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Influence of *Ulva* Meal Supplement to Diet on Plasma Lipoprotein of Black Sea Bream

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Effect of algae meal supplement improved lipid metabolism in black sea bream *Acanthopagrus schlegeli*[1,2] and other cultured fishes[3-7]. Assuming that the lipid accumulation and mobilization were controlled by dietary conditions, the metabolic change would arise in plasma lipoprotein besides body constituents. Fish serum constituents are highly variable in response to physiological condition. However, chemical and physiological properties of fish serum protein have been insufficiently explored. Despite that a theory concerning lipid metabolism in mammalian serum is directly inapplicable to fish[8], serum properties have been widely employed as health assessment of fish.

Accordingly, based on previous results[1,2], the paper described properties of plasma lipoproteins as indicators of improvement on lipid metabolism by dietary *Ulva* meal.

MATERIALS AND METHODS

Fish and Feeding Condition The fish used in the experiment was the same as that of previous works[1,2]. One hundred and sixty-six black sea bream *Acanthopagrus schlegeli* weighed 24g devided into two groups, were reared for 143 days in indoor tanks: one was control group feeding on commercial diet only, and the other was experimental group feeding on the diet of which 10% were replaced by *Ulva pertusa* meal.

Wintering of Fish After feeding experiment, the fish were kept wintering for 138 days without feeding. Water temperature during the wintering ranged from 7°C to 13°C.

Blood Analysis The fish ranged from 70 to 145g in body weight were subjected to blood sampling. The blood was withdrawn from the caudal peduncle into a heparinized watch glass.

The blood (5–15 samples in each group) were measured by hematological and plasmological methods. Hemoglobin and erythrocyte number were measured by a Compur M-1000 (Emes Co. Ltd.). The plasma obtained by centrifugation at 3,000 rpm for 10 min was stored in a freezer at −20°C until analysis. Plasma protein level was individually measured by Biuret method and lipid by Sulfo-phospho-vanillin method. Albumin/globulin (A/G) ratio was determined by turbidity after salting-out with

ammonium sulfate solution. Albumin content was calculated from plasma protein and A/G ratio. Triglycerides (TG), phospholipids (PL) and nonesterified fatty acids (NEFA) were measured by enzymatic assay.

Classification of Lipoprotein Pooled plasma obtained from 10 fish was mixed with an equal volume of saturated ammonium sulfate solution. After allowing to stand overnight in a cold room, the supernatant was obtained by centrifugation and designated albumin fraction. The precipitate was washed with an half saturated ammonium sulfate solution and designated globulin fraction. Protein solution was concentrated by Amicon filter(Amicon Co. Ltd.).

Electrophoresis Protein dialyzed against buffer solution was supplied to 7.5% polyacrylamide disc gel and 4–30% gradient polyacrylamide gel (PAG) electrophoreses. The plasma samples dissolved in 50% glycerin were applied on the top of gel. A constant voltage of 250V was charged for 360 min at 10°C in PAG and 2mA for 90–120 min in disc gel. Protein was visualized by Coomasie blue staining, and lipoprotein by Sudan black B staining. Proportion of lipoprotein was evaluated by 7% disc gel electrophoresis and its densitometry in five plasma samples of each group. Molecular weight was estimated by 4–30% PAG electrophoresis. As standard markers, an Electrophoretic Calibration Kit HWM (Pharmacia Fine Chemical Co. Ltd.) was used.

Lipid Analyses Prosthetic group lipid of lipoprotein was extracted with methanol-chloroform. Lipid class composition was analyzed by an Iatroscan (Iatron Co. Ltd.).

RESULTS

As described previously, *Ulva* meal supplement to commercial diet affected neither body weight gain, nor feed efficiency[1,2]. Although dietary protein level reduced by the supplementation, protein efficiency ratio elevated slightly. Consequently, the algae could save dietary protein.

Table. 1. Hematological and plasmological measurements of *Ulva*-meal fed black sea bream

Group	Hematocrit (%)	Hemoglobin (g/dl)	RBC (10^6)	Protein (g/dl)	Albumin (g/dl)	Plasma Lipid (g/dl)	TG/PL[*1] (10^3)	NEFA (μEq/l)
Before wintering								
Control	37.4	7.16	4.39	3.08	1.02	1.30	663	823
Experimental	38.6	7.33	4.05	2.93	1.09	1.04[*2]	416[*2]	783
After wintering								
Control	23.4	5.12	2.99	1.01	0.54	0.29	248	467
Experimental	21.8	5.21	3.43	1.20	0.45	0.28	235	654[*2]

Plasma constituents were expressed as the values in blood.
*1 Triglycerides/phospholipids ratio
*2 $p<0.01$ to control group
*3 $p<0.05$ to control group

Hematological and plasmological measurements of the *Ulva* meal fed fish are shown in Table 1. While the *Ulva* meal did not influence hematological indices, plasma lipid and TG/PL ratio decreased significantly. The decrease of plasma lipid was ascribed to the decrease of TG.

