Effect of fish meal replacement by soybean meal with or without phytase supplementation on the growth performance of the pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel) juvenile

Amal Biswas*, Biswajit K. Biswas, Junichi Ito and Kenji Takii
(Aquaculture Group)
Fisheries Laboratories, Kinki University

Recently, soybean meal becomes the most promising alternative of fish meal (FM) because of its availability and low cost.[1] Many studies have shown considerable success in partial or total replacement of FM by soybean meal and other plant products with or without phytase supplementation,[2,3] while others reported adverse effect on weight gain and feed efficiency.[4] This is attributed to the presence of several anti-nutritional factors in soybean meal and plant products that may disturb the digestive process.[5]

The Pacific bluefin tuna (PBT), *Thunnus orientalis* is one of the most popular fish in Japan because of its high meat quality and the popularity in 'sashimi' and 'sushi' markets. Due to its world-wide great demand, scientists have been tried to establish complete aquaculture in captivity during the last decades with limited success until 2002, when researchers from Kinki University, Japan obtained the successful completion of the PBT life cycle in captivity for the first time in the world.[6] Afterwards, a series of studies have been carried out to investigate the nutritional requirements of PBT at larval and juvenile stages.[7,8] Recently, artificial formulated diet has been established for PBT juveniles using enzyme treated FM as a main protein source.[9] However, it is necessary to search for alternative protein sources due to the price and unavailability of FM. As the growth rate of PBT at larval and juvenile stages is very fast, FM may not be replaced by soybean meal because of lower protein level and the presence of anti-nutritional factors.[10] However, this area needs to be clarified.

This study was therefore investigated whether FM can be replaced by soybean meal with or without phytase supplementation in the diet of PBT juvenile.

Materials and Methods

Diet preparation

Five isoenergetic diets (Table 1) were formulated as follows; FM 63.8% (S_0), FM 58.7% + soybean meal 10% (S_{10}), FM 49.7% + soybean meal 20% (S_{20}), and S_{10} and S_{20} were supplemented with phytase at 2000 FTU/ kg diet (S_{10}+P_{2000} and S_{20}+P_{2000}, respectively). Defatted enzyme treated FM (Profish S.A., Santiago, Chile) and soybean meal (Itochu Shoji, Tokyo, Japan) were used as main protein sources. One FTU is defined as the amount of enzyme that generates 1 μ-mole of inorganic phosphorus per min from an excess of sodium phytate at pH 5.5 and 37°C. For digestibility, 0.5% chromic oxide (Cr_2O_3) was included as an inert marker. The diets were pelleted by a laboratory pellet machine after mixing 100 parts of ingredients with 15 parts of tap water. Appropriate sizes were adjusted using sieves and were stored in a freezer at -20°C until used.

Table 1. Formulation and proximate composition of test diets

Ingredients (%)	S_0	S_{10}	$S_{10}+P_{2000}$	S_{20}	$S_{20}+P_{2000}$
Fish meal	63.80	58.69	58.69	49.69	49.69
Soybean meal	-	10.00	10.00	20.00	20.00
Salmon egg oil	7.54	7.54	7.54	7.54	7.54
α-starch	8.00	7.00	7.00	6.00	6.00
Vitamin mix[1]	5.00	5.00	5.00	5.00	5.00
Mineral mix[2]	5.00	5.00	5.00	5.00	5.00
Soybean lecithin	1.89	1.89	1.89	1.89	1.89
Taurine	2.00	2.00	2.00	2.00	2.00
Feeding stimulants[3]	0.50	0.50	0.50	0.50	0.50
Cellulose	3.89	0.00	0.00	0.00	0.00
Wheat gluten	2.26	2.26	2.26	2.26	2.26
Vitamin C	0.08	0.08	0.08	0.08	0.08
Vitamin E	0.04	0.04	0.04	0.04	0.04
Phytase (FTU/kg)			2000		2000
Proximate compositions (% dry weight basis)					
Crude protein	57.1	57.7	57.2	55.0	56.1
Crude lipid	15.8	15.6	15.6	15.0	15.2
Crude ash	7.4	8.4	7.7	8.2	8.0
Crude suger	10.3	11.7	11.9	12.7	13.2
Energy (kJ/g)	23.0	23.4	23.1	22.5	22.9
Phosphorus (g/kg diet)	24.7	16.2	15.8	15.3	14.6

