Changes of Body Composition During Starvation in *Chlorella*-extract Fed Ayu

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The effect of *Chlorella*-extract intake on ayu *Plecoglossus altivelis* was investigated in terms of its physiological merits. Fish of the control group were reared on commercial diet with 6% feed oil for 49 days. In the experimental group, 1% of *Chlorella*-extract was supplemented to the above diet.

The *Chlorella*-extract contributed to neither body weight gain nor elevation of feed conversion efficiency. The biological measurements and proximate composition of the muscle and viscera were not affected by the supplement. Serological measurements revealed a significant decrease in serum lipid and albumin levels in the experimental group fish. Similarly, analysis of body lipid revealed lipid class changes in visceral lipid, suggesting that *Chlorella*-extract contributed to the regulation of lipid metabolism. The experimental group exhibited a tolerance for hypoxic conditions, as well.

Upon starvation of the fish for 21 days, body weight loss was much lower in the experimental group. The muscle and visceral lipid levels decreased in the experimental group as compared to the control group.

Chlorella-extract can contribute to the mobilization of a lipid reserve and thus, suppress protein exhaustion due to energy metabolism. The necessity of ingestion of algae in ayu was discussed in terms of both physiological condition and food habit.

The efficiency of algal supplement to fish diet has been observed practically, but has not been scientifically established. The fact that *Chlorella* is suitable as a dietary protein source is well accepted but no attention has been paid to its physiological merits.

Chlorella-extract supplement in ayu *Plecoglossus altivelis* endowed resisting power against disease¹⁾ and tolerance to air dipping.²⁾ In addition, we recently reported that the supplement caused the depression of muscle lipid level and the study predicted possible improvement of lipid metabolism and well-being in cultured fish.²⁾

Cultivated ayu is generally fatty and lack of delicacy in taste and odor. The difference between wild and cultivated ayu could be resultant from differences in body constituents which might be influenced by the dietary regimen. Assuming that body constituents affect physiological condition, adequacy of the dietary regimen would appear as differences in the mode of energy metabolism.

As lipid reserves have been studied as part of energy metabolism during starvation,³⁻⁷⁾ we carried out starvation experiments in order to examine whether the mode of energy metabolism was influenced by *Chlorella*-extract supplement.

Material and Methods

Fish Culture System

Juvenile ayu *Plecoglossus altivelis* caught in the sea were preliminarily reared up to 10-15 g with commercial diet at the Wakayama Prefecture Inland Water Fisheries Center. Twenty thousand fish were divided and placed into two concrete tanks (10×10×0.7 m) and reared on different diets for 49 days (from Jun. 1 to Aug. 18, 1982). During this period the tanks were continuously supplied with fresh water, of which oxygen saturation was 70-80% and temperature ranged 15-20°C (average 17°C).

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Table 1. Effect of *Chlorella*-extract feeding on growth

Group	Initial body weight (g)	Body weight	Mortality	Growth rate	Feed conversion efficiency (%)
		(g)	(%)		
Control	15.4	52.4	1.1	3.21	72.3
Experimental	15.3	51.0	1.2	3.17	72.2

Table 2. Biological measurements of *Chlorella*-extract fed ayu

Group	Fatness	Viscerosomatic index (%)	Hepatosomatic index (%)	Intraperitoneal fat ratio (%)	Gonadosomatic index (%)	
					♂	♀
Control	16.6±1.0	13.5±1.9	1.32±0.38	6.51±2.35	3.47±2.21	0.83±0.31
Experimental	17.0±0.9	14.3±1.3	1.59±0.53	7.48±1.70	2.93±2.79	0.79±0.31

Diets

The control group was fed on a commercial diet with 6% feed oil. In the experimental group, 1% *Chlorella*-extract was supplemented to the above diet. The *Chlorella*-extract, which was prepared by hot water extraction from *Chlorella vulgaris* E-25, was kindly supplied by Kurimoto Iron Works Ltd. The fish were fed at approximately 4% of body weight, 4 times per day.

