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## REDUCING PHOSPHORUS AND NITROGEN EXCRETION IN YELLOWTAIL (*Seriola quinqueradiata*) NUTRITION: II. EFFECTS OF DIETARY FISHMEAL REPLACEMENT AND INCREASING ENERGY LEVEL

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### Abstract

Defatted soybean meal was replaced with fishmeal to evaluate its effect on growth and phosphorus/nitrogen excretion for young yellowtail (*Seriola quinqueradiata*). Soybean meal was replaced by fishmeal with the levels of 4 and 16% at 10, 16 and 20% dietary lipid level. The feeding trial continued for 80 days. Weight gain of fish significantly increased with increasing dietary lipid. Fishmeal (FM) replacement did not cause any negative effect on growth at any level of dietary lipid. Feed and protein efficiencies slightly decreased with increased level of FM replacement. Both parameters significantly ( $P<0.05$ ) improved with increasing dietary lipid. No significant interaction was determined between dietary lipid and SBM level on the any measured parameter. P and N excretion significantly ( $P<0.05$ ) decreased with increasing dietary lipid. In conclusion, based on growth and P/N excretion data, 10% FM replacement by defatted soybean meal with 20% lipid from the diet containing 43% protein could be recommended to obtain superior results considering decreasing detrimental effect of P and N to the environment in yellowtail nutrition weighing from ~420 g to ~900 g.

**Keywords:** Alternative protein, soybean meal, dietary energy, phosphorus and nitrogen excretion, Yellowtail, *Seriola quinqueradiata*

## Introduction

Aquaculture production has expanded at a rate of 15% per year and is predicted to continue to grow at this rate for at least the next decade. Demands on traditional fish feed ingredients, mainly fishmeal and oil, which are finite global resources, are increasing. At present, global fishmeal production averages 6 million tonnes per year, of which approximately 70–75% of global fishmeal production is used for aquaculture feeds (FAO, 2024). Clearly, expanded production of carnivorous species requiring high protein, high-energy feeds will further tax global fish meal and oil supplies. Suitable alternative feed ingredients will have to be utilized to provide the essential nutrients and energy needed to fuel the growth of aquaculture production (Hardy, 2000). One of the most effective methods to reduce the dependence on fishmeal in feeds used for feeding carnivorous fish species is to enable the use of more sustainable alternative raw materials and to popularize them globally. Especially plant sources with high protein content are remarkable in this respect. Soya products, one of these potential feedstuffs, are valuable and protein-rich by products from soybean oil extraction. Soya proteins have a wide range of application in the food industry where they are used for their good nutritional value, but the primary interests are their functional properties. Among others they are used as substitutes for animal protein and are particularly good animal protein replacements in aquaculture feeds. Presently, soybean meal (SBM) is the most important protein source as feed of productive farm animals and as partial or entire replacement of fishmeal. It is commonly used not only because of its high protein content but also due to its worldwide availability. The recommended inclusion levels for aquafeeds vary from 5 to 15% and 10 to 30% on carnivores and herbivores/omnivores fish species, respectively (Hertrampf and Piedad-Pascual, 2000). Yellowtail (*Seriola quinqueradiata*) is one of the economically important finfish for aquaculture in Japan. The total production of yellowtail in Japan was about 150,000 tons in 2004, representing about 70% of the total production of aquaculture marine finfish in Japan. Yellowtail culture had been expanded due to massive catches of low-cost fish used as food, such as sand-lance and sardine. However, in recent years, the catches of these fish have decreased, and their cost has therefore increased. This has forced many yellowtail producers to change from feeding fish to the use of a formulated diet (Masumoto, 2002, FAO, 2024). Since the main protein source in yellowtail diet is fishmeal, attempts have to be done to find a commonly available alternative protein sources to replace or decrease the substitution level of fish meal from the diets. Decreasing fishmeal might allow us to produce cheaper and more environmentally sound diet.

Therefore, the objectives of the present study are to evaluate the defatted SBM as a fishmeal replacer and to see the effect of increasing dietary lipid on growth, protein utilization and body composition in yellowtail.

## Materials and methods

### Test diets

The formulation of the experimental diets is shown in Table 1. The main protein source was fish fishmeal. Krill meal and corn gluten meal used to enhance the palatability and stability of the diets. Commercial SBM incorporated to replace 0 and 10% of dietary fishmeal. Each replacement level was represented at 3 different dietary lipid levels, low lipid (10%, LL), moderate lipid (16%, ML) and high lipid (20%, HL). Pollack liver oil served as the lipid source,  $\alpha$ -Starch and wheat flour were the carbohydrate sources in the diets.

To prepare diets, all dry ingredients were well mixed for 30min in a food mixer. Then pollack liver oil was added and mixed for 15min. Finally, water (35% of the dry weight of ingredients)

was put, and again mixed for 15min. The pH of the diets was adjusted to 7.0–7.5 with 4N sodium hydroxide. The pellets were (5 mm diameter) made with a meat grinder and dried in a dry-air mechanical convection oven (DK 400, Yamato Scientific, Japan) at 70°C for 2 to 4h to obtain approximately 10% moisture level. The experimental diets were stored at –30°C until used.