[1]Those of Halver (1957) excluding vitamin C and E

[2]Halver (1957)

[3]Mixture of alanine 13.7, glutamic acid 8.5, histidine 232.8, lysine 44.1 and inosine-5'-monophosphate Na_2 200.9 mg

Table 2. Fatty acid composition (% of total fatty acid) of test diets. Values are mean±SE (n=2)

Fatty acid	S_0	S_{10}	$S_{10}+P_{2000}$	S_{20}	$S_{20}+P_{2000}$
C14:0	4.2±0.1	4.1±0.3	3.9±0.1	3.8±0.1	3.8±0.1
C15:0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0
C16:0	15.2±0.1	15.9±1.1	15.2±0.3	15.3±0.1	15.1±0.2
C17:0	0.8±0.0	0.8±0.1	0.7±0.0	0.7±0.0	0.7±0.0
C18:0	4.6±0.1	5.0±0.4	4.8±0.1	4.8±0.1	4.7±0.1
Σsaturated	25.4	26.4	25.2	25.2	24.9
C16:1	6.2±0.1	6.2±0.5	5.9±0.1	5.8±0.1	5.7±0.2
C17:1	0.5±0.3	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
C18:1n-9(OA)	16.4±0.1	17.2±1.2	16.3±0.1	16.4±0.2	17.0±0.8
C18:1n-7	1.9±0.0	1.3±0.6	1.7±0.1	1.2±0.6	1.2±0.4
C20:1	2.2±0.2	2.5±0.2	1.8±0.9	2.4±0.1	2.3±0.2
Σmono-saturated	27.2	27.9	26.4	26.5	26.9
C18:2n-6 (LA)	6.6±0.1[a]	8.0±0.6[bc]	7.7±0.1[b]	9.1±0.0[c]	8.9±0.0[bc]
C20:4n-6 (AA)	1.1±0.0	1.2±0.1	1.4±0.4	1.1±0.0	1.1±0.0
C22:5n-6 (DPA)	3.3±0.0	3.5±0.3	3.2±0.1	3.2±0.0	3.2±0.1
Σn-6	11.0	12.7	12.3	13.4	13.2
C18:3n-3 (LNA)	1.4±0.0	1.5±0.1	1.3±0.2	1.6±0.0	1.6±0.0
C20:5n-3 (EPA)	10.8±0.1	11.2±0.8	10.5±0.1	10.3±0.1	10.4±0.1
C22:6n-3 (DHA)	14.1±0.2	15.2±1.2	14.5±0.5	13.9±0.2	13.8±0.2
Σn-3	26.3	27.9	26.3	25.8	25.8
n-3/n-6	2.4	2.2	2.1	1.9	2.0
DHA/EPA	1.3	1.4	1.4	1.3	1.3

Values in a row with different letters are significantly different (Tukey's tes, $P<0.05$)

Fish husbandry, experimental design, sampling, calculation and chemical analysis

Fertilized eggs of PBT were hatched out in the Fish Nursery Center, Kinki University, Uragami, Wakayama, Japan. The temperature and DO were maintained at 26.5°C and 7.7 mg/ l, respectively. When PBT juveniles were reached around 0.5 g body weight, each 400 fish were introduced into a 40 t circular tank for each diet. Initial fish were also sampled and kept frozen at -80°C until analysis. Juveniles were fed with test diets six times daily at 05:30, 08:00, 11:00, 14:00, 16:00 and 18:00 until apparent satiation for 10 days. Dead fish in each tank were counted and weighed during the rearing period. On 10^{th} day of rearing trial, fish were fed with Cr_2O_3 included diets for fecal collection to investigate the digestibility. All survived fish were sampled at 2.5 h after feeding with Cr_2O_3 included diets. Groups of fish were frozen at -80°C for proximate analysis and remaining fish were dissected to collect feces from the intestine for digestibility analysis and treated according to the methods described by Bureau et al. [11]

The data obtained were analyzed for weight gain (%), specific growth rate (SGR), feed conversion efficiency (FCE), condition factor (CF), retention efficiencies of protein, energy and phosphorus, phosphorus discharge and apparent digestibility coefficient (ADC).

