Growth and protein, energy and phosphorus utility in ayu, *Plecoglossus altivelis* when fish meal replaced by soybean meal

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Synopsis

To investigate the utility of soybean meal in ayu, four isoenergetic diets were formulated: fish meal 65% (FM, control), fish meal 47% + soybean meal 20% (SM20), fish meal 30% + soybean meal 40% (SM40) and fish meal 13% + soybean meal 58% (SM58). Thirty fish with mean body weight ca. 17.3g were randomly distributed into each of 12 tanks (300 L) in triplicate for each treatment. Fish were fed twice daily at 09:00 and 16:00, 6 days per week for 9 weeks. There were no significant differences in final mean body weight, specific growth rate, daily feeding rate and feed conversion efficiency between FM and SM20; however, SM40 and SM58 showed significantly lower values of those parameters. Retention efficiencies of protein and energy were significantly higher in FM and SM20 than those of SM40 and SM58. Phosphorus retention and discharge were significantly higher and lower, respectively in diet SM20 than others. The results suggested that 20% soybean meal can be included in ayu diet (around 30% fish meal replacement), which will help to achieve both economical and ecological benefits as it will reduce diet cost and phosphorus discharge to the environment.

Keywords: ayu, fish meal, soybean meal, growth, protein, energy, phosphorus

1 Introduction

Ayu, *Plecoglossus altivelis* (Osmeridae), is an amphidromous species[1] which inhabits in Japan, Korean Peninsula and the east of the continent of China[2]. Ayu usually live only for 1 year and die after spawning. They spawn at the lower reach of the river in autumn, drift downstream to the sea after hatching and remain there over the winter months[3]. Juveniles migrate to the upper and middle reach of the river in the next spring and feed on attached algae on rocks. In addition to the amphidromous ayu, a population of land-locked ayu inhabits Lake Biwa and several other lakes in Japan. Ayu is the most important species for freshwater fisheries in Japan, where juveniles are extensively stocked every year in many rivers. However, the supply of juveniles from lakes has been decreased substantially in recent years due to the mass mortality from the coldwater disease[4] or the bacterial hemorrhagic ascites[5]. Therefore, the demand for disease-free, hatchery-produced fish has been increasing as an alternative. For hatchery production, fish meal has been used in ayu diet. As fish meal is expensive and there has been growing concern over its future availability, it is important to find out less expensive and readily available protein source for ayu diet.

Among the alternative protein sources, soybean meal become the most promising one for fish feeds in terms of future availability and protein contents with a fairly well-balanced amino acid profile. Although soybean meal together with maize gluten facilitated only 22% decrease in the fish meal content of the feeds for rainbow trout[6], a combination of different plants protein together with krill meal, squid meal, krill water soluble and amino acids supplementation resulted in 75~100% fish meal replacement in rainbow trout[7,8] and tilapia[9]. However, there is no information on the utility of soybean meal in ayu. Since fish meal contains excess phosphorus, it can be anticipated that the increasing organic waste output from diet with fish meal alone as protein source will have a negative impact on the surrounding environment, which will in turn affect the sustainability of aquaculture. Although soybean meal supplementation has been reported to reduce phosphorus output[10], there is species-specific ability to utilize soybean meal. Therefore, it is necessary to find out the appropriate level of soybean meal to incorporate in a diet with the environmental concerns in mind as excess plant protein sources may not be well digested which will be leading to loss of nutrients either in feces or metabolic excretion.

This study therefore aims to investigate the optimum level of soybean meal replacing fish meal without compromising growth and affecting the surrounding environment. Since the feed company prefers the dietary formula as simple as possible and avoids using expensive supplementation to reduce diet cost, thinking about the practical implementation this study used soybean meal alone without amino acid or other supplementations to replace fish meal.