Tolerance Test in Hypoxia

Twenty nine fish (1.5 kg) were placed in a 30 l closed container which was filled with oxygen-saturated water, and kept for 18 min at 18.5°C. Oxygen saturation was gradually diminished by consumption. During continuous hypoxic condition, the oxygen tension and the number of the fish which lost equilibrium functions were monitored. After the experiment, the fish were immediately replaced in oxygen-saturated water and the survival rate was checked.

Starvation

Two hundred and ninety-one fish reared on different diets for 49 days were starved for 21 days and subsequential changes were measured. Fish were weighed every few days.

Growth and Biological Measurements

After 49 days rearing, body weight, feed conversion efficiency, mortality, and growth rate were determined. Five males and females each were randomly subjected to dissection and analysis of proximate composition. The viscera were quickly excised, and the liver, gonad, and intraperitoneal fat were weighed individually. Viscerosomatic index was calculated from the

visceral weight including all visceral organs except the kidney.

Blood Properties and Proximate Composition

The blood was withdrawn by cardiac puncture with a cylinge. Hematocrit value was evaluated by microcentrifugation. Hemoglobin was determined by the azide methemoglobin method. Serum protein was determined by Biuret method, serum lipid by sulfo-phospho-vanilline method,⁸⁾ serum albumin by salting-out method, and serum glucose by glucose oxidase method. Phospholipids and triglycerides were evaluated by enzymatic assay. The dorsal muscle and viscera were subjected to analysis of proximate composition. Crude protein was determined by Kjeldahl method. Lipid was extracted according to BLIGH and DYER,⁹⁾ and dried in an oven at 105–110°C.

Lipid Analyses

The lipid extracted with methanol-chloroform mixture was submitted to thin-layer chromatography. The lipid classes were separated on a precoated silica gel plate (E. Merck, Darmstadt). The lipid class composition was evaluated by a densitometer after visualization by spraying sulfuric acid-dichromate reagent and charring.

Results

Growth of the fish fed for 49 days is shown in Table 1. Both groups had good appetite during the feed period. *Chlorella*-extract had no effect on growth or feed conversion efficiency, in agreement with previous results¹⁾. In addition, none of the biological measurements were affected, as

Table 3. Effect of *Chlorella*-extract feeding on blood properties

Group	Hematocrit (%)	Hemoglobin (g/100 ml)	Serum protein* ¹ (g/100 ml)	Serum albumin* ¹ (g/100 ml)	Serum lipid* ¹ (mg/100 ml)	L/P* ² ($\times 10^{-4}$)	TG/PL* ³ (mg/100 ml)	Glucose* ¹
Control	40.9 $\pm$ 12.7	9.10 $\pm$ 1.58	4.67 $\pm$ 1.33	2.55 $\pm$ 0.99	235 $\pm$ 50	522 $\pm$ 130	186 $\pm$ 39	69.8 $\pm$ 11.7
Experimental	48.6 $\pm$ 8.6	10.3 $\pm$ 2.86	3.06 $\pm$ 0.82	1.59 $\pm$ 0.35* ⁴	168 $\pm$ 23* ⁵	550 $\pm$ 80	191 $\pm$ 34	74.9 $\pm$ 12.1

*¹ Values in 100 ml of blood.*² Serum lipid/serum protein ratio.*³ Triglycerides/phospholipids ratio.*⁴ $p < 0.05$ *⁵ $p < 0.01$ **Table 4.** Changes of biological measurements during starvation

Group	Body weight loss (%)	Fatness	Viscerosomatic index (%)	Hepatosomatic index (%)	Intraperitoneal fat ratio (%)	Gonadosomatic index (%)	
						♂	♀
Control	11.7	14.6 $\pm$ 0.7 (88.0)	11.3 $\pm$ 1.98 (83.7)	0.86 $\pm$ 0.25 (65.2)	4.15 $\pm$ 1.21 (63.7)	2.80 $\pm$ 1.25 (80.7)	3.55 $\pm$ 2.55 (428)
Experimental	8.8	14.9 $\pm$ 0.8 (91.2)	11.6 $\pm$ 2.29 (81.1)	0.89 $\pm$ 0.20 (56.0)	4.65 $\pm$ 1.19 (62.4)	3.43 $\pm$ 2.60 (117)	2.94 $\pm$ 2.50 (372)

Parentheses indicate the percentage when the values before starvation are expressed as 100.

