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Effect of Dietary *Sargassum* Meal on Growth and Body Composition of Ayu (*Plecoglossus altivelis*) Reared in Seawater

Sang-Min Lee*, Kyoung-Duck Kim, Huem Gi Park, Jong Kwan Lee[1] and Yong-Su Lim[1]

Faculty of Marine Bioscience and Technology, Kangnung National University, Gangneung 210-702, Korea

[1]National Fisheries Research and Development Institute, Busan 619-900, Korea

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This study was conducted to investigate the utilization of *Sargassum* meal in the diet on juvenile ayu (*Plecoglossus altivelis*) reared in seawater. White fish meal and wheat flour were used as the dietary protein and carbohydrate sources in the control diet. Wheat flour in the control diet was replaced with 5% and 10% *Sargassum* meal. Three replicate groups of fish average weighing 4.0 g were fed one of three isonitrogenous (45%) and isocaloric (14.5 MJ/kg diet) diets for 7 weeks. Survival of all groups were above 80%. Weight gain, feed efficiency and protein efficiency ratio were not significantly affected by dietary *Sargassum* meal levels ($P>0.05$). There were no significant differences ($P>0.05$) in moisture, crude protein, crude lipid, crude ash and fatty acid compositions of whole-body fish among groups. It is concluded that *Sargassum* meal could be used as a dietary additive or alternative low-cost dietary ingredient up to 10% for juvenile ayu reared in seawater.

Key words: Ayu, *Plecoglossus altivelis, Sargassum* meal

Introduction

Ayu (or called sweet fish or sweet smelt), *Plecoglossus altivelis,* is a salmonid distributed in Korea, Japan and China (Chyung, 1996). This species is a diadromous fish migrated from freshwater to brackish water to spawn, and hatched larvae migrated go to seawater and then back to freshwater for growing. In Asia, it has very high consumers demand due to its good taste and flavor. However, population of this species is currently decreasing because of river pollution, over-fishing and dam construction. Consequently, in order to increase the population resources, it is essential to develop aquaculture techniques such as artificial larval mass production and development of feed for optimal growth and high quality of fish. Development of nutritionally well-balanced and cost-effective feed is critical to increase the production of the fish.

Studies on nutrient requirement, dietary additive utilization and flesh quality improvement of ayu have been performed (Shimma et al., 1980; Takeuchi et al., 1981; Kanazawa et al., 1982; Hirano and Suyama, 1983; 1985; Nakagawa et al., 1984; Amano and Noda, 1985; Lee and Kim, 1999; Lee et al., 2000a; 2002). Dietary additives affect growth and body composition of fish. The possible utilization of algae as a dietary additive such as *Ulva, Undaria* or *Sargassum* for improvement of growth, flesh quality or physiological condition of marine fish and abalone has been reported (Nakagawa et al., 1985; Nakagawa and Kasahara, 1986; Yone et al., 1986a,b; Satoh et al., 1987; Yi and Chang, 1994; Lee et al., 1998; 2000b). Therefore, this study was conducted to investigate the influences of *Sargassum* meal in the diets on growth and body composition of ayu reared in seawater.

*Corresponding author: smlee@kangnung.ac.kr

Materials and Methods

Fish and rearing conditions

Juvenile ayu were obtained from Uljin Marine Hatchery (Uljin, Korea). After juvenile fish were acclimated to the experimental condition, 200 fish were randomly distributed into each 300 L fiber glass tank and fed a commercial feed containing 43% protein during pre-experimental period for 2 weeks. After the conditioning period, fish were weighed and 60 fish (initial mean weight: 4.0±0.1 g) were redistributed into each tank. After fish were stocked, each flow-through tank was covered with a plastic net to prevent them from escaping. Filtered seawater was supplied at a flow rate of 5 L/min into each tank. During the feeding trial, fish were exposed to natural photoperiod and water temperature was 15.4±0.93℃. Three replicate groups of fish were hand-fed to visual satiety three times daily (7 days a week) at 08:00, 12:00 and 17:00 h for 7 weeks. Pellet size was adjusted using a sieve and appropriate sized pellets were fed as the fish grew. Pellets were distributed slowly allowing all fish to eat.