2 Materials and methods

Diet preparation

Dietary composition and chemical analyses are shown in Table 1. Four isoenergetic diets were formulated with different levels of fish meal and soybean meal (Itochu Shoji, Tokyo, Japan) as follows: fish meal 65% (FM, control); fish meal 47% + soybean meal 20% (SM20); fish meal 30% + soybean meal 40% (SM40) and fish meal 13% + soybean meal 58% (SM58). This dietary formula provided the opportunity to replace around 30, 60 and 80% fish meal in diets SM20, SM40 and SM58, respectively. Vitamin and mineral mixtures were those of Halver[11]. The diets were made into pellets of 1 mm diameter by a laboratory pellet machine after mixing 100 parts of ingredients with 25 parts of tap water. The diets were freeze-dried and stored in a freezer at -20ºC until used. As mentioned earlier, the dietary formula was kept as simple as possible and no other protein source was used together with soybean meal to replace fish meal except corn gluten meal. Therefore, because of the lower protein content in soybean meal, there was a remarkable variation in protein content among the diets.

Fish and experimental conditions

Four hundred and eighty ayu juveniles (artificially produced) of mean body weight around 15 g were obtained from the Fisheries

Table 1. Dietary formula and proximate composition

Ingredients (%)	**F**	**SM20**	**SM40**	**SM58**
Fish meal	65.0	47.0	30.0	13.0
Soybean meal	0.0	20.0	40.0	58.0
Fish oil	4.0	5.0	6.0	7.0
α-Starch	10.0	9.0	6.0	4.0
Vitamin mixture*	4.0	4.0	4.0	5.0
Mineral mixture*	9.0	10.5	11.7	13.0
Cellulose	8.0	7.5	2.3	0.0
Proximate composition (dry basis)				
Crude protein (%)	51.4	50.5	47.0	43.4
Crude lipid (%)	9.5	9.7	10.1	10.1
Crude ash (%)	18.0	17.0	17.0	16.5
Crude sugar (%)	14.2	19.7	23.7	25.7
Phosphorus (g kg^{-1} diet)	19.5	13.6	12.7	10.5
Energy (kJ g^{-1})	19.6	19.4	19.4	19.3

*Halver[11]

Laboratories, Kinki University, Shingu, Japan and were randomly distributed among twelve 300-l tanks at 40 fish/tank where the fish were allowed to acclimate to the new rearing tanks for two weeks. The photoperiod was set at 12-h light :12-h dark and the tanks were supplied with well water at 10 L min^{-1}. During acclimation period, fish were fed to apparent satiation with a commercial diet (protein 45.7%, lipid 10.7%, Nihon Nousan, Yokohama, Japan), twice a day at 09:00 and 16:00 h.

At the end of acclimation period, fish were starved for 24 h, body length and weight were measured, and 30 fish (mean weight, ca 17.3 g) were stocked into each experimental tank. The excess number of fish during acclimation period provided the opportunity to keep the similar initial weight in all tanks. The experiment was designed so that triplicate tanks of each treatment were randomly assigned, thus reducing tank effect. At the beginning, a pooled sample of 30 fish was stored in a freezer for whole body proximate analyses. Fish were fed to apparent satiation with the experimental diets twice per day, at 09:00 and 16:00 h, 6 days per week, for 9 weeks. Individual body length and weight were measured at the 3rd and 6th week, and at the end of the experiment. Fish were deprived of food for 24 h before each weighing. At the end, all fish from each tank were frozen at -80°C for whole body proximate analysis.

Table 2. Growth performance in ayu fed with experimental diets for 9 weeks

Parameters	**F**	**SM20**	**SM40**	**SM58**
Initial weight (g)	17.3	17.3	17.3	17.3
Final weight (g)	37.7±2.7[a]	35.2±1.8[a]	29.9±1.6[b]	21.1±1.1[c]
SGR (%)	1.2±0.1[a]	1.1±0.1[a]	0.9±0.2[a]	0.3±0.1[b]
DFR (g/100g fish)	2.1±0.1[a]	2.4±0.1[ab]	2.7±0.1[b]	2.7±0.1[b]
FCE (%)	50.2±4.2[a]	40.6±3.9[b]	28.0±2.2[c]	8.6±2.9[d]
CF	1.3±0.1[a]	1.3±0.2[a]	1.0±0.1[ab]	0.9±0.1[b]
Survival rate (%)	82.2±3.9	85.6±8.4	84.4±11.7	82.2±1.9

Values in a row with different letters are significantly different (*P* <0.05).

