Soybean meal can replace enzyme treated fish meal partially in the diet of juvenile Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel)

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The high meat quality of Pacific bluefin tuna (PBT), *Thunnus orientalis* has been rewarded this species as one of the most preferable fish in the world. Recently, this species is not only famous in the 'sushi' and 'sashimi' market in Japan but also in other countries in the world. Therefore, the demand for PBT is gradually increased day by day. To cope with the demand, capture-based culture of PBT has increased sharply throughout the world over a short period using net cage (Nakahara 2004).[1] This practice has been created great pressure on the natural resources and threatened the PBT to become an endangered species. Therefore, researchers from the Fisheries Laboratories of Kinki University, Japan have been tried to establish complete aquaculture in captivity during the last decades and finally reached in goal for the first time in the world in 2002.[2] Afterwards, a series of studies have been carried out to investigate the nutritional requirements of PBT at larval and juvenile stages.[3-9] Recently, artificial formulated diet has been established for PBT juveniles using enzyme treated fish meal (EFM) as main protein source.[10,11] The EFM was used in an attempt to provide easily digestible protein for fast growing PBT juveniles. However, this EFM is very expensive and not available for industrial culture of PBT. In other species, alternative protein sources have been studied intensively during the last few decades because of the unavailability and high cost of fish meal, and also in an attempt to

formulate diets which minimize phosphorus excretion.[12,13] Therefore, it is necessary to sort out the alternative dietary protein source for PBT juveniles also.

As the global production of soybean meal (SM) has continued to increase over the last few decades, it became the most promising alternate protein source for fish feeds in terms of future availability.[12] SM has frequently been assessed as an inferior protein source to fish meal, citing adverse effect on growth performance in some species.[14-17] Whereas, it has been used to replace fish meal protein partially or fully with no subsequent decrease in weight gain in others.[18-23] Moreover, the effect of SM even varies depending on production lots. As EFM is reported to be effective for PBT growth performance, it is also necessary to investigate the effect of enzyme treated soybean meal (ESM) on the growth performance of this species.[9-11]

The main objective of this study is to investigate whether the costly and less available EFM can be partially replaced by ESM or normal SM.

Materials and methods

Experimental diets

Dietary formula and proximate composition of test diets are given in Table 1. The EFM (Profish S.A, Santiago, Chile), ESM (Chubu Feed Co. Ltd.,

Osaka, Japan) and normal SM (Chubu Feed Co. Ltd. Osaka, Japan) were used as protein sources. In this study, EFM was defatted using n-hexane at a ratio of 1:3 (w/v) to establish the practical diet for PBT juveniles. However, other protein sources were not defatted, as it was aimed to investigate the utility of the commercial products of both ESM and SM. Five diets were prepared: EFM 62.13% (EFM, control), EFM 56.13% + ESM 10% (ESM_{10}), EFM 46.13% + ESM 20% (ESM_{20}), EFM 56.13% + SM 10% (SM_{10}) and EFM 46.13% + SM 20% (SM_{20}). Salmon egg oil and fish oil were used as lipid sources. Previous study (unpublished) revealed that PBT has higher vitamin C requirement than the level included in the formula by Halver[24]. Therefore, we have included vitamin mixtures two times that of Halver,[24] except vitamin C. L-Ascorbyl-2-monophosphate magnesium salt, APM (46.46% vitamin C activity; Showa Denko K. K., Tokyo, Japan) was included at 1,200 ppm as a stable vitamin C derivative. Once moist pellets were prepared using a laboratory pellet machine, appropriate sizes were adjusted using sieves and were stored in a freezer at -20°C until used.