Experimental diets

Three experimental diets were formulated to contain 45% protein, 6.9% lipid and 14.5 MJ/kg diet according to results reported by Lee and Kim (1999) and Lee et al. (2002). Energy of the diets was estimated based on 16.7, 37.7 and 16.7 MJ/kg for protein, lipid and nitrogen-free extract, respectively (Garling and Wilson, 1976). White fish meal, squid liver oil and wheat flour were used as the primary protein, lipid and carbohydrate sources, respectively (Table 1). Wheat flour in the control diet was replaced with 5% and 10% *Sargassum* meal. All ingredients were mechanically mixed with water at the ratio of 3:1 and pressure-pelleted and dried at room temperature overnight. Experimental diets were stored at −30℃ until used.

Sample collection and chemical analysis

Sixty fish at the beginning and all surviving fish at the end of feeding trial were sacrificed for chemical analysis after 24 h starvation and stored at −75℃. Crude protein content was determined by Kjeldahl method using Auto Kjeldahl System (Buchi B-324/435/412; Flawil, Switzerland), and crude lipid content by ether-extraction method, and moisture content by a dry oven (105℃ for 24 hours), and crude fiber content by an automatic analyzer (Fibertec; Tecator, Hoganas, Sweden), and crude ash content by a furnace muffler (550℃ for 4 hours). Lipid was extracted by the method of Folch et al. (1957) and fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma

Table 1. Ingredients and nutrient contents of the experimental diets

	Sargassum meal levels (%)		
	0	5	10
Ingredients (g/100 g)			
White fish meal[1]	60.0	60.0	60.0
Wheat flour	25.0	20.0	15.0
Sargassum meal	0.0	5.0	10.0
Squid liver oil[2]	2.0	2.0	2.0
Vitamin premix[3]	2.2	2.2	2.2
Mineral premix[4]	4.0	4.0	4.0
Carboxymethyl cellulose	3.0	3.0	3.0
α-Cellulose[5]	3.0	3.0	3.0
Choline salt[5]	0.8	0.8	0.8
Nutrient contents (dry matter basis)			
Crude protein (%)	44.3	45.4	46.3
Crude lipid (%)	6.9	6.9	6.8
Crude ash (%)	17.1	17.8	18.2
Nitrogen-free extract (%)[6]	27.6	25.8	24.6
Estimated energy (MJ/kg)[7]	14.6	14.5	14.4
n-3 HUFA (%)[8]	1.7	1.7	1.7

[1]Produced by steam dry method.
[2]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[3]Vitamin mix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL--tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; nicin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.
[4]Mineral mix. contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; Na_2Se2O_3, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[5]Sigma Chemical, St. Louis, MO, USA.
[6]Calculated by difference (=100-crude protein-crude lipid-crude ash-crude fiber).
[7]Estimated energy was calculated (Garling and Wilson, 1976).
[8]Highly unsaturated fatty acids (C≥20) were calculated based on the contents of fish meal and squid liver oil.

Chemical Co., USA) for 30 min at 80℃. Fatty acid methyl esters were analyzed using a gas chromatography (HP-5890 II, USA) with flame ionization detector and equipped with HP-INNOWax capillary column (30 m×0.32 mm i.d., film thickness 0.5 μm, USA). Injector and detector temperatures were 250 and 270℃, respectively. The column temperature was programmed from 170℃ to 225℃ at a rate of 1℃/min. Helium was used as the carrier gas. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (Sigma Chemical Co., USA).

