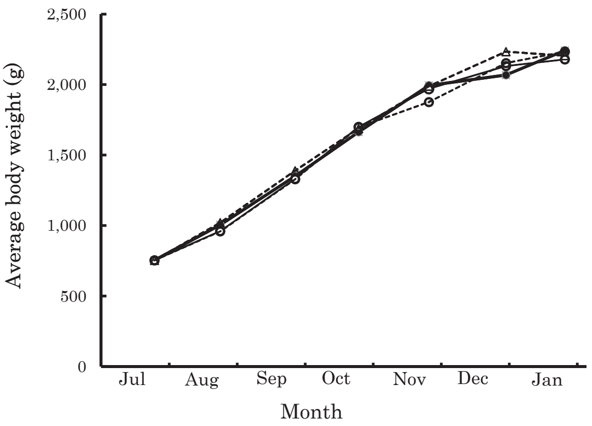

Fig. 5. Growth curves of yellowtail fed the experimental fish meal diet (FM : –●–) and non-fish meal diet (PP-SP : –⊖–, PP-FS : -⊝-, and AP–SP : -△-) in net cages for 6 months. Experimental diets are detailed in Table 1.

Plasma components measured included glucose, total protein, total cholesterol, glutamate pyruvate transaminase (GPL), and alkaline phosphatase (ALP). No significant difference was observed in plasma components between the treatments in the summer period. Total cholesterol exhibited a significant seasonal variability between the treatments with lower values of 246 and 266 mg/dl in PP-SP and PP-FS respectively ($p<0.05$) (Table 4).

Characteristics such as uptake, stomach evacuate rate, and disease resistance of fish fed the experimental diets: The uptake of protein from non-fish meal diets was reduced by 4–9 % compared to the FM control (Satoh *et al.*, 2013).

Evacuation of feed from fish stomachs was observed for all three diets. The order of the rate of evacuation was PP-SP > AP-SP ≈FM (Fig. 6).

Survival rate at 42 days after the challenge test was 80% in FM group, and 95% in PP-SP and AP-SP group (Ishida *et al.*, 2013).

Diet palatability: Fish spitting out feed was almost zero for single dry fish meal pellet. The spitting rate was higher in single dry pellet made from plant protein than those made from animal protein. To improve palatability of PP and AP pellets we washed the original materials with 80% ethanol to remove the extractive component and made each single dry pellet from those pre-treated materials. The spitting rate in single dry pellet made from pre-

Table 4. Changes in hepatosomatic index, heamatocrit value, and hematochemical characteristics in yellowtail (n=5) fed the experimental deits

Rearing season (days)	Initial	Summer (90 days)				Autumn (91 days)			
		FM	PP-SP	PP-FS	AP-SP	FM	PP-SP	PP-FS	AP-SP
Hepatosomatic index (%)*1	1.1 ± 0.0	1.4 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.2^{a}	1.3 ± 0.3^{a}	1.4 ± 0.2^{a}	1.3 ± 0.1^{a}	1.2 ± 0.0^{a}	1.2 ± 0.1^{a}
Hematocrit value (%)	53.6 ± 3.4	61.5 ± 7.4^{a}	50.8 ± 0.7^{a}	57.0 ± 4.9^{a}	55.3 ± 7.3^{a}	46.2 ± 2.1^{a}	45.7 ± 4.5^{a}	50.6 ± 6.5^{a}	48.0 ± 6.2^{a}
Heamatochemical characteristics									
Glucose (mg/*dl*)	144 ± 25.2	237.8 ± 51.8^{a}	262.6 ± 30.8^{a}	267.0 ± 41.3^{a}	280.8 ± 146.1^{a}	138.6 ± 10.5^{a}	127.6 ± 21.3^{a}	143.0 ± 12.1^{a}	130.0 ± 28.5^{a}
Total protein (g/*dl*)	4.0 ± 0.5	4.3 ± 0.7^{a}	3.7 ± 0.2^{a}	4.3 ± 1.5^{a}	4.8 ± 0.7^{a}	$4.1 \pm 0.5a^{b}$	3.7 ± 0.2^{b}	$3.9 \pm 0.5a^{b}$	4.4 ± 0.4^{a}
Total cholesterol (mg/*dl*)	349.4 ± 63.0	282.0 ± 30.8^{a}	203.4 ± 21.7^{a}	267.0 ± 79.5^{a}	276.4 ± 101.0^{a}	396.8 ± 72.3^{a}	245.6 ± 29.0^{b}	266.4 ± 61.0^{b}	346.0 ± 82.1^{ab}
GPT (IU/*l*)*2	25.4 ± 2.6	32.4 ± 4.2^{a}	59.2 ± 44.1^{a}	34.6 ± 14.5^{a}	44.4 ± 19.9^{a}	32.0 ± 13.4^{a}	30.8 ± 14.8^{a}	23.4 ± 8.6^{a}	33.8 ± 11.4^{a}
ALP (IU/*l*)*3	148.6 ± 33.8	156.4 ± 34.3^{a}	134.8 ± 14.4^{a}	181.0 ± 64.0^{a}	200.6 ± 22.9^{a}	143.4 ± 43.4^{a}	125.2 ± 16.9^{a}	126.5 ± 23.7^{a}	147.8 ± 42.3^{a}

