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Optimum Level of *Ulva* Meal Diet Supplement to Minimize Weight Loss During Wintering in Black Sea Bream *Acanthopagrus schlegeli* (Bleeker)

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Abstract

Feeding trials were conducted to establish the optimum supplementation level of *Ulva pertusa* meal in diets of black sea bream (*Acanthopagrus schlegeli*) with a view to minimizing body weight loss during wintering. Fish of 56 g in initial body weight were reared with formulated diets supplemented with various levels of *Ulva* meal (0, 2.5, 5, 10 and 15%) for 63 days in floating net cages.

The *Ulva* meal diets repressed lipid accumulation in intraperitoneal body fat without loss of growth and feed efficiency.

After the feeding experiment, the fish were wintered for 150 days without artificial feeding. The body weight of fish fed an *Ulva*-free diet and a 15% *Ulva* meal diet decreased significantly. Groups fed 2.5, 5 and 10% *Ulva* meal did not show significant body weight loss. The suppression of body weight loss would be ascribed to preferential mobilization of reserved lipids and retention of muscle protein.

Introduction

The black sea bream is omnivorous and its superior resistance to disease, environmental changes, wintering and water pollution makes it suitable for culture in the Inland Sea of Japan. However, weight loss during winter months is of serious concern in its culture.

The usefulness of *Ulva* meal as a feed supplement in the fish was previously confirmed in regard to lipid metabolism (Nakagawa et al. 1984b, 1986a, 1987). Lipid reserves play a significant role as an energy source in fish, especially under feed deprivation. A series of experiments where algae were fed showed improvement in lipid metabolism (Nakagawa et al. 1984a, 1986b, 1987; Amano and Noda 1985; Nakagawa and Kasahara 1986; Nakazoe et al. 1986; Yone et al. 1986).

Identification of efficacious substances and optimum supplementation level are still a matter of debate. This study was designed to assess the appropriate supplement level of *Ulva* meal in diets, with particular attention to body weight loss during wintering and lipid mobilization.

Materials and Methods

Black sea bream hatched at the Farming Fisheries Center, Okayama Prefecture, Japan, were transferred to the Okayama Prefecture Fisheries Experiment Station and reared in floating net cages (1 x 1 x 1 m). Fish of 56 g were divided into five groups of 50 fish and fed with experimental diets containing different levels of *Ulva* meal for 63 days. The water temperature throughout the feeding experiment was 19.1-27.0°C.

Five experimental diets formulated according to Yone and Toshima (1973) are shown in Table 1. The mixture of fish meal and dextrin was replaced by *Ulva pertusa* meal (0.2 mm) which was air-dried and pulverized with a Pulverizer (Retsch Co.). Fish were fed approximately 2% body weight per day.

Following the feeding experiments, fish were over-wintered in floating net cages without feeding from 12 December to 9 May (150 days). The lowest water temperature throughout the wintering period was 7.6°C.

Biological indices were calculated as follows:

Feed conversion efficiency (%) = body weight gain/diet fed x 100

Protein efficiency ratio = body weight gain/dry weight of protein feed

Hepatosomatic index (%) = liver weight/body weight x 100

Table 1. Test diets and their proximate composition.

Ingredient	Dietary group				
	1[1]	2	3	4	5
White fish meal	50.0	48.5	47.0	44.0	41.0
Dextrin	34.0	33.0	32.0	30.0	28.0
Vitamins[2]	3.0	3.0	3.0	3.0	3.0
Minerals[3]	8.0	8.0	8.0	8.0	8.0
Cuttlefish oil	5.0	5.0	5.0	5.0	5.0
Ulva meal	0	2.5	5.0	10.0	15.0
Proximate composition (%)					
Moisture	9.4	9.0	9.7	10.1	10.1
Ash	13.8	14.1	13.9	15.0	16.2
Crude protein	35.0	35.8	35.0	35.3	34.3
Lipid	6.9	6.6	6.6	6.8	6.5

[1]Yone and Toshima (1973)
[2]Halver's vitamin mixture
[3]ICN Nutritional Biochemical Salt Mixture No. 2

$$\text{Intraperitoneal body fat (IPF) ratio (\%)} = \text{IPF weight/body weight} \times 100$$

$$\text{Condition factor} = \text{body weight/body length}^3 \times 100$$

Blood samples were drawn by cutting through the caudal peduncle of 10 fish, and the hematocrit values were determined. The plasma was subjected to measurements of total protein (Biuret method), total lipid (sulfo-phospho-vanillin method) and nonesterified fatty acids (NEFA, enzymatic assay).