Statistical analysis

One-way ANOVA were applied to determine the significance of measurements. Where significant differences ($P<0.05$) were found, Duncan's multiple range test (Duncan, 1955) was used to rank groups using SPSS Version 7.5 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

Results and Discussion

Survival, weight gain and feed efficiency (Table 2) of ayu were not significantly different among the diets ($P>0.05$). Daily feed intake, protein efficiency ratio and protein retention (Table 3) were also not influenced by dietary *Sargassum* levels. A possible explanation for these no effects on growth and feed utilization of ayu fed the different dietary *Sargassum* levels is that *Sargassum* meal used in this study has low protein (21%) and high carbohydrate (58%) similar to those of wheat flour which is replaced by *Sargassum* meal in the diet. No beneficial growth effects have also been observed in ayu fed a diet containing *Chlorella*-extract (Nakagawa, 1985; Nematipour et al., 1987). This indicates that *Sargassum* meal can be used as a partial substitute for wheat flour up to 10% in this dietary formulation for growth of juvenile ayu reared in seawater. The *Sargassum* meal used in this study is not used as human food, although it include good nutrients such as essential amino acid (Lee et al., 2000b). Considering nutritive value of *Sargassum* meal and environmental pollution with disuse, the use of *Sargassum* meal as feed ingredient can reduce the production cost for fish culture.

The supply of high quality fish meal for aquaculture feeds has gradually decreased during the last decade, leading to an increased price in the product (Tacon, 1997). Therefore, numerous studies have investigated the potential of alternative and low-cost dietary ingredients (Kaushik et al., 1995; El-Sayed, 1999), and these studies have focused on utilization of available plant protein sources to replace fish meal. However, wheat flour in the control diet was replaced with *Sargassum* meal in this study. Therefore, more detailed study is needed to improve the utilization of *Sargassum* meal as a substitute for fish meal in the diet for ayu.

Weight gain and feed efficiency of ayu fed the experimental diets for 7 weeks in this study were relatively lower than those of ayu reared in freshwater (Nematipour et al., 1988; Lee and Kim, 1999). This could result from differences in experimental conditions, especially salinity. Weight gain of fish in this study is comparable to that obtained by Jeon et al. (1999) who observed lower growth rate of ayu with increase in salinity. Faster growth of anadromous or diadromous species of fish in freshwater than in seawater has been reported (Mckay and Gjerde, 1985; Morgan and Iwama, 1991).

There were no significant differences ($P>0.05$) in moisture, crude protein, crude lipid and crude ash contents (Table 4) of whole-body ayu fed the diets

Table 2. Weight gain, feed efficiency and survival of juvenile ayu fed the diets containing different *Sargassum* meal levels for 7 weeks[1]

Dietary *Sargassum* meal levels (%)	Initial weight (g/fish)	Survival (%)	Weight gain (g/fish)	Feed efficiency (%)[2]
0	3.9±0.12[ns]	94± 0.7[ns]	4.0±0.46[ns]	53.1±4.76[ns]
5	4.0±0.17	80±13.3	4.2±0.33	52.1±4.80
10	4.0±0.12	85± 7.7	3.9±0.17	49.8±2.81

[1]Values are mean±SEM of three replicates.
[2]Weight gain×100/Feed intake (dry matter).
[ns]Not significant ($P>0.05$).

Table 3. Feed intake and protein utilization of juvenile ayu fed the diets containing different *Sargassum* meal levels for 7 weeks[1]

Dietary *Sargassum* meal levels (%)	DFI[2]	DPI[2]	DLI[2]	PER[3]	PR[4]
0	2.51±0.03[ns]	1.11±0.01[ns]	0.17±0.002[ns]	1.20±0.11[ns]	17.8±1.82[ns]
5	2.46±0.09	1.12±0.04	0.17±0.006	1.15±0.11	17.3±1.39
10	2.56±0.04	1.19±0.02	0.17±0.003	1.08±0.06	16.0±0.90

[1]Values are mean±SEM of three replicates.
[2]Daily feed (protein or lipid) intake=Feed (protein or lipid) consumption×100/[(initial fish wt.+final fish wt.+dead fish wt.)×days fed/2].
Protein efficiency ratio=Fish wet weight gain/Protein intake.
[4]Protein retention=Body protein gain×100/Protein intake.
[ns]Not significant (P>0.05).