Samples (diets, fish and feces) were analyzed for dry matter, crude protein and ash using standard methods.[12] Lipid for fatty acid (FA) analysis was extracted according to Folch et al. [13] The fatty acid methyl esters were analyzed using methanolic 2M NaOH solution and methanolic 2M HCl solution according to Yoshinaka and Satoh[14] with a gas chromatograph (G-3000; Hitachi, Tokyo, Japan) equipped with an Ultra Alloy® capillary column (30 m x 0.25 mm ID; Frontier Laboratories, Fukushima, Japan) and a flame ionization detector. Tricosanoic acid methyl ester (Sigma-Aldrich, USA) was used as an internal standard. The column oven temperature was increased from 180 to 240°C at a rate of 4°C per min and then the temperature was maintained at 240 °C for 20 min. The carrier gas was nitrogen, and source and column head pressure at 5 and 1 kgf/ cm^2, respectively. The final temperatures for the injector and detector were 260 and 290°C, respectively. Peak quantification was performed with an integrator (D-2500; Hitachi, Japan). Phosphorus content of diet and fish whole body was determined using the ammonium-molybdate method described by Baginski et al.[15] after the digestion of samples with nitric and perchloric acid. Gross energy was analyzed using an automated oxygen bomb calorimeter (IKA-Werke, Staufen, Germany). Chromic oxide in the diet and feces was determined by a wet-acid digestion method.[16] All chemical analyses were performed in duplicate and averaged.

Statistical analyses

All statistical analyses were carried out using the SPSS program for Windows (v. 10.0). For growth parameters, there was only one value for each treatment, making post-hoc tests impossible. However, all chemical analyses for final whole body proximate composition were performed in duplicate, which were considered as two cases per treatment for the post-hoc test. Wherever possible, the means were compared using Tukey's test of multiple comparison with a 95% significance level.

Results

Table 3. Growth performance of *Thunnus orientalis* juvenile fed with different diets

Parameters	S_0	S_{10}	$S_{10}+P_{2000}$	S_{20}	$S_{20}+P_{2000}$
Initial body weight (g)	0.54	0.54	0.54	0.54	0.54
Final body weight (g)	4.2	3.1	3.3	3.3	3
Weight gain (%)	650.8	450.4	471.1	401.4	368.7
SGR (%)	20.6	17.4	18	18.2	17.3
Feed intake (g/100 g fish)	7.5	9.1	8.7	10.1	10.6
FCE (%)	133.4	110.4	114.9	98.6	94.6
CF	1.2	1.1	1.1	1.1	1.2
Survival rate (%)	31.8	36.0	31.0	53.0	51.3

All test diets contained similar proximate constituents except phosphorus, which was decreased gradually with increasing levels of soybean meal inclusion (Table 1). There was no major variation in fatty acid contents among the diets (Table 2).

Remarkable reduction in weight gain (%) and SGR were observed when FM was replaced partially by soybean meal, and phytase supplementation did not have great impact on the stimulation of both parameters (Table 3). For 100 g of fish in a day, feed intake was increased with increasing level of FM replacement by soybean meal. However, remarkable reduction in FCE was observed when FM was replaced partially by soybean meal, irrespective of phytase supplementation. Survival was remarkable higher in S_{20} and $S_{20}+P_{2000}$ than that of other treatments.

At 2.5 h after feeding, relative intestinal digesta weight was lowest in S_0 and increased with increasing soybean meal substitution (Fig. 1a). FM replacement resulted in reduced protein digestibility in diets S_{10} and S_{20}; however, phytase supplementation increased the digestibility marginally in diets $S_{10}P_{2000}$ and $S_{20}P_{2000}$ (Fig. 1b). No remarkable variation in final whole body proximate composition was observed among the treatments (Table 4). Partial replacement of FM reduced the retention efficiency of protein and energy; however, phosphorus retention was increased (Table 4) and resulted in lower phosphorus discharge soybean meal groups (Fig. 1c).