**Table 1.** Ingredient composition and proximate analysis of the experimental diets

Ingredient	Diets					
	LL4	LL16	ML4	ML16	HL4	HL16
Fishmeal	45.0	35.0	45.0	35.0	45.0	35.0
Krill meal	5.0	5.0	5.0	5.0	5.0	5.0
Corn gluten meal	5.0	5.9	5.0	5.9	5.0	5.9
Defatted soybean meal	4.0	16.4	4.0	16.4	4.0	16.4
Pollock liver oil	9.0	9.9	13.8	14.7	17.2	18.1
$\alpha$ -Starch	3.0	3.0	3.0	3.0	3.0	3.0
Wheat flour	10.0	6.0	10.0	5.7	10.0	6.0
Vitamin mix <sup>1</sup>	4.0	4.0	4.0	4.0	4.0	4.0
Mineral mix <sup>2</sup>	4.0	4.0	4.0	4.0	4.0	4.0
Stay C	0.1	0.1	0.1	0.1	0.1	0.1
Rice bran	11.0	10.8	6.2	6.3	2.8	2.6
<b>Proximate composition (% in dry diet)</b>						
Crude protein	43.0	43.9	43.4	42.9	42.4	42.4
Crude lipid	11.1	10.2	16.0	17.1	20.4	22.0
Crude ash	14.4	13.4	13.5	12.9	13.3	12.2
Gross energy (kcal/g) <sup>3</sup>	4.3	4.3	4.7	4.7	5.1	5.1
Total phosphorus	1.5	1.4	1.4	1.3	1.3	1.2

<sup>1</sup>Vitamin mixture (g 100 g dry diet):  $\beta$ -caroten 12.84; Vitamin D<sub>3</sub> 1.29; Menadione NaHSO<sub>4</sub> 6.11; DL- $\alpha$ -Tocopherol Acetate (E) 51.33; Thiamine-Nitrate (B<sub>1</sub>) 7.70; Riboflavin (B<sub>2</sub>) 25.65; Pyridoxine-HCl (B<sub>6</sub>) 6.11; Cyanocobalamine (B<sub>12</sub>) 0.01; d-Biotin 0.77; Inositol 513.22; Nicotinic acid 102.63; Ca Panthothenate 35.93; Folic acid 1.92; Choline Chloride 1049.24; Aminobenzoic acid 51.10; Cellulose 256.60;

<sup>2</sup>Mineral mixture (g/100 g dry diet): NaCl 143.72; MgSO<sub>4</sub>·7H<sub>2</sub>O 506.70; NaHPO<sub>4</sub>·2H<sub>2</sub>O 322.54; KH<sub>2</sub>PO<sub>4</sub> 886.93; Ca(H<sub>2</sub>PO<sub>4</sub>)·2H<sub>2</sub>O 502.26; Fe citrate 109.84; Ca Lactate 1209.47; Al(OH)<sub>3</sub> 0.69; ZnSO<sub>4</sub>·7H<sub>2</sub>O 13.20; CuSO<sub>4</sub> 0.37; MnSO<sub>4</sub>·7H<sub>2</sub>O 2.96; Ca(IO<sub>3</sub>)<sub>2</sub> 0.56; CoSO<sub>4</sub>·7H<sub>2</sub>O 3.69.

<sup>3</sup>Carbohydrate = 100 – (% protein + % lipid + % fiber + % ash)

<sup>4</sup>Gross energy is calculated based on protein, 5.65 kcal/g; lipid, 9.45 kcal/g; carbohydrate, 4.10 kcal/g

### Fish and experimental design

The experiment was conducted at Kagoshima Prefectural Fisheries Technology and Development Center (Ibusuki, Tenpozan, Kagoshima, Japan). Yellowtail (*Seriola quinqueradiata*) (419.2 ± 19.5g) were used as test animal and randomly allocated to 12 FRP circular tanks (1 ton/tank) with duplicate groups consisting of 15 fish each. The fish were fed the respective test diets at apparent satiation level, two times a day (morning and evening). Every 20 d all fish were anesthetized in eugeunol solution (20 mg/L) (Soto and Burhanuddin, 1995) weighed in bulk. The feeding trial conducted for 80 days. All rearing tanks were provided with continuous aeration and maintained under natural light/dark regime (12:12 h). Filtered seawater was continuously provided at a flow rate of 48 L/min. Water temperature and salinity ranged between 20.1°C ~ 25.2°C and 31.4 ~ 33.7‰, respectively, during the trial.

### Chemical analysis

Crude protein was determined by the Kjeldahl method with a Tecator Kjeltex System (1007 Digestion system, 1002 Distilling unit and Titration unit). Total lipid was determined using the Bligh and Dyer (1959) method. Ash and moisture contents were analyzed by standard methods (AOAC, 1990). P concentrations in the samples were determined photometrically by the method of Lowry and Lopez (1946).

### Statistical analysis

All data from the feeding trial and chemical analysis were tested using one or two-way analysis of variance (Package super-ANOVA, ver. 1.11, Abacus Concepts, Berkeley, CA, USA). Percentage survival data were arcsin-square-root transformed before statistical analysis. Significant differences between individual treatments ( $P < 0.05$ ) were evaluated by Duncan's new multiple range test (Package super-ANOVA, Abacus Concepts, Berkeley, CA, USA).

### Evaluation of the data from feeding trail

Feed intake was recorded by subtracting the amount of uneaten diet from total amount of diet fed on a dry weight basis. Uneaten diet was trapped from the outflow water by means of a net, freeze-dried and weighed. P and N excretion and retention calculated by the formulas provided in footnotes of tables.

## Results

Data on growth performances are presented in Table 2. At the end of the feeding trial (80 days), yellowtails attained weight gain of 57 – 114% with high survival. WG of the fish significantly increased with increasing dietary lipid level at both SBM level (Table 2, 3, Fig 1). FM replacement did not show any significant effect on WG. The highest WGs were obtained from the fish fed the diets containing the HL level at both SBM level. Feed intake (FI) was not significantly influenced by the dietary treatment. Feed (FE) and protein efficiencies (PE) significantly improved with increasing dietary lipid at both SBM levels (Table 2, Fig 2, 3). FM replacement caused a slight decrease on FE and PE ( $P > 0.05$ ) (Fig 4, 5). No significant interaction was determined between dietary protein source and lipid level on any growth parameters (Table 3).