Table 4. Proximate composition (%) of juvenile ayu fed the diets containing different *Sargassum* meal levels for 7 weeks[1]

Dietary *Sargassum* meal levels (%)	Moisture	Crude protein	Crude lipid	Crude ash
0	69.1±0.55[ns]	15.6±0.06[ns]	9.9±0.20[ns]	2.6±0.03[ns]
5	69.4±0.75	15.8±0.19	8.8±0.40	2.6±0.03
10	70.1±1.32	15.7±0.15	8.7±1.29	2.9±0.33

[1]Values are mean±SEM of three replicates.
[ns]Not significant (P>0.05).

containing different *Sargassum* meal levels. Fatty acids compositions (Table 5) were not affected by dietary *Sargassum* meal levels (P>0.05). This trend is not in accordance with data obtained from Nakagawa (1985) and Nematipour et al. (1987) who observed low lipid contents of ayu with *Chlorella*-extract supplemented diets. Ayu has a sweet smell such as aroma of watermelon. This sweet smell of ayu seems to be related to natural food in its habitants. However, the cultured ayu fed the formulated feed are lack in the smell and they have high lipid content compared to wild ayu (Hirano and Suyama, 1983). Generally, high lipid content of fish is believed to cause the low consumers demand probably because of palatability. Body lipid contents of ayu fed the diets containing of yeast or herb as an additives tended to decrease (Lee and Lim, 2000; Lee et al., 2000a; Nematipour et al., 1987). Lee et al. (2000a) reported that protoplasted *Kluyveromyces fragilis* supplement in micro-formulated diet can improve growth and reduce body lipid of larval ayu. However, supplemental *Sargassum* meal in the diet had no influence on growth and body lipid content of ayu in this study.

Theses results indicate that *Sargassum* meal could be used as a dietary additive or alternative low-cost dietary ingredient in the formulated diet for ayu.

Table 5. Major fatty acids composition (% of total fatty acids) of whole-body ayu fed the diets containing different *Sargassum* meal levels for 7 weeks[1]

	Initial	Dietary *Sargassum* meal levels (%)		
		0	5	10
Fatty acids				
14:0	3.3	4.5±0.50	5.5±0.15	4.7±0.44
16:0	21.3	22.2±2.35	25.9±0.42	23.5±0.78
16:1n-7	9.1	12.9±0.64	13.4±0.24	11.7±0.59
18:0	4.1	1.8±0.92	3.2±0.15	3.4±0.12
18:1n-(7+9)	32.8	25.0±3.12	25.5±0.40	25.4±0.91
18:2n-6	7.9	2.8±1.42	3.9±0.46	3.3±0.29
18:3n-3	0.9	0.5±0.03	0.4±0.00	0.4±0.03
20:1n-9	2.3	5.6±1.27	5.2±0.50	6.9±0.55
20:2n-6	0.3	0.7±0.59	0.2±0.00	0.3±0.10
20:3n-3	0.3	0.8±0.27	0.3±0.00	0.2±0.12
20:4n-6	0.5	0.7±0.44	0.4±0.03	0.7±0.15
20:5n-3	3.1	5.4±1.39	3.6±0.47	3.4±0.67
22:1n-9	3.7	3.7±0.42	3.4±0.19	2.9±1.13
22:2n-6	0.9	1.2±0.96	0.5±0.34	0.2±0.03
22:5n-3	1.1	2.1±0.54	1.0±0.12	1.0±0.19
22:6n-3	4.7	6.6±1.56	4.3±0.64	4.6±1.14
n-3 HUFA[2]	11.8	15.3±4.02	9.5±1.03	9.2±2.18
EPA+DHA	7.8	12.0±2.95	7.9±1.08	7.9±1.80

[1]Values are mean±SEM of there replicates.
[2]Highly unsaturated fatty acids (C≥20).

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