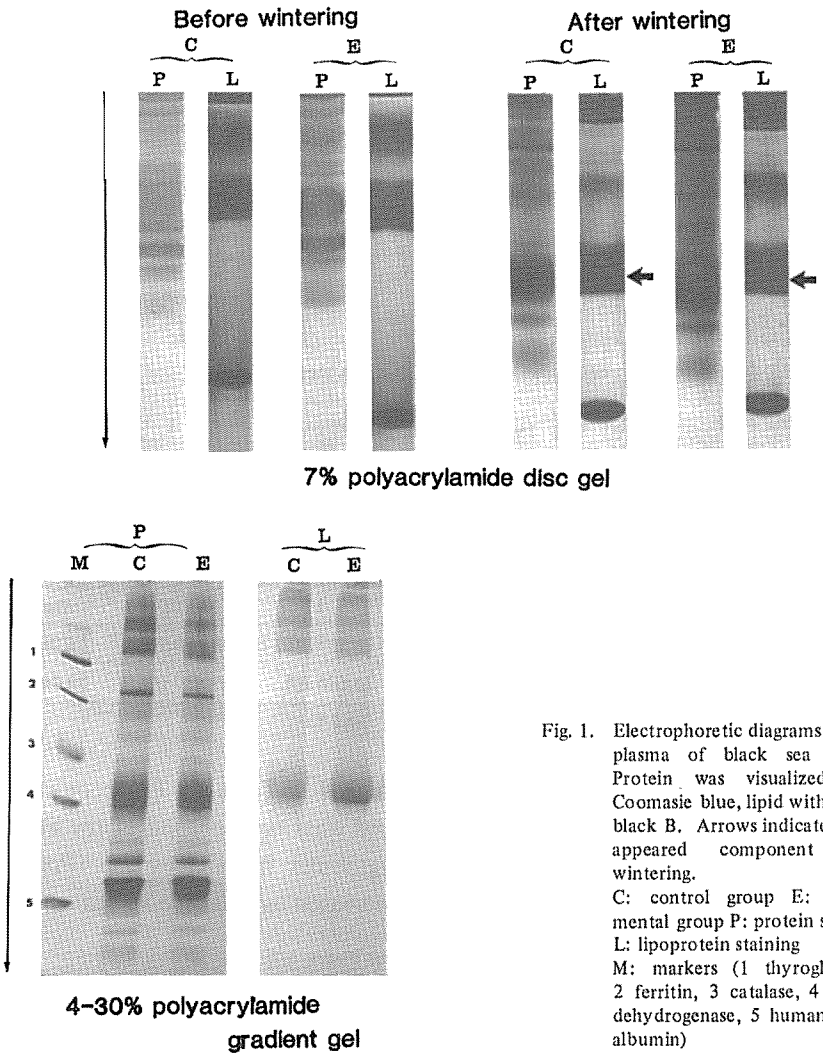

Fig. 1. Electrophoretic diagrams of plasma of black sea bream. Protein was visualized with Coomasie blue, lipid with Sudan black B. Arrows indicate newly appeared component after wintering.
C: control group E: experimental group P: protein staining L: lipoprotein staining
M: markers (1 thyroglobulin, 2 ferritin, 3 catalase, 4 lactate dehydrogenase, 5 human serum albumin)

Electrophoregram of the whole plasma represented 18 protein bands on 7% disc gel (Fig. 1). Nine bands were lipid-positive in them. Disc electrophoretic diagrams of the whole plasma and the classified proteins are schematically shown in Fig. 2. Albumin and globulin fractions were different in both protein and lipoprotein patterns. Protein bands were numbered from the origin to front. The albumin fraction showed only one lipoprotein band (Fr.12). Fr.12 was about 150,000 in molecular weight and positive for bromphenol blue (BPB)-binding test. The globulin fraction comprised 8 lipoproteins (Fr. 1, 3, 3', 4, 5, 6, 7 and 8). Their molecular weights were more than 7000,000.