**Table 2.** Growth parameters and feed utilization in yellowtail fed the test diets

Diets	Initial weight (g)	Final weight (g)	Weight gain (WG, %) <sup>1</sup>	Feed intake (g/fish)	Feed efficiency (FE) <sup>2</sup>	Protein efficiency (PE) <sup>3</sup>	Survival (%)
LL4	409.8±1.8	689.9±18.5	68.4±4.0	812.1±9.9	0.35±0.02	0.89±0.05	83.3
LL16	446.0±20.3	700.6±11.9	57.1±2.4	831.6±40.1	0.31±0.01	0.77±0.04	100.0
ML4	420.0±4.9	759.9±15.7	81.0±5.4	827.9±90.1	0.41±0.01	1.04±0.02	96.7
ML16	396.8±29.9	690.9±9.4	74.5±6.9	838.4±21.5	0.35±0.1	0.90±0.02	93.4
HL4	418.4±11.3	894.7±66.0	113.6±11.7	783.4±64.8	0.61±0.05	1.57±0.10	83.3
HL16	424.3±6.4	867.1±55.2	104.3±10.8	870.0±108.2	0.50±0.03	1.33±0.04	80.0

Values are means of duplicate groups ± S.E.

<sup>1</sup>WG, %: ((Final body weight (g) – Initial body weight (g)) / Initial body weight (g)) x 100

<sup>2</sup>FE: Wet weight gain (g) / dry feed intake (g)

<sup>3</sup>PE: Wet weight gain (g) / protein intake

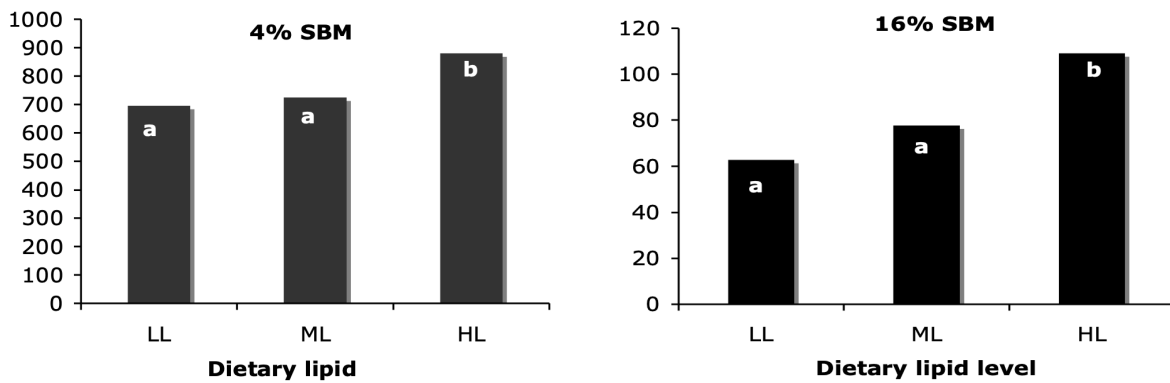
**Table 3.** Results of two-way ANOVA on growth parameters ( $P < 0.05$ ).

	Initial weight (g)	Final weight (g)	Weight gain (%) <sup>1</sup>	Feed intake	FE <sup>2</sup>	PE <sup>3</sup>	Survival (%)
Dietary lipid level	0.2825	<b>0.0050</b>	<b>0.0025</b>	0.9709	<b>0.0001</b>	<b>0.0001</b>	0.2257
Dietary SBM level	0.5165	0.3793	0.1988	0.3475	<b>0.0068</b>	<b>0.0065</b>	0.5813
Interaction	0.0975	0.5887	0.9525	0.6893	0.3253	0.4603	0.3322

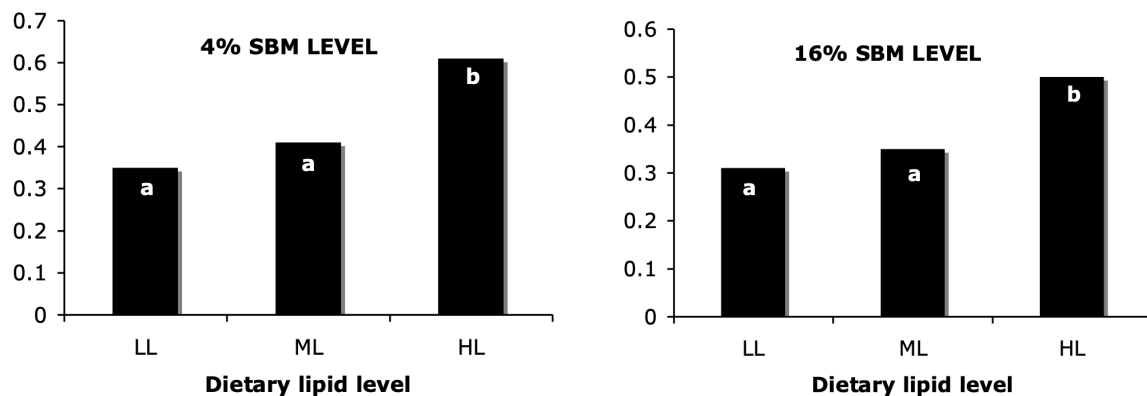
<sup>1</sup>WG, %: ((Final body weight (g) – Initial body weight (g)) / Initial body weight (g)) x 100