Table 5. Changes of blood properties during starvation

Group	Hematocrit (%)	Hemoglobin* ¹ (g/100 ml)	Serum protein* ¹ (g/100 ml)	Serum albumin* ¹ (g/100 ml)	Serum lipid* ¹ (mg/100 ml)	L/P* ² ($\times 10^{-4}$)	TG/PL* ³	Glucose* ¹ (mg/100 ml)
Control	53.0 $\pm$ 7.2	11.1 $\pm$ 1.99	1.55 $\pm$ 0.21	0.93 $\pm$ 0.26	194 $\pm$ 22	125 $\pm$ 17	304 $\pm$ 37	57.9 $\pm$ 12.3
Experimental	48.7 $\pm$ 5.0	11.4 $\pm$ 1.26	1.64 $\pm$ 0.19	0.93 $\pm$ 0.27	189 $\pm$ 37	115 $\pm$ 19	280 $\pm$ 70	66.2 $\pm$ 10.0

*¹ Values in 100 ml of blood.*² Serum lipid/serum protein ratio.*³ Triglycerides/phospholipids ratio.

Fig. 1. Endurance test of fish subjected to decreasing oxygen tension. Bars indicate mortality of fish after experiment.

seen in Table 2. Table 3 shows blood properties. Hematocrit, hemoglobin, serum protein, and glucose were not influenced by the supplement. However, serum albumin and lipid in the experimental group were significantly lower than those in the control group. They were consistent with the result reported previously.²⁾ Analyses of triglycerides/phospholipids ratio (TG/PL ratio) in the serum revealed that the abundance of lipid in the experimental group was attributed to high triglycerides.

Proximate compositions of the dorsal muscle and viscera are presented in Table 6. There were no significant differences between both groups. The results were not in agreement with the previous experiment which was carried out at the level of 4% in feed oil supplement.²⁾ There were some cases where *Chlorella*-extract did not always increase body lipid level, which varied in response to dietary lipid level. A slight difference was found in visceral lipid and moisture levels, which seemed to have counterbalanced each other.

Fig. 1 shows the results of tolerance test for hypoxia. The indicator of tolerance was designated as the number of fish which lost balance under continuously depressed oxygen tension. The process of oxygen consumption of the experimental group was different from that of the control group. It revealed acceleration of oxygen uptake by *Chlorella*-extract. The fish in the control group initially lost balance at 11 min. The oxygen saturation at which 50% of fish lost balance were 20% in the control group and 8% in the experimental group. Accordingly, the results showed that *Chlorella*-extract enhanced tolerance to hypoxic conditions.

The fish reared for 49 days were subsequently kept for 21 days without feeding. Changes of average body weight are shown in Fig. 2. At the

Fig. 2. Change of average body weight (%) during starvation.

6th day of starvation, simultaneously with a decrease of body weight, 44.3% of control group were affected by ulcerative dermal necrosis (UDN)-like ulcer disease. No symptoms were observed in the experimental group. Thereafter, fish densities were thinned to prevent occurrence of the disease. Body weight of the fish affected by the disease decreased remarkably when compared to that of nonaffected fish. Therefore only non-affected fish were weighed. *Chlorella*-extract was effective in preventing the disease and in suppressing the abrupt decrease of body weight during starvation. The changes in biological measurements of starved fish are listed in Table 4. Starvation resulted in a decline of body weight, fatness, and viscerosomatic index. All of the biological indices of the experimental group after 21 days starvation were not significantly distinctive from those of the control group. However, when