There was no significant variation in final whole body fatty acid composition among treatments except C20:1, C20:4n-6 and C18:3n-3 contents (Table 5). C20:1 contents in fish fed diets S_{10}, $S_{10}+P_{2000}$ and $S_{20}+P_{2000}$ were significantly higher than that of fish fed diet S_{20} ($P<0.05$). Fish fed diets S_0 and S_{20} had significantly higher both C20:4n-6 and C18:3n-3 than those fed with other diets ($P<0.05$). The total unsaturated and mono-saturated fatty acids of final whole body were increased remarkably whereas, total n-3 and n-6 fatty acids were decreased compared to those of initial fish (Table 5).

Discussion

It is acknowledged that the experimental design with a single tank for each treat ment may be considered as a weak point of this study.

However, the limitation in sufficient seedling production of PBT as well as enormous death due to trauma caused by collision in small tank[17] have induced us to set up this experimental design with large sized single tank for each treatment. Furthermore, the management for experiment with large sized tanks becomes very difficult if more replicates are set for each treatment. Therefore, this study was designed with a large 40 t tank for each treatment. In addition, a 10-day rearing period may not be considered short at least for PBT juvenile rearing as the final mean body weight was 6-8 times bigger than the initial mean body weight. Previous studies in PBT juveniles with 10 to 14 days rearing period provided reliable growth data.[7-9,18] Another studies with PBT juveniles demonstrated that the deficiency syndromes of vitamin C could be observed as early as from the

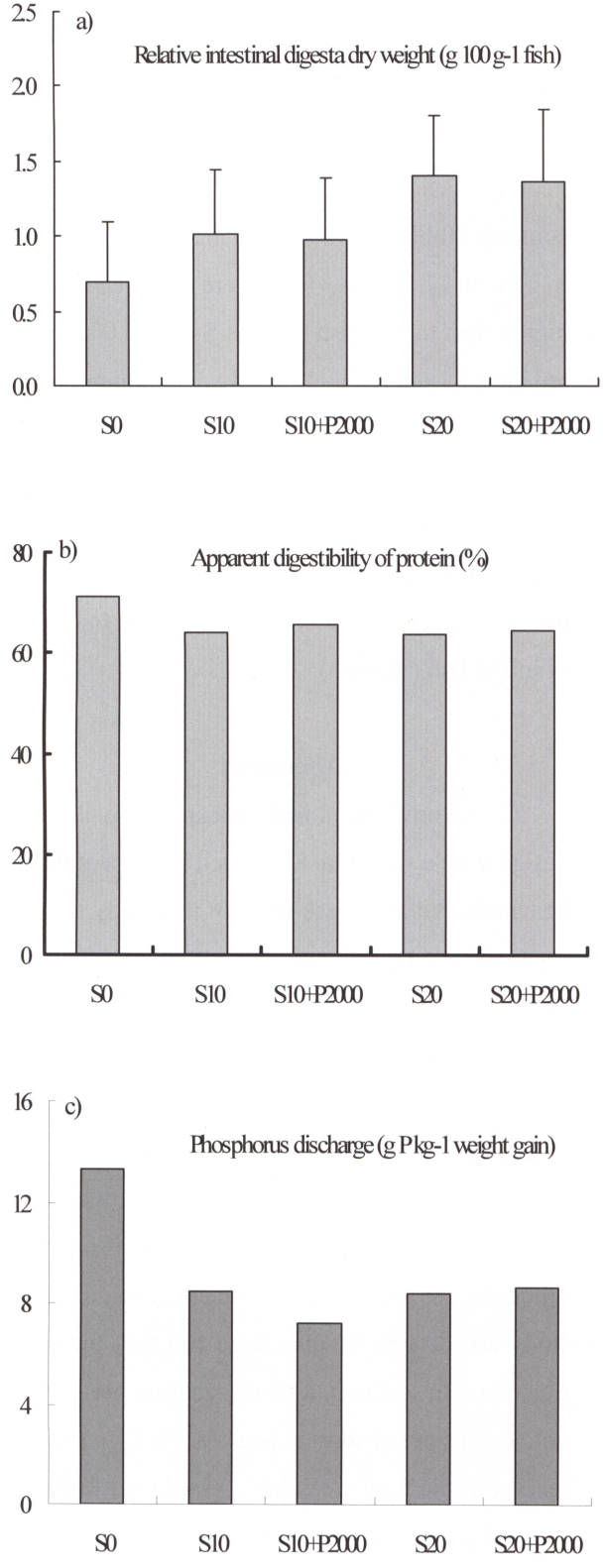

Fig. 1. Relative intestinal digesta dry weight at 2.5 hours after feeding (a), protein digestibility (b) and phosphorus discharge (c) in fish fed with different diets.