Fish, experimental design and sampling

Naturally spawned fertilized eggs were obtained from the Fish Nursery Center, Kinki University, Oshima, Wakayama. The eggs were reared in the Fish Nursery Center, Kinki University, Uragami, Wakayama and cultured until 27 day after hatch (DAH). Before transferring to the experimental tanks, PBT were started to feed the formulated diet together with hatched larvae of striped knifejaw from 22 DAH. On 28 DAH, 276 juveniles with an initial body weight of 0.38 g were randomly distributed into each of duplicate 15 m^3 tanks for each treatment. Initial fish were also sampled and kept frozen at -20 and -80°C until analysis. Test diets were fed to fish 6 times daily (5:30, 8:00, 11:00, 14:00, 1600 and 18:00) upto apparent satiety for 12 days. The tanks were illuminated for 24 h and flow rate of filtered seawater in each tank was 30 l/min. A clock-wise water circulation was created inside the tank. Bottom cleaning was performed two times a day (10:00 and 16:00), and dead fish were collected and weighed. The mean water temperature and DO were 26.8 ± 0.1°C and 6.5±0.4 mg/l, respectively.

At the end of the rearing trial, 30 fish from each tank were randomly selected to measure length and weight. Ten fish from each tank were kept under -20°C for carcass proximate analysis and another 10 fish were stored at -80°C for fatty acid (FA) analysis. Five fish from each tank were dissected to weigh viscera, liver, stomach and intestine. Liver was taken out from another 20 fish and stored at -80°C for hepatic aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities analyses. The remaining fish were stored at -80°C. Weight gain (%), specific growth rate (SGR), daily feeding rate, feed conversion efficiency (FCE), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), stomatosomatic index (SSI), intestosomatic index (ISI), protein retention efficiency (PRE), lipid retention efficiency (LRE), phosphorous retention efficiency and phosphorous discharge were calculated by the following formula.

Weight gain (%) = 100 × (average weight gain / average initial body weight), where average weight gain = {(final total weight + sampled fish weight) − initial total weight} / average of initial and final number of fish

Table 1. Feed formulation and proximate composition (g kg^{-1} dry diet)

Ingredients	EFM	ESM$_{10}$	ESM$_{20}$	SM$_{10}$	SM$_{20}$
Enzyme treated fish meal	621.3	561.3	461.3	561.3	461.3
Enzyme treated soybean meal	–	100	200	–	–
Soybean meal	–	–	–	100	200
Salmon egg oil	40	40	40	40	40
Fish oil	40	40	40	40	40
α-starch	80	80	80	80	80
Vitamin mixture*	50	50	50	50	50
Mineral mixture[1]	50	50	50	50	50
Soybean lecithin	14.3	14.3	14.3	14.3	14.3
Taurine	20	20	20	20	20
Feeding stimulants	5	5	5	5	5
Cellulose	56.8	16.7	16.7	16.7	16.7
Wheat gluten	22.6	22.7	22.7	22.7	22.7
Proximate composition (g kg^{-1} dry diet)					
Crude protein	552.0	555.0	542.0	535.0	522.0
Crude lipid	157.0	166.0	157.0	153.0	166.0
Sugar	125.0	134.0	157.0	137.0	167.0
Crude ash	81.0	87.0	88.0	83.0	88.0
Phosphorous (g kg^{-1} diet)	23.7	19.5	18.7	19.2	18.8

*Components in g kg^{-1} diet: thiamine-hydrochloride 0.12, riboflavin 0.4, pyridoxin hydrochloride 0.08, nicotininc acid 1.6, calcium pantothenate 0.56, inositol 8.0, biotin 0.012, folic acid 0.03, p-aminobenzoic acid 0.8, choline chloride 16.0, α-tocopherol 0.8, menadione 0.08, activated 7-de-hydrocholesterol 0.0001, cyanocobalamin 0.001 retinol 0.01376 and L-Ascorbyl-2-mono-phosphate magnesium salt, APM 1.2 (vitamic C derivative, 46.46% vitamin C activity; Showa Denko K. K., Tokyo, Japan)

[1]Halver (1957)