Each value is a mean±SE derived from 3 samples each of 30 fish for each treatment.

Calculation and chemical analyses

The data obtained were analyzed for specific growth rate (SGR), daily feeding rate (DFR), feed conversion efficiency (FCE), condition factor (CF), retention efficiencies of protein, energy and phosphorus, and phosphorus discharge using the following formula:

SGR (%) = 100 ´ ($\ln W_2$-$\ln W_1$)/time (days), where, W_1 and W_2 indicate the initial and final weight (g), respectively.

DFR (g/100g fish) = 100 ´ [{(initial weight + final weight) / 2} / feed intake ´ number of days]

FCE (%) = 100 ´ [wet weight gain (g) / dry feed intake (g)]

CF = 100 ´ (W / L^3), where, W = wet body weight (g) and L = body length (cm)

Retention efficiency of protein, energy and phosphorus (%) = 100 ´ [(final whole body protein, energy or phosphorus– initial whole body protein, energy or phosphorus)/total protein, energy or phosphorus intake]

Phosphorus discharge (g P kg^{-1} weight gain) = {phosphorus fed (g) – phosphorus deposited (g)} / weight gain (kg)

Samples (diets and fish) were analyzed for dry matter, crude protein and ash using standard methods[12]. Crude protein content was determined using semi micro-Kjeldahl, crude lipid by Soxhlet extraction with diethyl ether, moisture content by a dry oven (110°C for 24 h) and ash content by a muffle furnace (600°C for 24 h). Detary sugar was measured by the phenol-sulfuric acid method[13]. Phosphorus content of diet, fish whole body and feces was determined using the ammonium-molybdate method described by Baginski et al.[14] after the digestion of samples with nitric and perchloric acid. Gross energy was analyzed using an automated oxygen bomb calorimeter (IKA-Werke, Staufen, Germany). 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). Data were analyzed by a one-way analysis of variance

Table 3. Proximate composition and retention efficiencies in fish fed with different diets.

Parameters	F	SM20	SM40	SM58
Moisture (%)	70.5±0.6	71.0±0.7	71.2±1.5	71.2±0.2
Crude protein (%)	15.8±0.2^{a}	14.3±1.1^{ab}	13.6±1.1^{b}	14.3±0.3^{ab}
Crude lipid (%)	10.7±1.1	12.3±1.5	12.0±0.9	11.2±0.5
Crude ash (%)	3.2±0.4^{a}	2.9±0.2^{ab}	2.4±0.3^{b}	2.8±0.2^{ab}
Phosphorus (g kg^{-1} fish)	6.8±0.3^{a}	6.6±0.4^{a}	6.2±0.2^{ab}	5.7±0.2^{b}
Energy (kJ g^{-1} fish)	25.5±0.6	26.1±1.0	26.1±0.2	26.5±0.4
Retention efficiency (%)				
Protein	15.9±3.9^{a}	12.5±2.6^{ab}	8.5±0.6^{bc}	2.5±1.6^{c}
Lipid	51.6±11.4^{a}	66.0±18.9^{a}	47.6±16.1^{ab}	12.3±10.4^{b}
Energy	19.1±2.7^{a}	18.5±3.9^{a}	13.6±3.3^{a}	4.8±1.7^{b}

Values in a row with different letters are significantly different (P <0.05).

Each value is a mean±SE derived from 3 samples each of 30 fish for each treatment.

(ANOVA). When ANOVA indicated significant differences, the means within each treatment and among treatments were compared using Tukey's test of multiple comparison with a 95% significance level.