5th day of experiment (Biswas, unpubl. data), which suggests that the dietary effect can be achieved within short period in PBT juveniles.

The growth performance in terms of weight gain (%), SGR and FCE suggested that FM cannot be replaced by soybean meal irrespective of phytase supplementation. This finding parallels the findings in other species.[19] In contrast, it has been demonstrated that partial or full replacement of dietary FM by soybean meal is possible with or without phytase supplementation without any adverse consequence in terms of somatic growth or nutrient utilization.[2,3] The reduced growth performance from soybean meal groups may be attributed to the presence of anti-nutritional factors[20] and an adverse effect of phytate on growth performance and bioavailability of various dietary components.[21] It is also attributed to the reduced digestibility of protein as revealed in results, which is agreed with other studies.[3] Because of the lower digestibility of nutrients, fish fed with soybean meal supplemented diets showed higher intestinal digesta contents. This indicates that the fish try to keep the feed in intestine in an attempt to increase the digestibility of less digestible soybean meal supplemented diets. On the other hand, due to the lower digestibility of soybean meal supplemented diets, fish tried to compensate the energy by taking more feed as the feed intake per 100 g fish was increased with increasing levels of soybean meal substitution. However, those attempts in fish fed with soybean meal supplemented diets were not sufficient to sustain growth. This is due to the remarkable fast growth of PBT juvenile at early stages. It seems that PBT juvenile requires easily digestible diets to get more energy to meet their demand for fast growth. Although growth falls in soybean meal supplemented groups, remarkable higher survival rates were observed in fish fed diets S_{20} and

Table 4. Whole body proximate composition and retention efficiency in fish under different treatments

Parameters	Initial	S_0	S_{10}	$S_{10}+P_{2000}$	S_{20}	$S_{20}+P_{2000}$
Proximate compositions (% wet basis)*						
Crude Protein	8.1±0.0	12.6±0.3	11.6±0.1	11.6±0.7	12.9±0.6	13.3±0.4
Crude Lipid	1.1±0.1	1.7±0.1	1.9±0.2	1.5±0.2	2.1±0.1	2.1±0.1
Crude Ash	3.1±0.1	2.9±0.2	3.7±0.4	3.2±0.4	3.0±0.2	3.0±0.0
Moisture	82.7±0.3	81.3±0.0	81.9±0.5	81.6±0.1	80.7±0.1	79.8±0.1
Energy (kJ/g)	19.1	20.3	20.5	19.4	20.8	20.0
Retention efficiency (%)						
Protein		40.8	29.9	31.3	32.6	32.2
Energy		23.0	19.3	16.7	20.5	17.9
Phosphorus		28.1	42.1	47.7	46.0	44.5

*Values are mean±SE (n=2)

Table 5. Fatty acid composition (% of total fatty acid) of tuna juvenile fed with different diets. Values are mean±SE (n=2).