Table 2. Fatty acid composition (% of total fatty acid) of test diets

Fatty acid	EFM	ESM10	ESM20	SM10	SM20
C14:0	4.0 ± 0.2	4.0 ± 0.3	4.2 ± 0.4	4.1 ± 0.3	3.9 ± 0.2
C15:0	0.7 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
C16:0	15.0 ± 0.2	17.2 ± 0.9	17.7 ± 0.7	17.8 ± 1.0	18.3 ± 0.7
C17:0	0.9 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	0.7 ± 0.1
C18:0	4.3 ± 0.1	5.2 ± 0.6	4.7 ± 0.3	5.1 ± 0.3	4.9 ± 0.2
C16:1	5.2 ± 0.1	5.8 ± 0.2	5.7 ± 0.5	6.1 ± 0.4	5.9 ± 0.2
C17:1	0.5 ± 0.3	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
C18:1n-9(OA)	15.6 ± 0.2	16.5 ± 0.5	17.0 ± 0.9	17.4 ± 1.3	16.9 ± 0.5
C18:1n-7	2.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.4	1.2 ± 0.3	1.7 ± 0.5
C20:1	2.0 ± 0.1	1.9 ± 0.3	2.3 ± 0.3	2.4 ± 0.2	2.4 ± 0.3
C18:2n-6 (LA)	7.6 ± 0.1	7.8 ± 0.5	8.2 ± 0.6	7.9 ± 0.7	8.1 ± 0.4
C20:4n-6 (AA)	1.2 ± 0.0	1.4 ± 0.3	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
C22:5n-6 (DPA)	3.2 ± 0.1	3.2 ± 0.2	3.2 ± 0.3	3.4 ± 0.4	3.3 ± 0.1
C18:3n-3 (LNA)	1.3 ± 0.0	1.5 ± 0.2	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.1
C20:5n-3 (EPA)	11.2 ± 0.1	12.5 ± 0.6	12.4 ± 0.1	12.6 ± 0.9	12.3 ± 0.4
C22:6n-3 (DHA)	16.7 ± 0.5	13.5 ± 2.2	13.3 ± 1.8	13.7 ± 2.4	13.4 ± 2.2

Values are mean ± SE (n=2). Means in a row with different letters are significantly different (Tukey's test, P <0.05).

SGR (%) = 100 × (lnW$_2$-lnW$_1$) / time (days),

where, W$_1$ and W$_2$ denotes the initial and final

weight (g), respectively.

Daily feeding rate (%) = 100 × total feed intake (g)

/ {rearing period (day) × average of initial and

final fish number × average of initial and final fish

weight}

FCE (%) = 100 × [wet weight gain (g) /dry feed

intake (g)].

CF = 100 × (W / L^3), where W = wet body weight

(g) and L = fork length (cm).

VSI (%) = 100 × [wet weight of visceral organs

and associated fat tissue (g) / wet body weight (g)]

HSI (%) = 100 × [wet weight of liver (g) / wet

body weight (g)]

SSI (%) = 100 × [wet weight of stomach (g) / wet

body weight (g)]

ISI (%) = 100 × [wet weight of intestine including

pyloric cecae (g) / wet body weight (g)]

PRE, LRE and phosphorous retention efficiency

(%) = 100 × [(final whole body protein, lipid or

phosphorous - initial whole body protein, lipid or

phosphorous) / total protein, lipid or phosphorous

intake

Phosphorous discharge (g P/ kg weight gain) =

(phosphorous intake – phosphorous deposited)/kg

weight gain

Chemical analysis

Proximate composition of test diets and whole

body were analyzed by the standard AOAC

method.[25] Crude protein content was determined

using micro-Kjeldahl, crude lipid by Soxhlet

extraction with diethyl ether, moisture content by a

dry oven (110°C for 24 hrs) and ash content by a

muffle furnace (600°C for 24 hrs). Dietary sugar

was measured by the phenol-sulfuric acid method.

GOT and GPT activities were analyzed using

supernatant after centrifugation of hepatic

homogenate with deionized water by commercially

available kits (Wako Pure Chemical Ind. Ltd.,

Osaka, Japan).

Lipid for FA analysis was extracted according

to Folch et al.[26] The fatty acid methyl esters were

analyzed using M HCl solution according to

Yoshinaka and Satoh[27] 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°C to 240°C at a rate of

4°C/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 temperature for the

injector and detector were 260°C and 290°C,

respectively. Peak quantification was performed

with an integrator (D-2500; Hitachi, Japan).