Proximate analyses of muscle, liver and IPF were made in a pooled sample obtained from five fish. Crude protein was measured by Kjeldahl method. Lipid was extracted with a methanol-chloroform mixture using the method of Bligh and Dyer (1959). Lipid class composition was analyzed by an Iatroscan TH-10 (Iatron Co.).

Liver was fixed in Ca-formalin and/or Gendre's solution. Paraffin sections of 8 µm thickness were stained with Hematoxylin-Eosin and PAS. Frozen sections of 8 µm thickness were stained with Sudan III for the observation of lipid deposition after fixation with Ca-formalin.

The data were compared statistically with Duncan's multiple range test and t-test. Lipid class composition was evaluated by the method of Tamura and Osawa (1969).

Results

The effects of *Ulva* feeding and wintering on the body parameters are shown in Table 2. While feeding *Ulva* did not influence growth, it improved feed conversion efficiency and protein efficiency ratio by 5% and significantly depressed the hepatosomatic index and IPF ratio.

Body weight declined significantly by wintering in groups 1 and 5 ($P<0.05$), but the loss was significantly suppressed in the other groups. Wintering markedly reduced liver and IPF weight ($P<0.01$), but did not influence the condition factor. The percentage loss of IPF weight was relatively low in groups fed *Ulva* at 2.5-10% levels.

Table 3 presents changes in blood parameters of fed and overwintered fish. Diets containing *Ulva* significantly depressed plasma protein, lipid and NEFA. Overwintering caused some characteristic changes in response to the varying *Ulva* levels in the

Table 2. Effect of *Ulva* meal supplementation to diet on growth and body parameters[1] of black sea bream at the end of feeding (Feeding) and after overwintering (Winter).

		Dietary group				
		1	2	3	4	5
Feeding						
Body weight	(g)[2]	104±22.5	93.2±15.8	97.0±15.5	96.1±18.0	106±14.4
Feed conversion efficiency	(%)	47.5	48.1	50.3	43.3	42.7
Protein efficiency ratio		1.36	1.34	1.44	1.23	1.21
Condition factor		2.97±0.20	3.08±0.23	2.99±0.18	3.01±0.18	3.08±0.12
Hepatosomatic index	(%)	1.99±0.36[a]	1.69±0.25[bc]	1.79±0.28[ab]	1.47±0.32[c]	1.59±0.29[bc]
IPF ratio[3]	(%)	2.92±0.99[a]	1.34±0.67[c]	2.45±0.59[ab]	1.82±0.65[bc]	2.09±0.73[b]
Winter						
Body weight	(g)	87.3±14.4**	91.1±12.2	95.7±18.5	89.1±16.0	91.4±14.3*
Weight loss	(%)	10.5	2.2	1.3	7.3	13.8
Survival	(%)	97.1	92.7	92.1	89.7	94.9
Condition factor		2.96±0.37	3.18±0.41	2.99±0.36	2.90±0.13	2.93±0.18
Hepatosomatic index	(%)	1.02±0.16[a]	0.86±0.22[bc]*	0.75±0.10[c]*	0.93±0.13[ab]*	0.76±0.8[c]*
IPF ratio[3]	(%)	0.20±0.35[a]*	0.48±0.55[a]*	0.37±0.31[a]*	0.42±0.44[a]*	0.12±0.20[a]*

[1]Mean and SD
[2]Initial body weight 56 g
[3]Intraperitoneal body fat ratio
*Significantly different from the value before wintering ($P<0.01$).
**Significantly different from the value before wintering ($P<0.02$).
Values in the same line followed by different letters are significantly different ($P<0.01$).

Table 3. Blood constituents[1] of black sea bream fed a variety of *Ulva* meal levels at the end of feeding (F) and after overwintering (W).

		Dietary group				
		1	2	3	4	5
Hematocrit (%)	F	38.2±6.9[a]	38.4±6.5[a]	35.6±5.6[a]	35.4±8.4[a]	35.9±5.9[a]
	W	28.4±5.8[a*]	27.5±4.6[ab]	25.1±2.7[ab]	24.5±4.9[ab]	23.3±4.8[b]
Plasma protein (g/100 ml)	F	6.92±0.73[a]	5.04±1.97[b]	4.08±0.72[bc]	3.36±0.52[c]	3.56±0.96[c]
	OW	3.12±0.77[a*]	2.77±0.32[ab*]	2.74±0.28[ab*]	2.64±0.28[b*]	2.45±0.33[b*]
Plasma lipid (g/100 ml)	F	2.92±0.55[a]	2.00±0.47[b]	2.16±0.34[b]	1.95±0.72[b]	1.86±0.38[b]
	W	1.13±0.40[a*]	0.98±0.27[ab*]	0.91±0.31[ab*]	0.75±0.37[b*]	0.67±0.19[b*]
Plasma NEFA (mEq/l)[2]	F	1.15±0.51[a]	0.57±0.12[b]	0.49±0.15[b]	0.68±0.42[b]	0.54±0.11[b]
	W	0.68±0.23[a]	0.64±0.12[a]	0.62±0.22[a]	0.59±0.13[a]	0.74±0.17[a*]