	Initial	S_0	S_{10}	$S_{10}+P_{2000}$	S_{20}	$S_{20}+P_{2000}$
C14:0	0.7±0.0	2.8±1.0	3.5±0.1	3.5±0.0	3.4±0.1	3.3±0.0
C15:0	0.3±0.0	0.7±0.1	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0
C16:0	24.6±0.4	30.2±3.1	32.1±0.1	32.1±0.2	32.6±0.2	32.7±0.4
C17:0	0.7±0.0	1.3±0.1	1.4±0.0	1.4±0.0	1.4±0.0	1.3±0.0
C18:0	12.2±0.2	14.0±0.1	13.7±0.2	13.5±0.1	14.1±0.4	14.0±0.0
Σsaturated	38.5	49	51.5	51.3	52.3	52.1
C16:1	3.0±0.1	4.9±0.6	5.2±0.3	5.0±0.2	5.2±0.1	5.1±0.1
C17:1	0.4±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.5±0.2
C18:1n-9(OA)	12.7±0.2	22.0±0.0	18.7±0.8	18.3±0.0	20.4±2.5	20.9±2.0
C18:1n-7	2.9±0.2	0.7±0.1	0.9±0.1	3.2±0.0	2.0±1.8	2.0±1.7
C20:1	0.9±0.0	$1.1±0.7^{ab}$	$1.8±0.1^{a}$	$1.8±0.0^{a}$	$0.5±0.0^{b}$	$2.0±0.0^{a}$
Σmono-saturated	19.9	29.4	27.3	29	28.8	30.5
C18:2n-6 (LA)	2.6±0.1	1.4±0.6	2.5±0.1	2.5±0.0	2.9±0.0	3.0±0.0
C20:4n-6 (AA)	3.1±0.0	$1.3±0.0^{b}$	$0.7±0.0^{a}$	$0.7±0.0^{a}$	$1.4±0.0^{b}$	$0.6±0.1^{a}$
C22:5n-6 (DPA)	1.3±0.4	1.0±0.4	1.0±0.2	1.0±0.1	0.9±0.3	0.9±0.2
Σn-6	7	3.7	4.2	4.2	5.2	4.5
C18:3n-3 (LNA)	0.4±0.0	$2.6±0.8^{b}$	$0.3±0.0^{a}$	$0.3±0.0^{a}$	$1.8±0.0^{b}$	$0.2±0.1^{a}$
C20:5n-3 (EPA)	4.4±0.1	1.6±1.0	1.5±0.0	1.7±0.2	1.6±0.1	1.7±0.2
C22:6n-3 (DHA)	24.8±0.9	5.7±1.9	5.2±0.7	5.5±0.2	4.0±0.1	4.2±0.1
Σn-3	29.6	9.9	7	7.5	6.7	5.5
n-3/n-6	4.2	2.7	1.7	1.8	1.3	1.2
DHA/EPA	5.6	3.6	3.5	3.2	2.5	2.5

Values in a row with different letters are significantly different (Tukey's tes, $P<0.05$)

S_{20}+P_{2000}. This is due to some peculiar characteristics as noticed during the course of this study. It was observed that the bigger fish in all diets were started to die first. As the weight gains (%) from both S_{20} and S_{20}+P_{2000} were lower, the fish became smaller which resulted in lower mortality. However, the plausible cause behind this remains obscure which needs to be clarified.

The lower protein retention efficiency in fish fed with soybean meal supplemented diets is attributed to the reduction in protein digestibility as discussed earlier. In this study, phytase supplementation in soybean meal diets could not show remarkable stimulation of protein digestibility, which is disagreed with other studies where significant improvement in protein digestibility by phytase supplementation has been reported.[3] More interesting results were observed in the retention efficiency and discharge of phosphorus. The higher phosphorus retention from soybean meal groups may be attributed to the lower content of phosphorus in diets. Although phosphorus digestibility could not be calculated because of the insufficient samples, fish fed with soybean meal supplemented diets with lower phosphorus contents might be tried to digest and retain it properly which, in turn, resulted in lower phosphorus discharge. The lower phosphorus discharge from soybean meal supplemented diets might be considered a positive side as reducing phosphorus discharge is a critical factor in reducing environmental pollution from commercial fish production. Similar results of lower phosphorus discharge due to FM replacement were also reported in other studies.[3]

Final whole body fatty acid composition showed higher deposition of saturated and mono-saturated, and lower deposition of n-6 and n-3 specially DHA in all diets. These results are in contrast with previous studies[7,8,18]; however, the plausible cause behind this is not clear. Further investigations are required to clarify this area.

In conclusion, the remarkable reduction in growth performance in terms of weight gain (%), SGR and FCE suggests that even 10% fish meal also cannot be replaced by soybean meal in diets of PBT juvenile at least with the dietary formula used here. However, if the emphasis is given on survival rate and minimum phosphorus discharge to the environment, about 20% soybean meal can be included in juvenile PBT diets. Further studies are necessary to clarify whether other protein sources can be used to replace FM. It is important to investigate whether the manipulation of other ingredients levels such as vitamin and mineral mixtures can provide further opportunity to replace FM by soybean meal.

Acknowledgement

The expenses of this study were defrayed by the Global COE (Center of Excellence) program of the Ministry of Education, Culture, Sport, Science and Technology, Japan.