Mean ± standard deviation (n=5). Values having different superscript letters are significantly different ($P<0.05$).
*1 The ratio of liver weight to body weight.
*2 Glutamate-pyruvate transaminase.
*3 Alkaline phosphates.
Experimental diets are shown in Table 1.

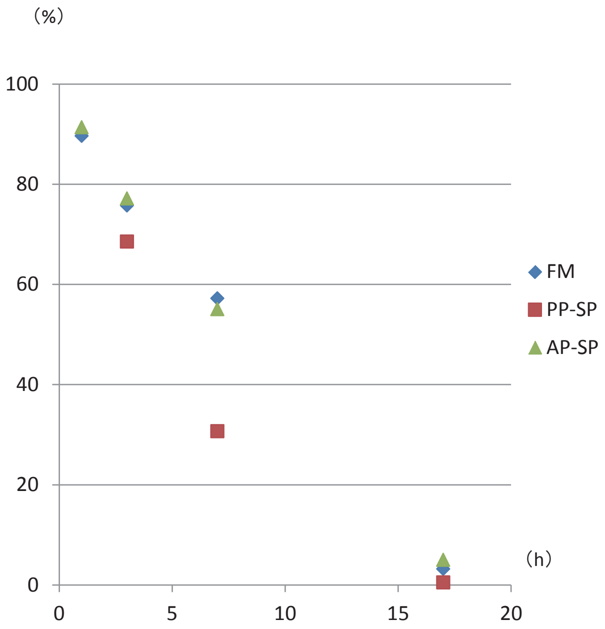

Fig. 6. Evacuation of the diet from the fish stomach after a single oral administration of diet at 2% body weight. Value are shown the percentage of nitrogen in the stomach residue per administered nitrogen. Experimental diets are shown in Table 1.

treated plant protein decreased. On the other hand, the opposite result was shown in pre-treated animal proteins that the spitting rate increased (Niinuma *et al.*, 2012, Yamauchi *et al.*, 2012).

Fish meat analysis: Table 5 shows the results of the fish meat analysis of cultured fish. In the proximate composition of all the diet groups, moisture content declined and fat content increased until the end of experiment. No difference was seen in the phosphorus content among the diet groups. The EPA, DHA and n3HUFA content of fish fed AP-SP pellets was always lower than in fish fed the other diets, at times significantly less (p<0.05) in both summer and autumn. These results were similar to the analysis of experimental diets.

Discussion

It has long been thought that fish meal was a necessary ingredient in fish feed. Generally, the formulated feeds for yellowtail contain about 50% fish meal. Recently, formulated feeds containing lower amounts of fish meal such as 30% have been produced as a commercial product. However a fish meal content of less than 30% would have considerable financial benefits for aquaculture enabling continued economically viable production. Therefore, we developed non-fish meal feeds and evaluated those in a field experiment with a similar aquaculture environment. We used one year old yellowtail which is an intermediate size during culturing and shows rapid growth requiring high feed costs. Experimental periods included the higher feeding activity season of summer and the lower feeding activity season of winter.

In the present study (Tsuzaki *et al.*, 2014), the plant protein based diets of PP-SP and PP-SP showed a similar daily feeding rate and daily weight gain, better feed conversion ratio and protein efficiency ratio than the control FM in summer season. On the other hand, the animal protein based diet of AP-SP showed a higher daily feeding rate and feed conversion ratio, lower protein efficiency ratio demonstrating lower protein uptake than others. We also studied uptake of nutrient in these non-fish meal diets and FM by analysis of yellowtail feces. It is clear that the uptake of protein in these non-fish meal diets were reduced by 4 – 9 % compared to the control FM.