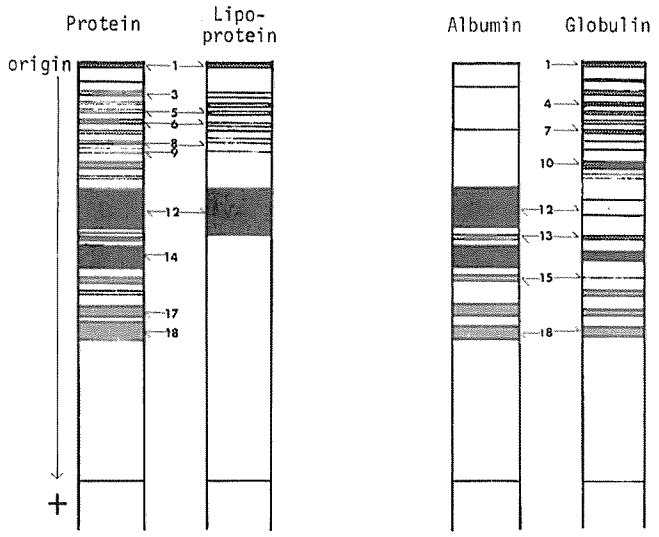

Fig. 2. Schematic disc electrophoregram of black sea bream plasma.

Electrophoretic diagrams of the two groups are shown in Fig. 1. Table 2 shows their proportion. The albumin fraction was higher in the experimental group.

Table 2. Plasma lipoprotein composition of black sea bream

Group	Before wintering		After wintering		
	Fr. 12	others*1	Fr. 12	Fr. 12'	others*1
Control	57.7±3.8%	42.3±3.8%	18.5±7.8%	48.3±3.7%	33.2±7.0%
Experimental	67.6±7.1*1	32.4±7.1%*2	21.6±5.0%	39.9±7.0%	38.4±3.0%

*1 Lipoproteins of globulin fraction (Fr. 1, 3, 3', 4, 5, 6, 7 and 8).
*2 $p<0.05$ to control group

Lipid class composition of lipid moiety is presented in Table 3. PL accounted for 55.8% in the lipid moiety of albumin fraction. Lipid class composition of the albumin fraction did not change as much as that of the globulin fraction by the *Ulva* meal. TG/PL ratio in the globulin fraction was markedly decreased by the *Ulva* meal.

Table 3. Lipid class composition of plasma lipoprotein in black sea bream

Lipid class	Control group		Experimental group	
	Albumin	Globulin	Albumin	Globulin
Cholesterol esters & wax esters	13.0%	17.9%	13.9%	17.9%
Triglycerides(TG)	20.0%	36.2%	16.7%	21.6%
Fatty acids	2.3%	2.4%	0.9%	0.9%
Cholesterol	8.8%	14.6%	9.9%	11.1%
Phospholipids (PL)	55.8%	28.8%	58.5%	48.5%
TG/PL ratio	0.36	1.26	0.29	0.45

Table 1 shows hematological and plasmological parameters after wintering. The all parameters decreased clearly. However, the decrease of NEFA level was a few in the experimental group as compared with the control group. The wintering caused great changes in body constituents[2], as well as plasma constituents. In addition, the *Ulva*

meal supplement affected lipid metabolism such as improvement of lipid accumulation and mobilization[2]. Accordingly, great changes by the wintering appeared on the disc gel. A new lipoprotein designated Fr. 12' was separated from Fr. 12. However, PAG electrophoresis did not show any changes. The wintering increased the proportion of albumin fraction in the control group (57.7% to 66.8%) but, on the contrary, decreased in that of the experimental group (67.6% to 61.5%). As a result, lipoprotein composition was not different between both dietary groups after wintering.

DISCUSSION

Unlikely mammalian serum, fish serum was characterized by that dominant lipoprotein was albumin-like protein. Amino acid composition of the dominant lipoprotein of carp plasma somewhat resembles both human serum albumin and high density lipoprotein (HDL)[9]. Molecular weight of the lipoprotein of albumin fraction (150,000) in the black sea bream coincided with that of carp *Cyprinus carpio*[10], yellowtail *Seriola quinqueradiata*[11] and ayu *Plecoglossus altivelis** was equivalent to double of human serum albumin. The liporpotein had an ability to bind to BPB which is an important definition of mammalian serum albumin[12]. As suggested by MILLS and TAILLER[13], lipid transport mechanism develops during the evolution of phylum. There is much variation among fish species in the extent of lipid metabolism. It was suggested that lipid transport in fish might not be specialized as highly as that of mammal[9]. HLD predominates in fish serum lipoprotein[14–18]. The level of a plasma lipoprotein corresponding to Fr. 12 of the fish or HDL fractuated in response to physiological condition. Fr. 12 might function as the mammalian albumin and HDL on lipid metabolism. While lipoprotein of albumin fraction seemed active for lipid transport and sensitive for physiological condition, the function of that of globulin was not yet clear.