References

1) Hardy RW. Current issues in salmonid nutrition. In: Nutrition and Utilization Technology in Aquaculture (ed. P C. E. Lim and D. J. Sessa), 1995; pp. 26-35. AOCS Press, Champaign, IL, USA.

2) Kaushik SJ, Covès D, Dutto G., Blanc D. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 2004; **230**: 391-404.

3) Biswas AK, Seoka M, Takii K, Kumai H. Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. *Aquaculture* 2007; **267:**284-291.

4) Krogdahl A, Bakke-McKellep AM, Baeverfjord G. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquacult. Nutr*. 2003; **9:** 361–371.

5) Storebakken T, Refstie S, Ruyter B. Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Soy in Animal Nutrition (ed. J.K. Drackley), 2000; pp. 127–170. Federal Animal Science Society, Savoy, IL, USA.

6) Sawada Y, Okada T, Miyashita S, Murata O, Kumai H. Completion of the Pacific bluefin tuna *Thunnus orientalis* (Temminck et Schlegel) life cycle. *Aquacult. Res*. 2005; **36**: 413-421.

7) Biswas AK, Nozaki J, Kurata M, Takii K, Kumai H, Seoka M. Effect of enrichment on the growth and survival of Pacific bluefin tuna *Thunnus orientalis* (Temminck and Schlegel) larvae. *Aquacult. Res*. 2006; **37:**1662-1670.

8) Takii K, Seoka M, Ohara N, Nasu T, Oda S, Miyashita S, Ukawa M., Shimeno S., Hosokawa H. Dietary utility of Chilean fish meal and pollack liver oil for juvenile Pacific bluefin tuna. *Aquacult. Sci*. 2007; **55:** 579-585.

9) Biswas B. K., Ji S. C., Biswas A. K., Seoka M., Kim Y. S., Kawasaki K., Takii K. Dietary protein and lipid requirements for the Pacific bluefin tuna *Thunnus orientalis* juvenile. *Aquaculture* 2009; **288**:114-119.

10) Liu K. (1997) Soybeans: chemistry, technology, and utilization. Chapman and Hall, New York, USA.

11) Bureau DP, Harris AM, Cho CY. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 1999; **180:** 345-358.

12) AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis of AOAC International,17th edition. AOAC International, Gaithersburg, Maryland, USA.

13) Folch J, Lees M, Sloane GH. Simple method for isolation and purification of total lipids from animal tissues. *J. Biol.Chem.* 1957; **226:** 497-507.

14) Yoshinaka R., M. Satoh. (1989) Chemistry for Fisheries. Kouseisya-kouseikaku, Tokyo, Japan.

15) Baginski ES, Slawa SM, Zak B. Phosphate, inorganic. In: Selected Methods of Clinical Chemistry. American Association of Clinical Chemistry (ed. ES Baginski), 1982; pp. 313-316. Washington DC, USA.

16) Furukawa A, Tsukahara H. On the acid digestion for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bulletin of Japanese Society for *Fish. Sci.* 1996; **32:** 502-506.

17) Miyashita S, Sawada Y, Hattori N, Nakatsukasa H, Okada T, Murata O, Kumai H. Mortality of northern bluefin tuna (*Thunnus thynnus*) due to trauma caused by collision during early growout culture. *J. World Aquacult. Soc.* 2000; **31:** 632-639.

18) Seoka M, Kurata M, Tamagawa R, Biswas AK, Biswas BK, Yong ASK, Kim YS, Ji SC, Takii K, Kumai H. Dietary supplementation of salmon roe phospholipid enhances the growth and survival of Pacific bluefin tuna *Thunnus*

orientalis larvae and juveniles. *Aquaculture* 2008; **275**: 225-234.

19) Yoo G.Y, Wang X, Choi S, Han K, Kang JC, Bai SC. Dietary microbial phytase increased the phosphorus digestibility in juvenile Korean rockfish *Sebastes schlegeli* fed diets containing soybean meal. *Aquaculture* 2005; **243:** 315-322.

20) Liener IE. Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr.* 1994; **34**: 31-67.

22) Satoh S, Poe WE, Wilson RP. Effect of supplemental phytate and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish. *Aquaculture* 1989; **80**: 155-161.