3 Results

At the end of experiment, there were significant differences in final mean body weight, total weight gain, SGR, DFR, FCE and CF among the treatments (Table 2). Final body weight and weight gain showed similar pattern where both diets FM and SM20 had significantly higher values than other diets ($P < 0.05$). Again, diets FM and SM20 showed significantly higher SGR and CF than those of diet SM58. Both diets SM40 and SM58 had significantly higher DFR than FM. However, FCE was significantly higher in fish fed with diets FM and SM20 than that of diets SM40 and SM58.

Although fish fed with diets SM40 and SM58 had significantly lower final whole body crude protein content than that of FM, there were no major differences in other parameters among the treatments (Table 3). Whole body phosphorus content in fish fed with diet SM58 was significantly lower than that of diets FM and SM20. Both protein and energy retention efficiency showed similar pattern among the treatments where diets FM and SM20 had significantly higher values than other diets. Phosphorus retention efficiency and discharge to the environment are given in Fig. 1. Diet SM20 showed significantly higher phosphorus retention efficiency than other diets, which resulted in significantly lower phosphorus discharge from SM20 followed by FM, SM40 and SM58.

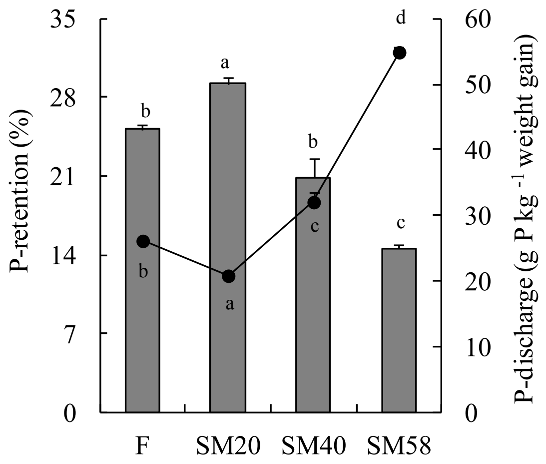

Fig. 1. Phosphorus retention efficiency (line) and discharge (bars) in ayu fed with experimental diets. Bars and points on line with different letters are significantly different (P<0.05). P-retention, phosporus retention; P-discharge, phosphorus discharge.

4 Discussion

Ayu fed with diet SM20 (20% soybean meal) showed good growth, FCE and nutrients retention efficiency comparable to those of fish fed with fish meal based diet (FM). However, a linear reduction in growth and nutrient utilization was observed at higher substitution levels in diets SM40 and SM58 (40 and 58% soybean meal, respectively). This suggests that the maximum inclusion level of soybean meal in ayu diet will be 20% which is equivalent to about 30% fish meal replacement. This replacement level is lower compared with some other freshwater species, such as rainbow trout [7,8], carp[15] and tilapia[9]; and either similar or lower than some marine species, such as yellowtail[16], gilthead sea bream[17] and red sea bream[10]. As ayu feed on algae in their natural habitat, it is reasonable to assume that they are able to digest the less digestible carbohydrate present in the cell wall of algae, which may afford them to utilize more soybean meal as alternate plant protein source. However, the larger amounts of anti-nutritional factor and phytate from increasing soybean meal supplemented diets were beyond the limit of ayu to handle, which resulted in significantly lower growth performance and retention efficiency of nutrients and energy in diets SM40 and SM58. The variation in soybean meal utility among the fish species is due to the difference in fish behavior, nutritional requirement and physiological ability to use soybean meal 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[18-20]. Since this study was tried to keep the dietary formula as simple as possible for practical implementation, the protein contents in soybean meal diets were lower, which resulted in lower growth performance. Further studies are necessary to investigate the optimal replacement level of fish meal by the mixture of soybean meal and other products through providing similar protein contents.