<sup>2</sup>FE: Wet weight gain (g) / dry feed intake (g)

<sup>3</sup>PE: Wet weight gain (g) / protein intake



**Figure 1.** Final weight and weight gain at 4 and 16% SBM level.



**Figure 2.** FE at 4 and 16% SBM level

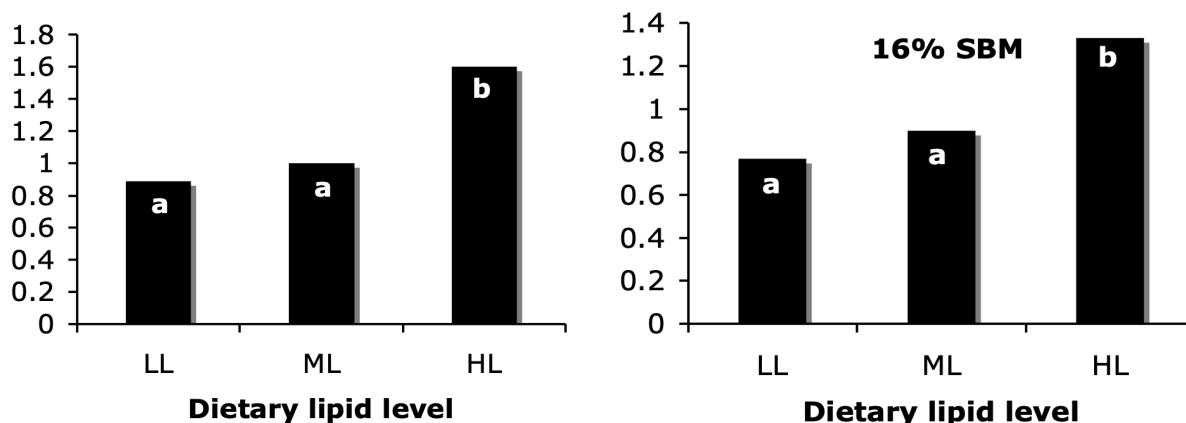


Figure 3. PE at 4 and 16% SBM level.

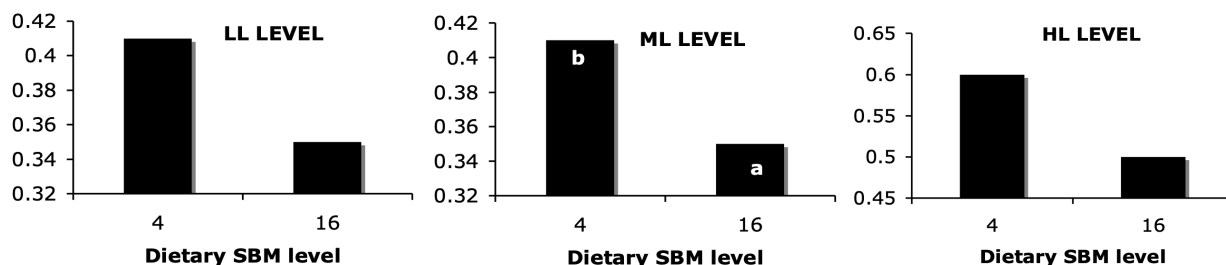


Figure 4. FE at varying dietary lipid level. Absence of letters indicates no significant difference between treatments.

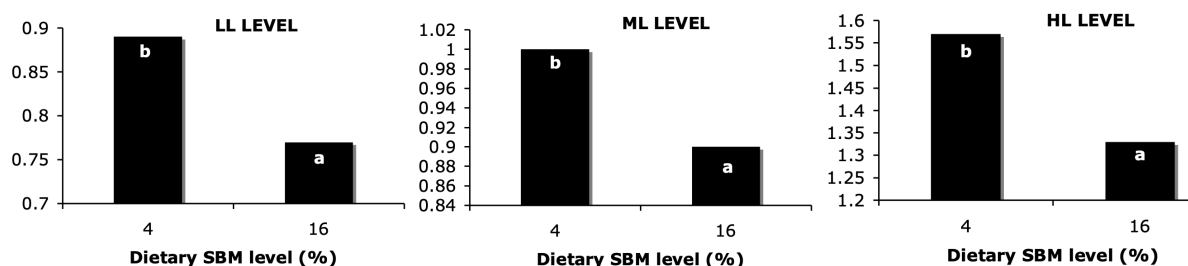


Figure 5. PE at varying dietary lipid level.

The proximate compositions of whole body of yellowtails at the end of the feeding trial are shown in Table 4. No significant differences were detected in ash and total phosphorus levels among all treatments (Table 5). Moisture and lipid content were significantly affected only by dietary lipid level. Lipid content of whole body significantly increased with increasing dietary lipid level while moisture content significantly decreased (Fig 6).

**Table 4.** Whole body proximate composition (% in wet sample) of yellowtail fed the test diets

Diets	Moisture	Crude protein	Crude lipid	Ash	Total P
LL4	68.0 ± 0.4	19.6 ± 0.3	5.9 ± 0.1	3.0 ± 0.2	0.54 ± 0.0
LL16	67.0 ± 0.7	18.4 ± 0.1	8.0 ± 0.6	2.6 ± 0.2	0.52 ± 0.1
ML4	65.3 ± 0.6	18.9 ± 0.0	8.9 ± 0.3	2.7 ± 0.0	0.57 ± 0.1
ML16	65.3 ± 0.0	19.2 ± 0.0	8.4 ± 0.0	2.8 ± 0.0	0.55 ± 0.0
HL4	63.5 ± 0.2	18.6 ± 0.2	10.9 ± 0.1	2.6 ± 0.1	0.55 ± 0.1
HL16	64.4 ± 1.0	19.0 ± 0.0	10.1 ± 0.9	2.8 ± 0.2	0.54 ± 0.0

Values are means of duplicate groups ± S.E. Within a row, means with the same letters are not significantly ( $P>0.05$ ) different. Absence of letters indicates no significant difference between treatments.