Table 6. Body composition before and after starvation

		Before starvation		After starvation	
		Control	Experimental	Control	Experimental
Dorsal muscle	Moisture	71.5±1.6%	71.4±2.4%	72.4±3.3%	72.1±1.8%
	Ash	1.2±0.1%	1.2±0.1%	1.3±0.1%	0.9±0.1%
	Crude protein	16.5±1.7%	15.7±2.0%	18.3±2.2%	20.8±2.8%
	Lipid	10.8±3.0%	11.7±3.7%	8.1±4.3%	6.2±2.0%
Viscera	Moisture	40.6±7.4%	45.0±1.5%	42.3±2.5%	39.8±4.9%
	Ash	1.0±0.3%	1.2±0.4%	1.3±0.4%	1.6±0.7%
	Crude protein	8.8±2.7%	8.1±0.3%	7.9±0.7%	14.1±0.4%*
	Lipid	49.6±9.1%	45.7±2.0%	48.6±3.6%	44.6±5.9%

* $p < 0.05$ to control.**Table 7.** Changes of lipid class of dorsal muscle during starvation

Lipid class	Before starvation		After starvation	
	Control	Experimental	Control	Experimental
Nonpolar lipids	trace	trace	trace	trace
Triglycerides	66.7±6.8%	72.1±4.1%	69.8±8.7%	76.7±6.2%
Fatty acids	6.7±0.4%	6.5±0.5%	8.9±3.4%	3.6±2.5%
Cholesterol	8.9±2.5%	7.6±2.6%	8.6±2.0%	5.0±1.9%
Partial glycerides	3.2±1.4%	1.3±1.3%	3.0±4.7%	2.4±1.1%
Phospholipids	14.6±4.0%	12.6±3.6%	9.7±3.1%	12.3±3.4%

Table 8. Changes of lipid class of viscera during starvation

Lipid class	Before starvation		After starvation	
	Control	Experimental	Control	Experimental
Nonpolar lipids	trace	trace	trace	trace
Triglycerides	73.6±5.2%	56.9±4.0%	71.0±4.4%	58.6±4.2%
Fatty acids	17.9±4.0%	14.6±1.8%	12.0±0.4%	17.0±10.1%
Cholesterol	6.2±5.2%	14.9±1.7%	9.8±5.7%	11.9±4.6%
Partial glycerides	1.9±1.2%	3.7±1.0%	2.2±0.9%	4.0±0.1%
Phospholipids	0.5±0.8%	9.9±2.6%	5.0±1.9%	8.4±1.6%

expressed as percentage loss (Table 4), the viscera trended to decrease somewhat greater in the experimental group. The viscerosomatic index calculated from visceral weight without gonad were 7.83 ± 1.14 in the control group and 7.99 ± 1.29 in the experimental group.

Blood properties of starved fish are shown in Table 5. Serum protein, albumin, and glucose levels decreased by starvation, nevertheless, serum lipid of experimental group did not decrease. The serum albumin level of starved fish showed no distinctions between both groups. The increase of TG/PL ratio was due to the preferential decrease in phospholipids in the serum of starved fish.

The proximate compositions of the dorsal muscle and viscera are shown in Table 6. Variation of crude protein and lipid were of interest in regard to energy metabolism. While lipid of the dorsal muscle in the experimental group clearly decreased, a relative increase in crude protein was found in both groups after starvation. As the visceral protein was reduced in the control group, a large part of body weight loss could be attributed to loss of the proteins. Despite the fact that the intraperitoneal fat ratio in the experimental group did not abruptly decrease, the visceral lipid decreased apparently.

The lipid class compositions of the dorsal muscle and viscera are presented in Tables 7 and 8. The

lipid was fractionated into 6 lipid classes. Triglyceride portion was always dominant. Non-polar lipid level were immeasurably low. The difference between control and experimental groups was not great. While muscle lipid decreased markedly in the experimental group, lipid composition did not change as much. The muscle phospholipids of the control group tended to degrade preferentially under starvation.

The visceral lipid was characterized by dominance of triglycerides, which were attributed to the intraperitoneal fat. Triglycerides were distinctly dominant in the control group. The supplement yielded alteration in visceral lipid composition, in spite of the fact that the fish were fed on identical dietary lipid. Therefore the visceral lipid composition was shown to be regulated by the *Chlorella*-extract supplement. However, all visceral lipid classes were equally consumed during starvation, as was the case for muscle lipid.