[1]Mean and SD
[2]Nonesterified fatty acids (mili equivalent/l)
*Significantly different from the value before wintering (P<0.01).
Values in the same line followed by different letters are significantly different (P<0.01).

diet. Plasma protein and lipid were significantly lowered (P<0.01). The NEFA of group 1 decreased but increased significantly in group 5.

Table 4 shows the proximate composition of muscle, liver and IPF. *Ulva* feeding slightly elevated muscle lipid, but decreased lipid levels in liver and IPF. Overwintering lowered lipid levels in the muscle, liver and IPF, as well as muscle protein.

Table 5 shows lipid class composition. The effect on lipid class composition of muscle was highly variable depending on the supplementation level. Muscle triglycerides (TGS) decreased in the wintering period, and induced a relative increase in phospholipids. Wintering elevated muscle free fatty acids in the *Ulva*-free group but depressed them in *Ulva*-fed groups.

Lipid class composition of liver and IPF was not appreciably influenced in the *Ulva*-fed groups. TGS were the main constituent in both liver (>52%) and IPF (>98%). The liver was characterized by high free fatty acids. Wintering liberated fatty acids from TGS in the groups fed *Ulva*, but the value of the group fed *Ulva*-free diet remained constant. In the IPF, free fatty acids which were increased slightly by *Ulva* feeding were further liberated by wintering.

The amount of reserved lipids is shown in Fig. 1. The TGS of IPF were preferentially utilized during wintering. The group fed an

Table 4. Proximate composition (%)[1] of dorsal muscle, liver and interaperitoneal body fat of sea bream at the end of feeding (Feeding) and after overwintering (Winter).

		Dietary group				
		1	2	3	4	5
Dorsal muscle						
Moisture	Feeding	75.6	75.9	75.0	76.1	75.8
	Winter	79.1	78.3	77.3	79.7	77.6
Ash	Feeding	1.3	1.4	1.5	1.5	1.6
	Winter	1.5	1.3	1.4	1.5	1.5
Crude protein	Feeding	22.1	21.8	22.3	21.4	21.8
	Winter	19.0	19.7	20.6	18.2	20.3
Lipid	Feeding	1.0	0.9	1.2	1.1	0.8
	Winter	0.5	0.7	0.8	0.6	0.6
Liver						
Lipid	Feeding	10.8	7.8	7.8	8.7	7.2
	Winter	3.2	1.6	2.3	3.2	2.9
Intraperitoneal body fat						
Lipid	Feeding	65.7	57.5	60.9	61.4	58.3
	Winter	23.0	31.4	28.7	44.0	26.8

[1]Average value of three analyses.

Ulva-free diet nearly exhausted TGS, but the *Ulva*-fed groups retained much of them.

Histological observation revealed depositions of glycogen and TGS in the liver. However, there were marked histological differences after wintering. Groups 4 and 5 retained liver glycogen even after wintering, but the other groups exhausted it. Staining with Sudan III showed distinct differences among the groups after wintering. Wintering did not influence liver TGS in the *Ulva*-fed group. Histological observation of energy storage exhibited the same trend as biological and biochemical determinations.

Discussion

Reserved lipids are preferentially mobilized as an energy source prior to muscle protein. As a result, body weight loss is eventually

Table 5. Lipid class compositions (%) of dorsal muscle and liver of black sea bream at the end of feeding (Feeding) and after overwintering (Winter). (tr = trace). Figures with different subscripts are significantly different ($P<0.01$).