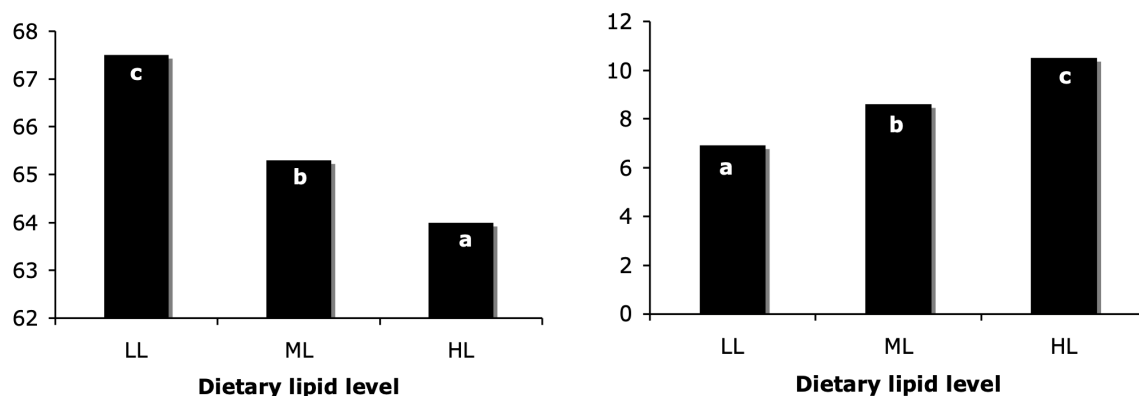
**Table 5.** Results of two-way ANOVA on whole body proximate composition ( $P<0.05$ )

	Moisture	Crude protein	Crude lipid	Crude ash	Total P
Dietary lipid level	<b>0.0032</b>	0.1711	<b>0.0007</b>	0.7106	0.2377
Dietary SBM level	0.9611	0.1188	0.4955	0.9250	0.2015
Interaction	0.3896	0.7508	0.6457	0.2855	0.9583

Phosphorus and nitrogen budget (kg P or N/t BW gain) and nutrient retention of experimental fish are shown in Table 6. FM replacement and dietary lipid level were significant factors on P/N intake, retention and excretion (Table 7). P intake was only affected by the dietary lipid level and significantly decreased with increasing dietary lipid level (Fig 7).

P excretion significantly decreased with increasing dietary lipid level at both SBM level. At the same dietary lipid level, significantly lower P retention was obtained from fish fed the diet containing 16% SBM compared to those consumed 4% SBM containing diet (Fig 8). P and N retention improved with increasing dietary lipid level at both SBM level (Table 6, 7, Fig 9, 10).

N intake and excretion significantly decreased, and P retention significantly improved with increasing dietary lipid level at both SBM level (Fig 10). Based on the same dietary lipid level, intake and excretion of N significantly decreased with increasing SBM level in diets (Fig 11) while N retention decreased. No significant interaction was observed between dietary SBM and lipid level on the P/N retention and excretion values (Table 7).



**Figure 6.** Whole body moisture and lipid levels.

**Table 6.** Phosphorus and nitrogen budget per unit body weight gain and nutrient retention in yellowtail fed the test over 80 days

Nutrient	Diets					
	LL4	LL16	ML4	ML16	HL4	HL16
<b>Phosphorus (kg P/t BW gain)</b>						
Intake <sup>1</sup>	43.6±2.3	45.8±2.0	34.1±0.7	37.1±0.8	21.6±1.4	23.7±0.7
Accumulation <sup>2</sup>	5.4±0.1	5.2±0.1	5.8±0.5	5.5±0.0	5.6±0.1	5.4±0.0
Excretion <sup>3</sup>	38.2±2.2	40.7±2.0	28.4±0.3	31.6±0.8	16.0±1.3	18.3±0.7
<b>Nitrogen (kg N/t BW gain)</b>						
Intake	200.1±10.5	229.5±10.1	169.0±3.5	195.7±4.3	112.4±7.2	133.7±3.6
Accumulation	31.0±0.5	30.0±0.0	30.0±0.0	31.0±0.0	30.0±0.0	30.0±0.0
Excretion	169.1±10.5	199.5±10.1	139.0±3.5	164.7±4.3	82.4±7.2	103.7±3.6
<b>Retention<sup>4</sup> (% of intake)</b>						
Phosphorus	12.4±0.4	11.3±0.4	16.9±1.0	14.9±0.4	25.9±1.5	22.9±0.7
Nitrogen	15.6±0.9	13.1±0.6	17.8±0.4	15.9±0.4	26.8±1.7	22.5±0.6

Values are means ± SE. Within a row, means with the same letters are not significantly different ( $P > 0.05$ ).

Absence of letters indicates no significant difference between treatments.