The *Ulva* meal did not influence on plasma albumin level, but lipoprotein composition and plasma lipid level were markedly different from the control group. The *Ulva* meal changed lipid class composition of lipoprotein. Especially, the change of globulin fraction was clear. The differences were ascribed to mechanical change of lipid accumulation. MCKAY and LEE[18] found that HDL of channel catfish remained relatively unchanged, and the amounts of the other lipoprotein classes were closely connected with food intake.

When dietary source was ceased, NEFA should be released from the reserved lipids into the plasma. In the human serum, NEFA are conjugated with serum albumin. In carp plasma lipoprotein, albumin fraction contained relatively high NEFA[10]. There were no differences in the proportion of free fatty acids between albumin and globulin fractions in the fish before wintering. NEFA derived from the reserved lipids were suggested to be incorporated with the lipoprotein of albumin fraction, because it might be active site for free fatty acid carrier. Relatively high NEFA level found in the experimental group after wintering might show that fatty acids were actively released from the reserved lipids. Although the dietary history of *Ulva* ingestion was suggested to

* NAKAGAWA, H. *et al.* unpublished data

influence on lipid mobilization lipoprotein composition and lipid level after wintering did not seemingly differenciate between the two groups.

The appearance of a new lipoprotein after wintering could be explained as follows. Fish plasma lipoproteins are subject to bind preferentially to exogeneously added lipids and to fasten electrophoretic mobility[19]. Nevertheless, identity of molecular weight of a new lipoprotein and Fr. 12 suggested that their apoproteins were essentially homogeneous. Therefore, the mobility change might be due to NEFA released from the reserved lipids by lipolysis.

SUMMARY

Effect of *Ulva pertusa* meal supplement to diet on improvement in lipid metabolism was determined in regard with plasma lipoprotein in black sea bream *Acanthopagrus schlegeli.*

1) At least 9 lipoproteins were electrophoretically detected. These lipoproteins were classified into albumin and globulin by salting-out. A lipoprotein classified as albumin having 150,000 in molecular weight was accounted for 57% in the total lipoproteins of control group. The *Ulva* meal increased its proportion (67.6%).

2) Molecular weight of lipoproteins in globulin fraction was more than 700,000. While lipid class composition of albumin fraction did not changed, that of globulin fraction was highly variable under the influence of the dietary *Ulva* meal.

3) Wintering without feeding caused great changes in electrophoretic behavior of lipoprotein. However, no differences were found in lipoprotein composition between both groups after wintering.

4 The improvement of lipid metabolism by the *Ulva* meal reflected on the plasma lipoprotein.

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クロダイの血漿リポタンパク質に及ぼすアオサ粉末添加飼料の影響

中川平介・笠原正五郎・杉山瑛之

クロダイ *Acanthopagrus schlegeli* の脂質代謝の改善に及ぼすアオサ *Ulva pertusa* 粉末添加飼料の効果を，血漿リポタンパク質の挙動から調べた。

1）電気泳動的に9成分のリポタンパク質を認め，これらを硫安塩析でアルブミンとグロブリンに分画した。全リポタンパク質の57%は分子量150,000のアルブミン性リポタンパク質が占める。他の8成分は分子量70,000以上のグロブリン性であった。

2）アオサ粉末の投与により，血漿リポタンパク質に影響がみられ，アルブミン性リポタンパク質の割合が増加した。アルブミン性リポタンパク質の脂質クラス組成はアオサ投与により変化はなかったが，グロブリン性リポタンパク質の脂質クラスに大きな変化がみられ，Triglycerides が減少しリン脂質が相対的に増加した。

3）無給餌による越冬で両区のアルブミン性リポタンパク質が電気泳動的に変化したが，越冬後の泳動像は両区で差異は認められなかった。

4）アオサの投与により生じた脂質代謝の改善は，血漿リポタンパク質の挙動にも影響を与えることを観察した。