Statistical analysis

All statistical analyses were carried out using

the SPSS program for Windows (v. 12.0, Chicago,

IL, USA). Data were expressed as the mean±SE of

two replicates. Where significant differences were

found, the means among treatments were

compared using Tukey's test of multiple

comparison with a 95% level of significance.

Results

Although there were no significant differences

in FA compositions among the diets, total saturated

FA levels in ESM and SM groups were remarkable

higher than the control group (Table 2).

Table 3. Growth performance and relative organ weight of fish fed with different diets

	EFM	ESM$_{10}$	ESM$_{20}$	SM$_{10}$	SM$_{20}$
Initial weight (g)	0.38	0.38	0.38	0.38	0.38
Final weight (g)	7.2 ± 1.9^a	5.6 ± 1.1^{bc}	4.6 ± 1.0^c	7.2 ± 1.7^a	6.6 ± 1.6^{ab}
Weight gain (%)	1672.6^a	1243.4^{bc}	976.2^c	1566.4^a	1369.0^{ab}
SGR (%)	13.7 ± 0.5	13.1 ± 0.1	12.6 ± 0.3	13.5 ± 0.4	13.1 ± 0.1
Daily feeding rate (%)	11.9 ± 0.1^a	13.4 ± 0.2^b	15.2 ± 0.2^c	13.2 ± 0.3^b	13.4 ± 0.1^b
FCE(%)	135.8 ± 4.1^a	121.7 ± 2.2^b	103.0 ± 0.9^c	128.5 ± 2.4^{ab}	120.8 ± 0.3^b
CF	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.0
Survival rate (%)	69.2 ± 3.1	65.2 ± 4.2	65.6 ± 5.1	70.1 ± 6.1	63.3 ± 3.0
Relative organ weight (%)					
VSI	8.7 ± 0.9^a	10.1 ± 1.3^c	10.6 ± 2.4^d	9.0 ± 0.9^{ab}	9.4 ± 1.2^b
HSI	2.3 ± 0.3^b	2.9 ± 0.6^c	3.1 ± 0.8^c	1.9 ± 0.4^a	2.0 ± 0.4^{ab}
SSI	1.4 ± 0.3	1.5 ± 0.3	1.6 ± 0.4	1.4 ± 0.3	1.5 ± 0.3
ISI	5.3 ± 0.7	5.6 ± 0.8	6.0 ± 1.5	5.4 ± 0.6	5.8 ± 0.9

Values are mean ± SE (n=2). Means in a row with different superscripts are significantly different (P<0.05).

Table 4. Proximate composition liver GOT and GPT and retention efficiency in fish fed with different diets

	Initial	EFM	ESM$_{10}$	ESM$_{20}$	SM$_{10}$	SM$_{20}$
Proximate composition (g kg^{-1} fish)						
Moisture	807 ± 4	791 ± 6	785 ± 5	794 ± 3	798 ± 6	794 ± 4
Crude protein	133 ± 1	151 ± 4	154 ± 3	154 ± 3	153 ± 1	158 ± 2
Crude lipid	16 ± 1	13 ± 2	18 ± 3	15 ± 2	15 ± 1	15 ± 3
Ash	29 ± 1	31 ± 5	28 ± 1	28 ± 3	28 ± 2	28 ± 2
Liver						
GOT IU 100g^{-1} body weight		48.8 ± 5.3^a	61.1 ± 3.9^{ab}	60.6 ± 10.4^{ab}	56.7 ± 4.5^{ab}	68.7 ± 11.6^b
GPT IU 100g^{-1} body weight		4.1 ± 1.2^a	7.6 ± 2.0^{ab}	10.6 ± 3.6^{bc}	7.8 ± 1.5^{ab}	15.8 ± 4.1^c
Retention efficiency (%)						
PRE		37.2 ± 1.2^a	34.3 ± 0.1^a	29.7 ± 0.2^b	37.0 ± 0.9^a	37.0 ± 0.1^a
LRE		11.3 ± 1.4	13.5 ± 2.6	10.1 ± 0.5	12.3 ± 0.6	10.7 ± 3.0
Phosphorus		27.7 ± 0.6^b	30.7 ± 0.4^a	26.8 ± 0.8^b	33.2 ± 1.1^a	31.9 ± 0.1^a

Values are mean ± SE (n=2). Values in a row with different superscripts are significantly different (P<0.05).