<sup>1</sup>{(Feed intake (g/fish) x P or N concentration in diet (%) / 100) / (Mean body weight gain (g))} x 1000

<sup>2</sup>{(Final mean body weight (g) x Final wholebody P or N concentration (%) / 100) – (Initial mean body weight (g) x Initial wholebody P or N concentration (%) / 100)} / (Mean body weight gain (g)) x 1000

<sup>3</sup>P or N intake (g/kg body weight gain) – P or N accumulation (g/kg body weight gain)

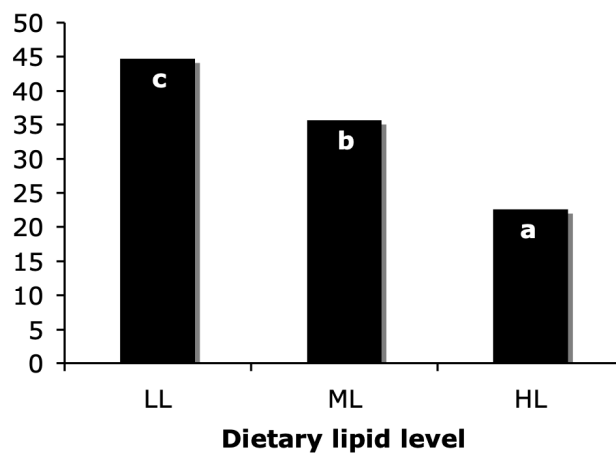
<sup>4</sup>P or N accumulation (g/kg body weight gain) x 100) / P or N intake (g/kg body weight gain)



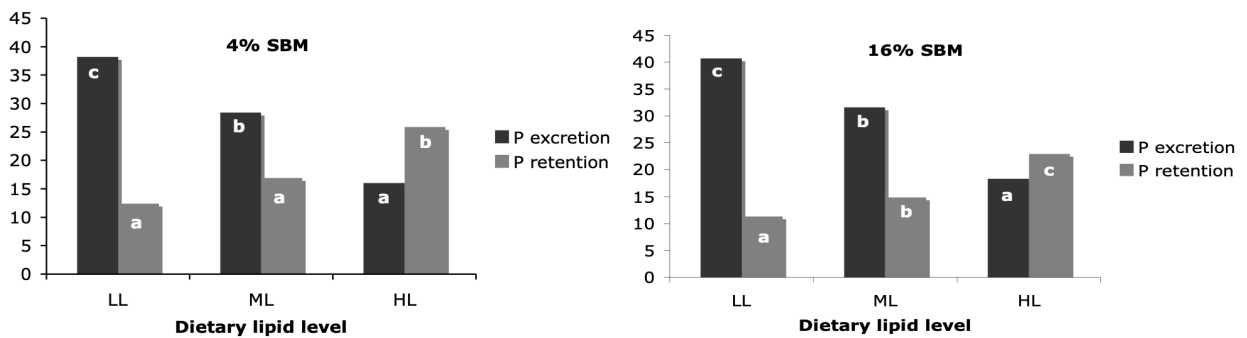
**Table 7.** Two-way ANOVA results: *P* values of the experimental factors on the P and N intake, accumulation, excretion and retention values ( $P < 0.05$ ).

	Phosphorus (kg P/t BW gain)				Nitrogen (kg N/t BW gain)			
	INT	ACCU	EXC	RET	INT	ACCU	EXC	RET
Dietary lipid level	<b>0.0001</b>	0.2599	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.2599	<b>0.0001</b>	<b>0.0001</b>
Dietary SBM level	0.0861	0.2128	0.0572	<b>0.0211</b>	0.2599	0.2128	<b>0.0045</b>	<b>0.0068</b>
Interaction	0.9445	0.9554	0.9301	0.5329	0.8515	0.9554	0.8232	0.4107

INT: intake; ACCU: accumulation; RET: retention; EXC: excretion



**Figure 7.** P intake of fish fed increasing level of dietary lipid.



**Figure 8.** P excretion and retention at 4 and 16% SBM level.

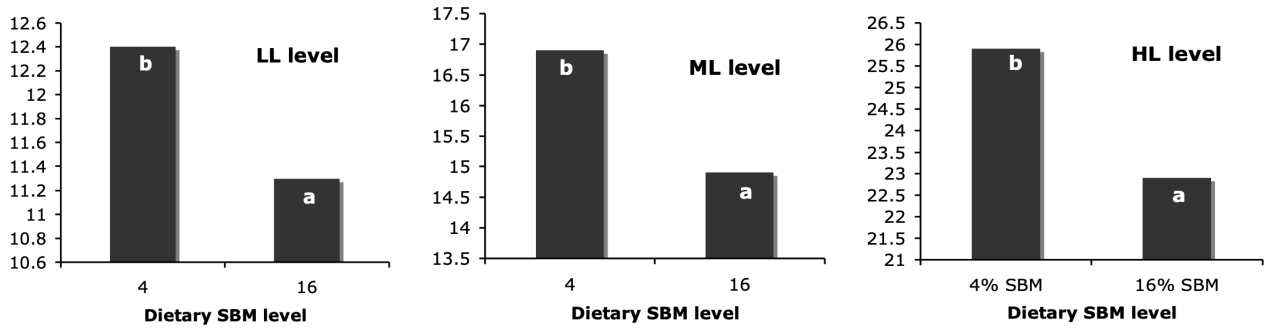


Figure 9. P retention values at 4 and 16% SBM level.