Discussion

In a previous study, a *Chlorella*-extract supplemented diet depressed muscle lipid levels in addition to eliciting physiological effects,²⁾ when feed oil was added at the level of 4%. In general, body lipid content of the cultivated ayu was higher than that of wild ayu.^{10,11)} Owing to the *Chlorella*-extract, the lipid level approached closely the value for wild ayu. Differing from the previous results,²⁾ the present study showed that high dietary lipid level had seemingly no influence on body lipid level. Thus, inclusion of algal substance in the diet can exert differential effects on lipid metabolism in response to differing feed oil levels.

The relationship between serum lipid and body lipid is useful in elucidating the mechanism of endogenous lipid mobilization. Suitable criterion for normal physiological condition in terms of hematological and serological parameters have not been established for fish. *Chlorella*-extract acted to depress serum albumin and serum lipid levels. As the serum is an important lipid-carrier for fish,¹²⁻¹⁴⁾ the decrease of the albumin, which seemed to accompany the decrease of lipid in *Chlorella*-extract fed fish, might be ascribed as an adaptation to serum lipid levels.

While *Chlorella*-extract was seemingly not effective in altering biological measurements and body composition, it had an effect on the tolerance for hypoxia, indicating an increase in anaerobic

metabolism under this condition.

Chlorella-extract, which acted to suppress the body weight loss during starvation, might play a more or less prominent role in energy metabolism. Moreover, the differentiation of visceral lipid composition was precisely due to *Chlorella*-extract, which related to the lipid deposit. Since fish can resist prolonged starvation imposed by wintering and migration, the lipid reserve has been well reviewed as part of energy metabolism during starvation.⁸⁻⁷⁾ JEZIERSKA *et al.*⁷⁾ suggested that visceral lipid was more mobile and made a substantial contribution to energy utilization during starvation in rainbow trout. As the decreasing rate of muscle lipid was higher in the experimental group, it might be concluded that *Chlorella*-extract acted to enhance lipid utilization to energy and to suppress the consumption of protein. Although the depression of body lipid is generally accompanied with a relative increase of moisture,^{8,10,11)} the decrease of lipid which occurred under the supplement did not result in a direct increase of moisture. In the control group, the marked loss of body weight might be ascribed to consumption of the muscle protein in place of the lipid deposit. Thus, this phenomena would be in accord with the effect of dietary inorganic phosphate observed by SAKAMOTO and YONE,¹⁰⁾ and TAKEUCHI and NAKAZOE.¹⁶⁾ The lipid reserved in inorganic phosphate-deficient fish was retained in the body without being consumed as an energy source. The accumulated energy as the lipid reserve should be effectively released as required.

As lipid is good source of energy storage, feed oil is employed for the purpose of prompting growth. However, excess dietary lipid occasionally contributes to various physiological disorders. In response to dietary lipid, body lipid of ayu increased and was metabolized in a different mode.^{17,18)} Lipid metabolism was influenced by dietary composition,¹⁹⁻²¹⁾ and eventually resulted in different responses to starvation.²⁰⁻²²⁾ WOOD *et al.*^{23,24)} suggested that the body lipid level related to survival rate in salmon reared over a long period. Physiological disorders did not always relate to the quantity of body lipid, but to the availability of the lipid for mobilization.

Ayu of more than *ca.* 6 cm in body length is herbivorous, feeding on algae; diatom, blue green algae, and green algae in freshwater habitat. Cultivation on ayu depends on commercial diet mainly composed of animal and grain ingredients.

Nutritional value in terms of growth rate, availability of diet, and economical aspects have been tested. However, the adequacy of nutrient requirement should be attested to be involved endowment of physiological activity. Availability of wholesome nutrients which controls physiological activity has not yet been theoretically proved. It would consequently enhance the survival rate that *Chlorella*-extract was directly related to lipid metabolism and controlled energy metabolism. The observations that physiological merits were indebted to such algal substances as *Chlorella* might support the importance of food habit of alga-ayu association in fish nutrition.

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