Table 5. Fatty acid composition (% of total fatty acid) of tuna juvenile fed with different diets

	Initial	EFM	ESM10	ESM20	SM10	SM20
C14:0	0.7 ± 0.0	2.7 ± 0.7	3.0 ± 0.2	3.3 ± 0.6	3.2 ± 0.2	3.4 ± 0.5
C15:0	0.3 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
C16:0	24.6 ± 0.4	20.2 ± 5.1	21.1 ± 4.3	20.7 ± 4.4	21.1 ± 4.4	21.6 ± 5.2
C17:0	0.7 ± 0.0	1.2 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
C18:0	12.2 ± 0.2	14.0 ± 0.5	13.5 ± 0.8	14.0 ± 0.8	13.7 ± 0.7	14.3 ± 0.5
C16:1	3.0 ± 0.1	3.8 ± 0.5	4.0 ± 0.3	4.8 ± 0.2	4.1 ± 0.3	4.2 ± 0.6
C17:1	0.4 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
C18:1n-9(OA)	12.7 ± 0.2	14.0 ± 1.2	15.8 ± 2.0	16.4 ± 2.9	15.6 ± 0.9	16.4 ± 3.5
C18:1n-7	2.9 ± 0.2	2.7 ± 0.3	3.2 ± 0.5	3.0 ± 1.7	2.9 ± 0.3	2.7 ± 1.2
C20:1	0.9 ± 0.0	1.4 ± 0.5	1.3 ± 0.7	1.8 ± 0.5	1.8 ± 0.4	1.5 ± 0.4
C18:2n-6 (LA)	2.6 ± 0.1	3.4 ± 0.6	3.5 ± 0.4	3.0 ± 0.6	3.5 ± 0.3	2.9 ± 0.3
C20:4n-6 (AA)	3.1 ± 0.0	2.3 ± 0.3	2.7 ± 0.4	2.6 ± 0.5	2.7 ± 0.8	2.4 ± 0.7
C22:5n-6 (DPA)	1.8 ± 0.4	3.0 ± 0.4	3.0 ± 0.3	2.9 ± 0.2	2.9 ± 0.2	2.9 ± 0.4
C18:3n-3 (LNA)	0.4 ± 0.0	1.6 ± 0.8	1.3 ± 0.5	1.2 ± 0.4	1.3 ± 0.2	1.3 ± 0.4
C20:5n-3 (EPA)	6.4 ± 0.1	7.6 ± 1.1	7.7 ± 1.2	7.7 ± 0.9	8.5 ± 0.9	8.1 ± 1.7
C22:6n-3 (DHA)	22.8 ± 0.9	18.7 ± 1.9	14.9 ± 2.2	14.2 ± 3.1	14.2 ± 1.9	14.0 ± 2.0

Values (mean±SE, n=2) in a row with different letters are significantly different (Tukey's test, P<0.05)

Docosahexaenoic acid (DHA) content was higher in control group than other diets, giving a higher DHA/EPA ratio. There was no significant difference in mean final body weight among juveniles fed diets EFM, SM_{10} and SM_{20}, although the values in those diets were significantly higher than that of diet ESM_{20} ($P<0.05$, Table 3). Similar pattern was also observed in weight gain (%). The daily feeding rate was significantly higher in diet ESM_{20} followed by ESM_{10}, SM_{20}, SM_{10} and EFM. In contrast, FCE was significantly higher in diet EFM followed by SM_{10}, ESM_{10}, SM_{20} and ESM_{20}. There were no significant differences in SGR, CF and survival rate among the treatments ($P>0.05$). VSI was significantly higher in diets ESM_{20} and ESM_{10} than that of other diets, and similar pattern was also observed in HSI. There were no significant differences in SSI and ISI among the treatments.