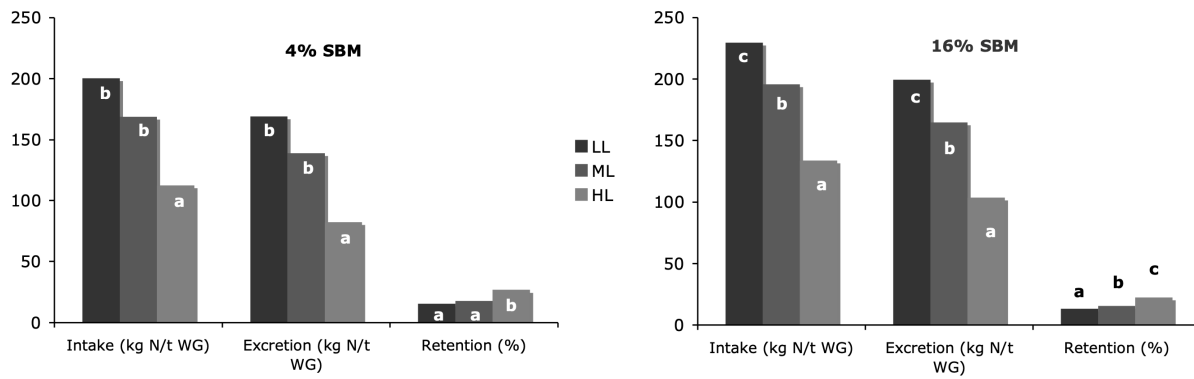


Figure 10. Intake, excretion and retention values of N at 4 and 16% SBM level.

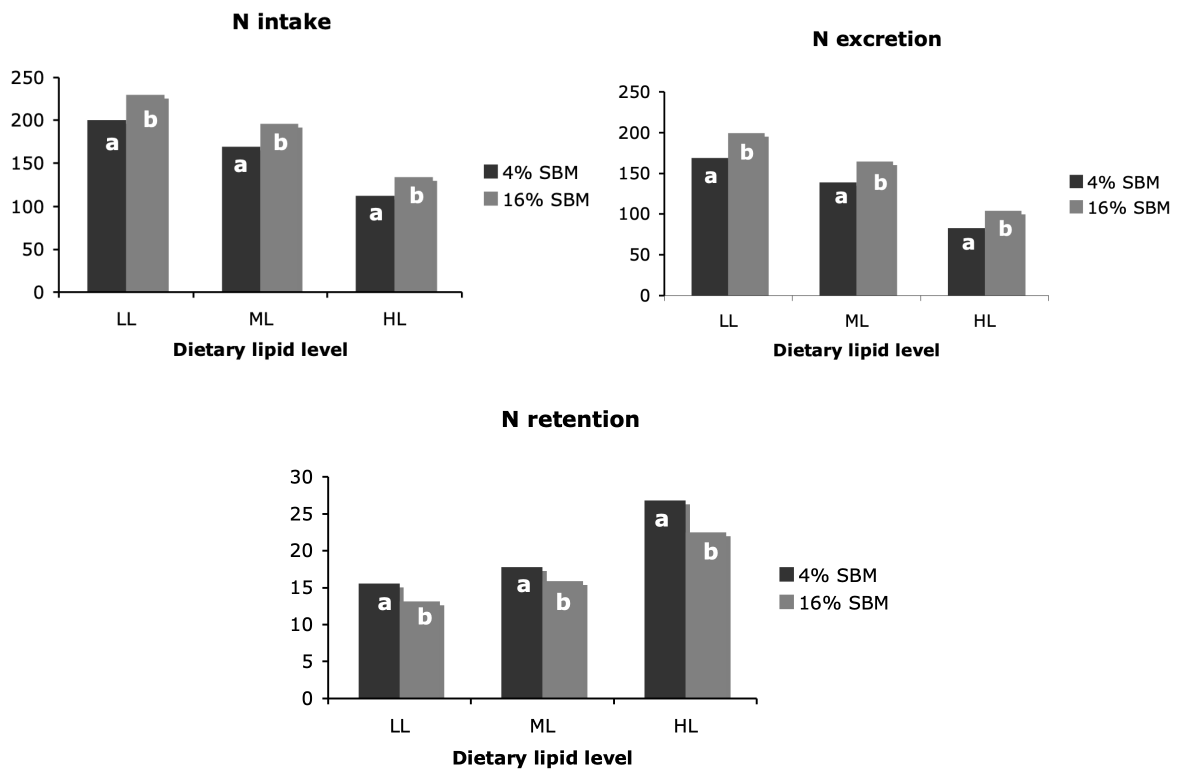


Figure 11. Intake, excretion and retention of N based on each dietary lipid level.

## Discussion

One of the major factors limiting the expansion of aquaculture is the development of nutritionally adequate, cost-effective diets. Feeds and feeding can contribute up to 70% of the total operating cost for fish and shrimp farm. The most expensive component of pelleted feeds is protein, of which 25-55% is required, depending upon whether the species is herbivorous, omnivorous or carnivorous (NRC, 1993; Lovell, 1989). The major protein source for most aquaculture diets is fishmeal (Lovell, 1989), which is a limited source, and formulated diets can contain up to 60% fishmeal. Therefore, development of plant-based or fishmeal minimized aquafeeds will provide economic and environmental benefits to the aquaculture industry.

Lipid is the major energy source in fish diets. In general fat-enriched high-energy diets result in rapid growth and favorable feed conversion. Low protein-high energy diets bring about low nitrogen excretion that is important for maintenance water quality. Another benefit of increasing lipid in fish diet is to decrease the amount of fishmeal using as protein source. At the previous study, the lower dietary protein with high lipid produced the highest growth rate proving the high utilization ability of lipid in yellowtail. Based on these findings, we tried to make a further decrease in dietary protein from 46 to 42 at varying lipid level with or without replacement of FM by SBM at the present study. The result of the present study demonstrated that yellowtail has a great ability to utilize lipid from the diets, and fish can tolerate the decreasing protein at the presence of high lipid. The highest WG was obtained from the fish fed the diet containing HL level and SBM level did not affect on WG at the present study. Since there was no dietary treatment containing higher dietary lipid level than the HL level at the present study, it seems that further increment in dietary lipid and decrease in protein could be possible.