Then, in the autumn season, the plant protein based diets of PP-SP and PP-FS showed a low performance because of the higher daily feeding rate and feed conversion ratio, lower daily weight gain. These results are thought to be due to the physical properties of the diets. Because these plant protein based diets did not sink appropriately and flowed out from the net cage compared to the other diets. From these results, more studies are needed for the improvement of uptake for animal protein based diets and adjustment of the physical characteristics for plant protein based diets. However, these results demonstrate that non-fish meal diets have the potential to support the growth of one year old

Table 5. Proximate composition, phoshorus conntent, and fatty acid composition of muscle in yellowtail (n=5) fed the experimental diets

Rearing season (days)		Summer (90 days)				Autumn (91 days)			
	Initial	FM	PP-SP	PP-FS	AP-SP	FM	PP-SP	PP-FS	AP-SP
Proximate composition (%)									
Moisture	71.9 ± 0.8	64.7 ± 1.7	64.5 ± 1.4	64.8 ± 1.7	65.6 ± 4.1	60.6 ± 4.6	60.0 ± 3.0	60.3 ± 1.3	62.5 ± 0.9
Crude ash	2.3 ± 0.5	1.3 ± 0.3	1.3 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.1
Crude protein	22.1 ± 0.2	21.4 ± 0.6	20.3 ± 1.1	20.5 ± 0.8	20.7 ± 1.3	20.1 ± 2.3	20.5 ± 1.6	22.0 ± 1.0	22.0 ± 0.5
Crude lipid	3.7 ± 0.9	11.5 ± 2.1	12.8 ± 2.6	10.6 ± 1.2	10.1 ± 3.3	16.0 ± 6.2	17.9 ± 4.7	16.0 ± 1.2	14.3 ± 1.5
Phosphorus content									
P (mg/g)	15.5 ± 4.4	4.2 ± 1.7	5.5 ± 2.8	5.0 ± 1.5	4.3 ± 2.5	4.6 ± 1.1	4.5 ± 1.6	4.4 ± 0.7	4.7 ± 1.4
Fatty acid composition (%)									
14: 0	4.2 ± 0.4	4.0 ± 0.3	3.1 ± 0.6	3.4 ± 0.7	3.9 ± 0.2	4.3 ± 0.2	3.9 ± 0.4	3.8 ± 0.3	4.2 ± 0.2
16: 0	19.6 ± 0.9	18.0 ±0.8	16.2 ± 1.3	16.8 ± 1.1	16.1 ± 0.3	16.4 ± 0.4	16.5 ± 0.7	15.6 ± 0.6	16.0 ± 0.5
18: 0	5.6 ± 0.5	4.2 ± 0.3	4.0 ± 0.1	4.2 ± 0.5	4.5 ± 0.4	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.9 ± 0.0
16: 1n-7	5.9 ± 0.8	6.6 ± 0.3	5.8 ± 0.5	5.9 ± 0.4	5.9 ± 0.3	7.0 ± 0.3	6.3 ± 0.2	6.2 ± 0.2	6.6 ± 0.3
18: 1n-9	21.4 ± 1.4	23.2 ± 2.2	25.0 ± 0.8	25.6 ± 1.3	25.0 ± 1.9	20.5 ± 1.9	23.5 ± 1.6	22.6 ± 2.0	22.1 ± 0.3
20: 1n-9	3.1 ± 0.8	5.1 ± 0.3	5.4 ± 0.3	5.7 ± 0.5	9.4 ± 1.4	5.6 ± 1.2	5.3 ± 0.1	5.6 ± 0.3	6.5 ± 0.2
20: 1n-11	0.6 ± 0.9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.9	0.2 ± 0.0	0.2 ± 0.0	2.9 ± 0.1
22: 1n-11	2.7 ± 0.3	3.4 ± 0.4	3.7 ± 0.5	4.0 ± 0.6	8.7 ± 0.7	3.6 ± 0.6	3.1 ± 0.1	3.7 ± 0.4	6.3 ± 0.4
18: 2n-6	5.6 ± 0.2	5.8 ± 0.2	8.6 ± 0.3	8.6 ± 0.1	7.2 ± 0.2	5.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	7.4 ± 0.2
20: 4n-6	1.2 ± 0.2	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.4 ± 0.0
18: 3n-3	0.6 ± 0.1	1.1 ± 0.0	1.3 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	0.8 ± 0.0
18: 4n-3	0.9 ± 0.1	1.6 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.6 ± 0.3	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.0
20: 4n-3	0.5 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
20: 5n-3 (EPA)	5.3 ± 0.2	6.2 ± 0.5^{a}	5.3 ± 0.4^{ab}	4.8 ± 0.9^{ab}	4.6 ± 1.1^{b}	6.6 ± 0.2^{a}	5.0 ± 0.3^{bc}	5.1 ± 0.3^{b}	4.7 ± 0.1^{c}
22: 5n-3 (DPA)	2.2 ± 0.2	2.4 ± 0.1^{a}	2.2 ± 0.2^{ab}	2.1 ± 0.2^{b}	1.5 ± 0.1^{c}	2.4 ± 0.2^{a}	2.0 ± 0.1^{b}	2.1 ± 0.1^{b}	1.4 ± 0.1^{c}
22: 6n-3 (DHA)	11.4 ± 1.6	10.9^{a}	11.3 ± 1.0^{a}	9.4 ± 3.1^{a}	4.9 ± 1.2^{b}	11.4 ± 0.6^{a}	10.2 ± 0.8^{a}	10.5 ± 1.2^{a}	5.1 ± 0.5^{b}
Others	9.2 ± 2.8	5.9 ± 1.5	5.0 ± 0.3	5.2 ± 1.0	4.5 ± 2.4	8.9 ± 1.9	7.9 ± 2.0	8.5 ± 2.0	9.8 ± 0.2
n-3 HUFA	20.3 ± 1.4	21.7 ±2.5^{a}	20.9 ± 1.6^{a}	18.3 ± 4.3^{ab}	13.2 ± 2.9^{b}	22.7 ± 0.6^{a}	19·2 ± 1.2^{b}	19.8 ± 1.5^{b}	13.2 ± 0.6^{c}