	Feeding					Winter				
Dietary group	1	2	3	4	5	1	2	3	4	5
Dorsal muscle										
Sterol esters	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Triglycerides	41.2	29.5	46.0	31.7	28.6	11.0	12.1	15.5	14.4	8.5
Flatty acids	13.7	17.7	10.0	16.4	13.2	21.6	6.5	6.9	13.3	7.2
Cholesterol	5.8	6.0	5.4	5.4	5.6	4.9	6.0	6.4	3.6	5.4
Phospholipids	39.3	46.8	38.6	46.5	52.6	62.5	75.4	71.2	68.7	78.9
	a	b	c	b	d	a	b,c	c	d	b
Liver										
Sterol esters	0.7	0.7	0.8	tr	tr	0.6	1.3	0.6	1.0	tr
Triglycerides	62.2	59.1	63.3	61.1	52.1	36.1	8.6	2.0	21.1	3.1
Fatty acids	24.8	29.3	21.4	25.4	28.4	23.1	40.2	34.9	34.2	47.4
Cholesterol	4.7	4.1	4.0	3.7	3.7	5.5	5.2	7.9	7.0	10.1
Phospholipids	7.6	6.8	10.5	9.8	15.8	34.7	44.7	54.6	36.7	39.4
	a	a,b,c	a,b	a,b,c	c	a	a	a	a	a
Intraperitoneal body fat										
Sterol esters	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Triglycerides	99.5	98.7	98.9	99.0	98.4	98.9	97.7	96.7	97.3	96.0
Fatty acids	tr	0.8	0.5	0.5	0.8	tr	0.8	1.4	0.9	1.5
Cholesterol	0.5	0.6	0.6	0.5	0.8	0.5	0.8	1.1	0.9	1.3
Phospholipids	tr	tr	tr	tr	tr	0.7	0.7	0.8	0.9	1.2
	a	a	a	a	a	a	a	a	a	a

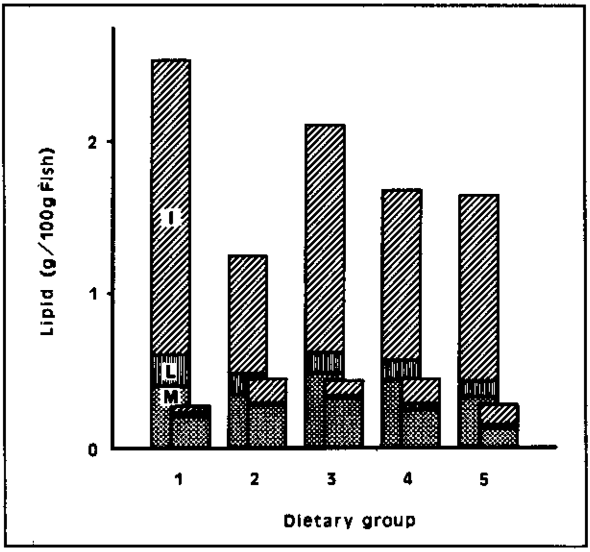

Fig. 1. Effect of various levels of dietary *Ulva*-meal on lipid accumulation in muscle (M), liver (L) and intraperitoneal body fat (I) of black sea bream at the end of feeding (left bar) and after overwintering (right bar). The dietary groups are as in Table 1.

minimized under food deprivation. The phenomenon offers a practical use of *Ulva* meal as a feed additive.

Despite marked increase in muscle lipid in the previous experiment (Nakagawa et al. 1987), the supplementation of *Ulva* meal did not seem to affect body constituents. It might be explained that the dietary algae improved absorption and assimilation of dietary protein and the spared dietary energy was converted to lipid reserves. Accordingly, algae feeding will indirectly influence muscle lipid level. While lipid metabolism could be activated with the increasing algae level, the use of the algae was limited by the decrease of dietary protein.

The effects of *Ulva* were distinct in the overwintered fish. Energy retention after overwintering was associated with low body weight loss. The phenomenon might provide efficient utilization of reserved lipids and repression of muscle protein loss.

Jezierska et al. (1982) suggested that the ability of lipid mobilization might depend on TGS structure. Fatty acid composition was highly variable in response to dietary algae (Amano and Noda 1985; Nakagawa and Kasahara 1986; Nakazoe et al. 1986; Nematipour et al. 1987). However, TGS conformation would not always be responsible to lipolysis. In addition to susceptibility of TGS in mobilization, the activation of the lipolysis system means that endocrine secretion is improved by dietary algae, as suggested by Nematipour et al. (1990).

Feeding algae favorably influenced feed conversion efficiency and protein efficiency ratio. But these values decreased as supplementation levels of algae increased to 10% or higher. As to lipid mobilization, a 5% supplementation level of *Ulva* meal appeared to be optimum for the fish. However, an optimum algae level would depend upon the species of algae and fish. In yellowtail (*Seriola quinqueradiata*), 0.5% *Undaria* meal supplementation to fresh bait was a profitable way to improve physiological condition (Nakagawa et al. 1985; Nakagawa et al. 1986b). That dietary fiber (Furuichi et al. 1983) and *Ulva* extract (Nakagawa et al. 1984a) contributed to improve physiological condition suggests that the effect is derived from multiple algal substances.

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