The daily feeding rate (g/100g fish) in this study was increased with increasing levels of fish meal replacement, in contrast with the results observed in Asian seabass[21]. This increasing feeding rate may be indicated that the ayu tried to compensate the energy by eating more feed as the soybean meal has lower digestibility[10] due to the presence of anti-nutritional factors as discussed earlier. Moreover, the lowest growth from diets SM40 and SM58 may also be due to the poor amino acid balance of these diets. Although soybean meal considered as the best plant protein source with regard to amino acid profile, the higher inclusion levels of soybean meal without any amino acid supplementation possibly caused methionine deficiency resulting in lower growth through reduced FCE, and protein and energy utilization. A similar response was demonstrated in olive flounder[22] and Asian seabass[21]. Unfortunately, the amino acid composition was not analyzed in this study; however, growth and protein utilization of ayu would possibly be improved if the diets were supplemented with amino acids which were present at below requirement levels.

Reducing phosphorus discharge is a critical factor in reducing environmental pollution from commercial fish production, because it is the most important pollution source. In this point of view, the significantly higher phosphorus retention and lower discharge to the environment from fish fed with diet SM20 is an interesting finding from this study. The higher phosphorus discharge from diet FM than SM20 is corresponded to the higher amount of dietary phosphorus. These findings are in line with those observed in rainbow trout[23] and Asian seabass[21]. However, diets SM40 and SM58 showed the lowest phosphorus retention and highest discharge despite the lower dietary phosphorus content. This indicated that ayu cannot utilize phosphorus properly at higher soybean meal supplementation levels. Although we did not investigate the stomach or intestinal disorders, there is possibility that the presence of more anti-nutritional factors at higher supplementation level caused intestinal enteritis [24,25] and ulcer-like lesions in the stomach[26]. These disorders caused by anti-nutritional factors in the plant protein have presented problems such as reduced intestinal absorptive ability[27,28]. However, these areas need to be further investigated in ayu. It has been demonstrated in some species that the phytase supplementation in soybean meal diet could help to increase replacement level of fish meal[10,29,30]. Therefore, it is also necessary to investigate whether phytase supplementation can help to increase soybean meal supplementation level in ayu.

The results from this study suggested that 20% soybean meal (around 30% fish meal replacement) can be included in ayu diet as all growth parameters in fish fed with diet SM20 were similar to those of diet FM. Since this study aimed to keep dietary formula as simple as possible and didn't use other protein sources or combination of different amino acids, the lower protein content and imbalance in essential amino acids resulted in lower growth performance in higher inclusion levels. Therefore, further studies are necessary to investigate the possibility of higher fish meal replacement with limiting amino acids and phytase supplementation. However, around 30% fish meal replacement will allow a decrease in diet costs for producers and potentially increase profits, as well the ecological benefit could be achieved as 20% soybean meal inclusion has provided the opportunity to decrease about 20% phosphorus discharge to the environment compared to fish meal based diet.

Acknowledgements

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.

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飼料魚粉の大豆粕代替とアユの成長とタンパク質，エネルギーおよびリン利用率

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要　約

アユの大豆粕に対する利用能を，等エネルギーの 65%魚粉（FM，対照），47%魚粉+20%大豆粕（SM20），30%魚粉+40%大豆粕（SM40）および 13%魚粉+58%大豆粕（SM58）飼料を用いて調べた。平均体重 17.3 g のアユ稚魚を 30 尾ずつ収容した 3 反復試験区に，1 日 2 回，1 週間に 6 日所定の飼料を与えて 9 週間飼育したところ，終了時平均体重，成長率，日間摂餌率，飼料効率，タンパク質・エネルギー蓄積率などは，FM および SM20 に区間差はなく，SM40 と SM58 ではいずれも有意に低下した。一方，SM20 は他区に比べてリン蓄積率は有意に高く，リン排泄量は有意に低かった。アユ稚魚に 20%大豆粕飼料（魚粉代替率 30%）を給与することで，生産コストや環境負荷の低減につながることが示された。