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Effects of *Ascophyllum* and *Spirulina* Meal as Feed Additives on Growth Performance and Feed Utilization of Red Sea Bream, *Pagrus major*

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Abstract Following up on findings that algae as feed additives improve the physiological condition of cultured fish, the efficacy of *Ascophyllum* and *Spirulina* as feed additives was investigated in one year old red sea bream, *Pagrus major* (ca. 85 g in body weight). Control feed was supplemented with 5% cellulose. In experimental feeds, cellulose was replaced by 5% algae meal. These feeds were provided for 77 days and the effects on growth, feed utilization, and body composition were monitored. Feeding on both *Ascophyllum* and *Spirulina* elevated growth rates and improved feed conversion efficiency, protein efficiency ratio, and muscle protein deposition without negative effects. *Spirulina* showed the most pronounced effects on growth and feed utilization, compared to those macroalgae which have been tried so far.
Key words: algae, feed additive, growth, feed utilization, *Pagrus major,* red sea bream

INTRODUCTION

There have been several studies of algae meal as a dietary protein source for fish (NOSE, 1960; STANLEY and JONES, 1976; MATTY and SMITH, 1978; TSAI, 1979; APPLER, 1985). In addition, it has been confirmed that small amounts of algae added to fish feed exert pronounced effects on growth (YONE *et al.,* 1986; XU *et al.,* 1993), lipid metabolism (NAKAGAWA, 1985; NAKAGAWA and KASAHARA, 1986), body composition (NAKAGAWA *et al.,* 1987), and disease resistance (SATOH *et al.,* 1987). Even though the mechanism of action of algae as a feed additive is not clear, the practical use of algae in fish diet has been increasing considerably in Japan.

The present study examines the effects of *Ascophyllum nodosum* and *Spirulina maxima* as feed additives on growth and feed utilization in the red sea bream, *Pagrus major.*

MATERIALS AND METHODS

Fish and rearing conditions

One year old red sea bream, *Pagrus major,* having an average body weight of 85±15 g were divided into 9 lots, 3 control and 3 each for the two experimental treatments and reared for 77 days in one ton plastic tanks at the Fisheries Laboratory of Hiroshima University. Each tank contained 28 fish. The temperature and salinity of the water rang-

Table 1. Feed composition for red sea bream

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
Ingredients (%)			
White fish meal	75	75	75
Dextrin	10	10	10
Pollack liver oil	5	5	5
Vitamin mixture*1	3	3	3
Mineral mixture*2	2	2	2
Cellulose	5	0	0
Ascophyllum meal	0	5	0
Spirulina meal	0	0	5
Proximate composition (%)			
Moisture	10.6	10.8	10.9
Crude protein	48.7	47.8	50.6
Lipid	8.9	8.5	8.7
Ash	14.6	15.2	15.5

*1 Halver's vitamin mixture
*2 Salt mixture No. 2 (ICN Nutritional Biochemical)

ed 20.2-26.5°C and 32.0-33.5 ppt, respectively.

The control group was fed with dry diet consisting of white fish meal, dextrin, pollack liver oil, vitamins, minerals, and cellulose, as shown in Table 1. In the experimental groups, the cellulose was replaced by one of two kinds of algae meal. *Ascophyllum nodosum* and *Spirulina maxima* meal were produced by Algea Produkter A/S in Norway and Dainippon Ink & Chemicals, Inc. in Japan, respectively. The proximate compositions of the diets are shown in Table 1. Hand feeding was carried out to encourage the fish to satiate twice per day (7:00 and 17:00).

Biometric analyses

Morphological and anatomical measurements were carried on 12 fish from each group. The condition factor, viscerosomatic index (VSI), hepatosomatic index (HSI), muscle ratio (MR), intraperitoneal fat body (IPF) ratio, and muscle protein deposition (MPD) were calculated from the following equations:

$$\text{Condition factor} = \text{body weight}/\text{body length}^3 \times 10^5$$
$$\text{VSI (\%)} = \text{viscera weight}/\text{body weight} \times 100$$
$$\text{HSI (\%)} = \text{liver weight}/\text{body weight} \times 100$$
$$\text{MR (\%)} = \text{muscle weight}/\text{body weight} \times 100$$
$$\text{IPF ratio (\%)} = \text{IPF weight}/\text{body weight} \times 100$$
$$\text{MPD} = \text{muscle protein gain}/\text{total protein fed} \times 100$$

Blood analyses

Blood collected from caudal artery was subjected for the hematological and serological determinations. For hematocrit value, the blood drawn in to a capillary tube was centrifuged for 10 min. at 11,000 rpm in a micro-hematocrit centrifuge. Hemoglobin and erythrocytes were measured according to cyanmethemoglobin method and turbidity method by a Compur M-1000 (Emes Co. Ltd.). The blood after coagulation was centrifuged at 3000 rpm for 10 min and the serum was stored at −20°C until serological analyses. Total serum protein and lipid were determined by Biuret and sulfo-phospho-vanillin methods, respectively. Nonesterified fatty acids (NEFA) was measured by enzymatic assay with NEFA C test kit (Wako Pure Chem. Co. Ltd.). Albumin and globulin ratio was estimated by ammonium sulfate salting-out method (Al-Glo ratio test kit, Kyokuto Seiyaku Kogyo Co. Ltd).