FM replacement did not cause any growth retardation on WG at the any dietary lipid level at the present study. It reveals that defatted soybean meal can be used to replace of 26% of dietary fishmeal protein (or 10% fishmeal) based on the present experimental conditions. Previous studies showed that soybean meal is one of the most promising alternative protein sources in yellowtail diets. Jover et al. (1999) reported in the study of *Seriola dumerilii*, the inclusion of around 20% soybean meal has given excellent growth results, which agrees with Shimeno et al. (1992, 1993a, b), Viyakarn et al. (1992) and Watanabe et al. (1992), who obtained similar growth in Japanese yellowtail fed 0 and 20% soybean meal. Compared to these studies, the inclusion level of the SBM (10% of FM) is relatively low at the present study, since our main objective is to develop such a kind of diet lowering the P excretion by means of partially FM replacement with increasing lipid level, rather than to maximize substitution level of soybean meal. Therefore, inclusion level of soybean meal was minimized in the present study.

FM replacement by SBM did not produce any significant effect of FE and PE, although there was a slight declining trend with increased level of SBM, which might show that 10% FM replacement by SBM cause a negligible level of imbalance in dietary amino acid structure. Increasing N excretion with replacement compared to FM based diet (Table 6) could also support to this speculation. Poor feed utilization and decreasing tendency in PE have been commonly demonstrated when dietary imbalance of amino acid is encountered in fish. However the effect was very limited, and no significant effect occurred on FE, PE and even on FI. FE and PE significantly improved with the increasing dietary lipid level, revealing that dietary protein was spared by dietary lipid. Improving PE shows that dietary protein was utilized to build body protein rather than to be used as energy source, and main energy source had come from dietary lipid, which is called as protein-sparing effect of lipid.

In the present study, the content of moisture and lipid in were significantly affected by dietary treatments. Lipid accumulation in the body increased with dietary lipid level. Whole body moisture decreased while dry matter increased as lipid level increased. This trend has been described by Jobling (1994). He pointed out that there was a strong negative correlation between the percentage of body lipid and moisture in fish. No significant alteration was observed on whole body ash and phosphorus level. This finding indicates that dietary treatments did not cause any negative effect on bone or wholebody mineralization of fish.

Lowering the P excretion is one of the major goals of the present study. In order to decrease the amount of P excretion, the most applicable strategy is to decrease P intake of the fish through replacing the fish meal by the low P containing protein source (Lall, 1991; Talbot and Hole, 1994), to increase the availability of plant originated P (such as phytate P) supplementing the diet with phytase to decrease the dietary protein level as much as possible, and to increase the energy which allows to decrease amount of fishmeal used in the diet. In the present study, we focused on a combined strategy, such as FM replacement and increasing dietary lipid level. Within the same lipid level, FM replacement did not produce any significant decrease on P intake because FM replacement level was very limited, hence, dietary P level could not effectively be decreased. However, P level of test diets and P intake of the fish decreased with increasing dietary lipid. The possible explanation is that the increasing dietary lipid was managed by decreasing rice bran, which contains relatively higher amount of P compared to other fillers such as cellulose. Therefore, the substitution level of rice bran would be responsible on the P intake. Accumulation of P in fish body did not significantly differ from each other. It revealed that utilization level of P was not influenced by the dietary treatments. Because of stable P accumulation and decreasing P intake, P excretion significantly decreased. Considering P excretion, diet 5 and diet 6 gave the best results. P retention values significantly improved with increasing dietary lipid level, but not the level of FM replacement. Since no significant alteration was identified among the wholebody P level (revealing no effect on P utilization), the only reason of improving P retention could be P intake decrease.

Earlier findings have shown that a decrease of dietary fish meal replaced with alternative protein sources, such as plant proteins (Storebakken et al., 2000; Kaushik et al., 2004), fisheries by-catch and by-product meals (Uyan et al., 2007; Uyan et al., 2009) or natural food based diets (Sumagaysay-Chavosa, 2003) containing lower P, resulted in 30 – 60% reductions of P loading without any significant growth retardation in fish. In this aspect, the present results revealed that increasing dietary lipid level could be used to decrease P loading in yellowtail diets. The fish fed containing 20.4 and 22.0% lipid (diet 5 and 6) showed around 40-60 % less P discharge into water environment compared to other diets. One of the most obvious benefits of increasing lipid was observed as decreasing N intake (Table 6). When growth improved with increasing dietary lipid level, N intake decreased because the amount of protein in digested feed also decreased. However, this condition did not cause any growth retardation (because of lower protein intake) since dietary protein was fully utilized, and energy demand was supplied by the dietary lipid (protein sparing). Improving PE values with increasing lipid clearly supported to this explanation, namely the highest PE/protein retention and the lowest N excretion were obtained from the fish diet containing highest lipid level.

## Conclusion

In conclusion, based on growth and P/N excretion data, 10% FM replacement by defatted soybean meal with 20% lipid from the diet containing 43% protein could be recommended to

obtain superior results considering decreasing detrimental effect of P and N to the environment in yellowtail nutrition.

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### Informed consent

Not available

### Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

### Conflicts of interest

There is no conflict of interests for publishing this study.

### Contribution of authors

Orhan UYAN: Conceptualization, Data curation, Formal analysis, Writing original draft

Shunsuke KOSHIO: Funding acquisition, Investigation, Methodology

Manabu ISHIKAWA: Project administration, Resources, Supervision, Validation

Saichiro YOKOYAMA: Resources, Supervision, Validation, Visualization

“All authors have read and agreed to the published version of the manuscript.”

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