Carcass proximate composition, liver GOT and GPT activities, and retention efficiencies of protein, lipid and phosphorus are presented in Table 4. There was no significant difference in final whole body proximate composition among the treatments. GOT activity was significantly higher in diet SM_{20} than that of EFM. However, there was no significant difference among other diets. GPT activity was also showed similar pattern as of GOT with little exception. PRE was significantly lower in ESM_{20} than other treatments. There was no significant variation in LRE among the treatments. Phosphorus retention efficiency was significantly higher in juveniles fed variation in LRE among the treatments. Phosphorus retention efficiency was significantly higher in juveniles fed diets SM_{10}, SM_{20} and ESM_{10} than that of EFM and ESM_{20} ($P<0.05$). In contrast, phosphorous discharge was significantly lower in juveniles fed the former diets

than the later diets ($P<0.05$, Fig. 1).

Carcass FA composition of PBT juveniles is given in Table 5. Dietary FA profile has well reflected in carcass FA composition. Although little bit higher total saturated and mono-saturated FA, and lower total n-3 highly unsaturated fatty acid (HUFA) were observed in ESM and SM groups, there were no significant differences among the dietary treatments.
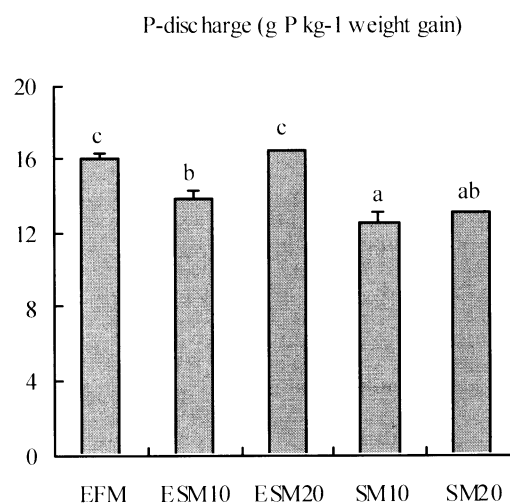
P-discharge (g P kg-1 weight gain)

Fig. 1. Phosphorus discharge in PBT juveniles fed with different diets (n=2). Bars with different letters are significantly different ($P<0.05$).

Discussion

In this study, the final mean body weight was 10-17 times bigger than the initial mean body weight only in 12 days, which indicates that the perception for all diets by PBT juveniles was good and a 12-day rearing period may not be considered short at least for PBT juvenile rearing. Previous studies in PBT juveniles with 10 to 14 days rearing period provided reliable growth data.[3-11] Another studies with PBT juveniles demonstrated that the deficiency syndromes of vitamin C could be observed as early as from the 5th day of experiment

(unpublished), which suggests that the dietary effect can be achieved within short period in PBT juveniles. Therefore, the rearing period used in this study may be considered as sufficient to get reliable data.

The lack of significant variation in most of the growth parameters among diets EFM, SM_{10} and SM_{20} suggests that 20% SM can be supplemented (around 26% EFM replacement) in diet of PBT juveniles without major adverse consequences. Ji et al.[9] reported better growth performance in PBT juveniles when fed diet made from EFM. Therefore, it was assumed that ESM would have produced better results over SM diets in this study; however, the results showed significantly higher growth performance from SM groups. The ESM used in this study is a commercial product (DaBomb, Bayer Medical, Tokyo, Japan), which was produced by fermentation with lactic acid producing bacteria to reduce trypsin inhibiter, antigen like glycinine, less digestible oligosaccharide, and to increase protein digestibility in order to use in pig feeds. The plausible reason, why ESM could not produce good result in PBT juveniles, is not yet clear. It may be possible that the enzyme treatment in ESM caused an early absorption of oligopeptide amino acids and this timing was not coincided with post-prandial metabolism in PBT juveniles. Therefore, the absorbed oligopeptide may be drained out before proper use in the body which, in turn, resulted in lower PRE from ESM diets. However, this area needs to be clarified further.