Biochemical analyses

The dorsal white muscle was removed from five fish in each group, frozen in liquid nitrogen, and stored at −80°C until the nucleic acid and acid protease analyses were carried out. Muscle DNA and RNA were determined according to MUNRO and FLECK (1966). Muscle acid protease activity was measured by the method of MAKINODAN *et al.* (1982). Activity was expressed as nanomoles of tyrosine released from substrate per hour. Protein was measured by the Folin method (LOWRY *et al.,* 1951).

Crude protein was measured by the Kjeldahl method. Lipid was extracted with methanol-chloroform according to BLIGH and DYER (1959). Glycogen content of the liver was measured by anthrone reagent (CORROLL *et al.,* 1956).

Statistical analyses

The data were analyzed for significance using Duncan's new multiple range test. Probabilities of 0.05 or less were considered statistically significant.

RESULTS

The feed supplemented with algae meal was well ingested by the fish. The effects of algae as a feed additive on growth and feed utilization are shown in Table 2. The survival rate was more than 98%. Both *Ascophyllum* and *Spirulina* supplements significantly elevated body weight gain and daily growth rate. In addition, there was also improvement in feed conversion efficiency, protein efficiency ratio, and muscle protein deposition. The effects were markedly higher in the *Spirulina*-fed group than in the group fed with *Ascophyllum.*

Table 2. Effects of *Ascophyllum* and *Spirulina* as feed additive on growth performance and feed utilization in red sea bream

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
Total feed provided (g)	6727[a,b]	7119[a]	6589[b]
Initial weight (g)	2423[a]	2395[a]	2305[b]
Final weight (g)	5932[a]	6343[b]	6336[b]
Biomass production (g)	3508[a]	3949[b]	4030[b]
Survival (%)	98.8	100	98.8
Feed conversion efficiency (%)	52.2[a]	55.4[b]	62.3[c]
Protein efficiency ratio	1.07[a]	1.16[b]	1.23[c]
Muscle protein deposition (%)	12.4[a]	12.9[b]	14.5[c]
Daily growth rate (%)	1.19[a]	1.28[b]	1.35[c]

Different letters as superscripts on the same row indicate significant differences ($p<0.05$).

Table 3. Effects of *Ascophyllum* and *Spirulina* as feed additive on biological characteristics in red sea bream

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
Mean body weight (g)	214±35 [a]	227±39 [b]	230±37 [b]
Mean body length (cm)	184±11	187±12	185±11
Condition factor*1	3.40±0.26 [a]	3.47±0.23 [a]	3.60±0.25 [b]
Viscerosomatic index (%)	7.63±1.44 [a]	7.77±1.44 [a]	9.07±1.47 [b]
Hepatosomatic index (%)	1.63±0.36 [a, b]	1.43±0.32 [a]	1.81±0.40 [b]
Muscle ratio (%)	46.4±3.3	46.8±1.6	47.3±1.5
IPF ratio (%)*2	3.36±1.07 [a, b]	2.94±1.13 [a]	4.21±1.28 [b]

Mean±SD

Different letters superscript on the same row indicate significant differences ($p<0.01$).

*1 Body weight/body length$^3 \times 10^5$

*2 Intraperitoneal fat body ratio

Table 4. Blood and serum properties of red sea bream fed *Ascophyllum* and *Spirulina* as feed additive

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
Hematocrit (%)	39.2±7.1	39.9±5.7	37.7±7.5
Hemoglobin (g/d*l*)	6.83±1.16	6.79±1.25	6.32±1.23
Erythrocytes (10^6/mm^3)	4.17±1.14	4.25±0.80	4.02±0.78
Total protein (g/d*l*)	3.76±0.17 [a]	3.95±0.26 [a, b]	4.11±0.28 [b]
A/G ratio*1	0.64±0.09 [a]	0.53±0.09 [b]	0.52±0.06 [b]
Albumin (g/d*l*)	1.46±0.13	1.36±0.21	1.40±0.09
Total lipid (g/d*l*)	1.34±0.28	1.23±0.16	1.17±0.27
Glucose (mg/d*l*)	97.0±83.9	82.0±50.5	101±76.1
NEFA*2 (mEq/*l*)	0.16±0.09	0.10±0.03	0.11±0.04

Mean±SD

Different letters as superscripts on the same row indicate significant differences ($p<0.01$).

*1 Nonesterified fatty acids

*2 Albumin/globulin ratio

The biological characteristics of the fish are shown in Table 3. Mean body weight was higher in algae-fed groups than the control group. While the other parameters were not influenced by *Ascophyllum,* condition factor, and VSI were significantly increased by *Spirulina.*

Table 4 shows some blood properties in control and two experimental groups. Feeding both *Spirulina* and *Ascophyllum* did not affect hematological parameters. Except total protein and albumin/globulin ratio, other serological parameters such as total lipid, NEFA, and albumin content were not also affected by the algae feeding.