Mean ± standard deviation (n=5). Values having different superscript letters are significantly different (P<0.05).
Experimental diets are shown in Table 1.
Fatty acid compositions are shown in Table 2.

yellowtail compared to the results of Takagi *et al.* (2013).

For substitute materials, plant protein material such as soy bean concentrate has been studied (Aoki *et al.*, 2000a, 2000b). We also used soy bean concentrate and confirmed its performance as a substitute protein for fish meal in the present study. In the field, it is sometimes believed that plant protein materials stay longer in the stomach than fish meal. However, in our experiment, the plant protein based diets did not stay longer among the diet groups. Fish meal based diet was the first to be evacuated from the stomach. In addition, it is sometime believed that plant protein fed yellowtail is more susceptible to epidemic disease than fish meal based diet. However, in our experiment, those phenomena were not seen (Ishida *et al.*, 2013). We also compared the stomach evacuation rate and the *in vitro* digestion using only one protein material diet, and found minimal difference (Tohata *et al.*, 2012). More studies are needed in future using low cost materials such as defatted soy bean meal, corn gluten meal and canola oil.

As for substitute animal protein materials, there are meat meal, chicken meal, feather meal, and pork meal. Feather meal and meat meal were reported to be useful for yellowtail (Shimeno *et al.*, 1993a, 1993b, 2000). However, the animal protein studies are fewer than plant protein studies. There is potential to use chicken meal and pork meal. As there are few studies about pork meal, we tried to use pork meal in the present study. Pork meal has less DHA, DPA and n3HUFA of fatty acid than FM. A similar tendency was seen in the whole fish body and the meat of yellowtail fed by pork meal based diet. However, there is no significant effect on growth and survival. Only slightly less protein digestion in pork meal based diet was seen than the plant protein based diet. And feeding activity on pork meal based diets was active. From these results, pork meal was thought to be available as a substitute material.

The biggest problem has been shown to be the reduction of feeding activity when fish are fed low fish meal content diets (Aoki *et al*., 2000a, 2000b, 2000c). However, it is reported that an added palatability ingredients improved the feeding activity in eel (Takii *et al*., 1984), yellowtail (Takii *et al*., 1994), and tiger puffer (Takii *et al*., 1998). In the present study, we found that fish did not especially prefer plant protein to animal protein and fish meal. However, after washing the feed ingredients to remove the extractive component, the spitting ratio decreased in plant ingredient feeds and increased in animal protein feeds, which suggested that the extractive components might affect feeding activity (Niinuma *et al*., 2012, Yamauchi *et al*., 2012). Then, we added some palatability ingredients to the plant based diet, fish swallowing ratio was increased and spitting ratio was decreased. From these results, we used the skipjack peptide (SP) and fish sauce (FS) as palatability ingredients in order to improve the feeding activity. In the results, higher palatability was seen when using both ingredients. The biggest problem of reduction of feeding activity in non-fish meal diets was thought to be almost resolved by the addition of palatability ingredients.

The meat quality of yellowtail fed with non-fish meal diets were compared to control yellowtail by a panel test, which showed a difference in color and smell. Then we used colorimeter and Electronic Nose System of Alpha MOS (http://www.alpha-mos.co.jp/products/sensory.html). The fish fed by non-fish meal diets had whiter muscle and a less fishy smell (Tohata *et al*., 2013). Now, the effect of non-fish meal diets on fish meat quality and as a method of enhancing the meat quality control is being determined.

As described above, we alternated fish meal to plant or animal protein materials to make non-fish meal diets, and fed them to one year old yellowtail, and got similar growth, body and blood compositions. The main reason for the similar growth rates was thought to be the addition of palatability ingredients and enzyme mixtures (Table 1). As we used both plant and animal materials, we suggest the possibility of many materials for non-fish meal diets. More study is needed to improve low cost diets and produce higher quality meat.

This work was supported by Research and Development Projects for application in promoting new policy of agriculture, forestry and fisheries from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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