Although a previous study (unpublished) demonstrated significantly lower growth performance from SM diets, the growth performance from this study was comparable to that of control diet (EFM) until 20% inclusion of

SM as discussed earlier. This may be, in part, attributed to the variation in vitamin mixtures between two studies. The extra amount of vitamin mixtures might be somehow played an important role in this study, as vitamins are known to act as antioxidant, reductants, cofactors etc., and play role in energy metabolism, many biosynthetic and catabolic reactions.[28] The quality of juveniles might be another factor, as the juveniles from previous study showed inferior quality with high mortality even from the control group (unpublished). Although the rearing period is different, there have also been so many reports where SM was used to replace fish meal partially or fully in some species without any adverse consequence in terms of somatic growth or nutrient utilization.[18,19] In contrast, it has been demonstrated that fish meal replacement by SM was resulted in lower growth performance in other species.[15,16] This variation is due to the difference in fish behavior, nutritional requirement and physiological ability to use SM diet as it has been reported that distinct fish species and size-related differences in nutrient requirements and tolerance to dietary anti-nutritional factors exist.[4,5]

The significantly higher daily feeding rate in SM supplemented groups is indicated that the PBT juveniles tried to compensate the energy by taking more feed as the SM has lower digestibility[21,22] due to the presence of anti-nutritional factors.[28] The survival rate among the treatments didn't differ but was remarkably lower than other cultured species.[30,31] This is attributed to their panicking response to external stimuli, inevitable trauma caused by flash collision against the walls and biting on caudal peduncles, resulting in the imbalance morphological development,[32] and may be due to some other unknown reasons. However,

the present study was maintained higher survival than our previous studies on PBT juveniles.[3-5,9-11]

The higher liver GOT and GPT activities in SM supplemented groups may be due to the deflection of essential amino acid balance in SM diets itself, the adverse effect of phytate on the physiological activity of PBT juveniles[33] and the presence of anti-nutritional factors as discussed earlier.　However, the similar growth between EFM and SM groups may indicate that PBT juveniles somehow managed to maintain their growth. As in dietary proximate composition, SM supplemented diets had lower phosphorus content. Therefore, the PBT juveniles tried to use the available phosphorus properly from the SM diets, which resulted in higher retention efficiency and lower phosphorus discharge from SM supplemented diets except ESM_{20}. This is an interesting finding from SM supplemented diets as phosphorus discharge in the environment is of increasing concern in commercial fish production, because it is the most important pollution source. Therefore, reducing phosphorus discharge is a critical factor in reducing environmental pollution from commercial fish production.

The lack of significant difference in carcass FA profile among treatments indicates that the fish were able to fulfill their FA acid requirement from SM diets. For marine fish species, essential fatty acid requirements are chiefly met by dietary n-3 HUFA, EPA and DHA,[34] which were almost similar among the diets except a little higher DHA content in control diet. The importance of DHA/EPA ratio in test diets for optimal growth has been reported in different species.[35] It has been demonstrated that the ratio above 1.0 is better for normal growth of PBT juveniles,[3,8,10] which is fulfilled by the FA profile of test diets from this study.

The results demonstrated that it is possible to include 20% SM (around 26% EFM replacement) in the diet of PBT juveniles without major adverse consequences in the growth performance at least at the experimental design used in this study. However, further studies are required to investigate the effect of SM inclusion in long term rearing of PBT juveniles. In addition, the SM supplemented diets will help to reduce phosphorus discharge in the environment. As a rough estimate, this replacement provided opportunity to reduce feed cost about 30% (price for EFM and SM is about USD 5 and 1.5, respectively). However, this diet is still expensive compared to the diets of other fishes. Further studies are required to investigate the possibility of replacing other costly ingredients such as taurine and feeding stimulant from the diet of PBT juveniles.

Acknowledgements

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