Table 5 shows the nucleic acids, protein, and acid protease activity in muscle. Muscle protein content was not affected by the algae, but there tended to be a decrease in acid protease activity. No significant effects of the algae supplements were observed in the cellular growth parameters.

Table 5. Muscle constituents of red sea bream fed *Ascophyllum* and *Spirulina* as feed additive

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
RNA (μg/g)	1390±190	1440±110	1550±130
DNA (μg/g)	435±53	439±25	478±18
Protein (mg/g)	202±6[a, b]	199±7[b]	209±4[a]
RNA/DNA	3.19±0.33	3.28±0.22	3.25±0.25
Protein/DNA	0.47±0.06	0.46±0.02	0.45±0.02
Acid protease activity			
(nmol Tyr/h/g tissue)	364±85	293±35	326±83
(nmol Tyr/h/mg protein)	3.33±0.56	2.58±0.31	2.89±0.84

Mean±SD (n=5)
Different letters as superscripts on the same row indicate significant differences ($p<0.01$).

Table 6. Proximate composition of red sea bream fed *Ascophyllum* and *Spirulina* as feed additive

	Dietary group		
	Control	*Ascophyllum*	*Spirulina*
Muscle (n=10)			
Moisture	74.1±0.8[a]	73.2±1.7[a, b]	72.3±1.2[b]
Crude protein	21.5±0.8	21.0±0.6	21.9±0.6
Lipid	3.0±0.5[a]	4.1±1.7[b]	4.3±1.0[b]
Ash	1.5±0.1[a]	1.7±0.3[b]	1.6±0.1[a, b]
Liver (n=5)			
Lipid	21.8±4.5	25.1±6.4	27.3±3.9
Glycogen	3.8±1.9	1.9±1.7	2.7±1.5
Intraperitoneal fat body (n=5)			
Lipid	72.4±8.5	70.1±9.1	65.7±5.4

Mean±SD
Different letters as superscripts on the same row indicate significant differences ($p<0.01$).

Proximate compositions of muscle, liver, and IPF are shown in Table 6. Although muscle protein was not affected by the algae, muscle lipid was significantly elevated. The algae slightly elevated liver lipid and depressed liver glycogen. Although *Spirulina* increased the IPF ratio, the lipid content of the IPF was decreased.

DISCUSSION

Effects of algae on growth performance have been reported in red sea bream (YONE *et al.*, 1986a; MUSTAFA *et al.*, 1994c), Japanese flounder *Paralichthys olivaceus* (XU *et al.*, 1993), and rainbow trout (SOMMER *et al.*, 1992). In the present study, provision of *Ascophyllum* and *Spirulina* as feed additive to red sea bream, *Pagrus major*, produced remarkable effects on growth, feed utilization, and protein deposition. Feeding *Spirulina* activated protein synthesis and somatic growth in red sea bream (MUSTAFA et al., 1994b). Furthermore, the supplementation of feed with macroalgae resulted in a considerable elevation of biomass production (MUSTAFA *et al.*, 1994c). As to effective substances, algal supplements are recognized to delay the absorption of dietary nutrients and to improve carbohydrate and protein utilization (YONE *et al.,* 1986b). In addition, there were several algal factors which might activate metabolism and act as growth stimulants (NAKAJIMA, 1991).

While the algae elevated the muscle lipid content of the red sea bream, the mode of lipid deposition is apparently different among fish species and rearing condition (NAKAGAWA and KASAHARA, 1986; NAKAGAWA *et al.,* 1987; MUSTAFA *et al.,* 1994a). Digestibility by fish is fairly different among algae species (MONTGOMERY and GERKING, 1980). Neavertheless, the present comparison of the effects of two algae revealed common positive effects on growth, feed utilization, and body composition. *Ascophyllum nodosum* is widely distributed in northern Atlantic and is used as a feed additive for animals and fish. While macroalgae are more or less non-toxic, microalgae are occasionally toxic (BECKER, 1986). According to hematological and serological parameters, physiological condition was unaffected by feeding *Spirulina* and *Ascophyllum.* Supplementation of fish feed with *Spirulina* at a high level might produce physiologically negative effects (WATANABE *et al.,* 1990). Nevertheless, the results of this experiment showed only superior effects and improvement of growth and feed utilization in red sea bream.

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マダイの成長，飼料利用率に及ぼす飼料添加物としての *Ascophyllum* と *Spirulina* の効果

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養魚飼料への藻類添加は生理状態の向上に有効であることが明らかにされているので，本研究ではマダイについて *Ascophyllum* と *Spirulina* の効果をみた。対照区には配合飼料に5％のセルロース，*Ascophyllum* 区，*Spirulina* 区にはそれぞれ藻類の粉末を5％添加した。初期体重 85 g のマダイ1年魚を77日間飼育し，成長，飼料利用率，生物学的性状，体成分に及ぼす効果をみた。

いずれの藻類の添加においても成長，飼料効率，タンパク質効率，筋肉タンパク質蓄積量が向上し，筋肉成分では脂質蓄積量に増加がみられた。*Spirulina* の投与では悪影響は認められず，各項目において *Ascophyllum* より優れた効果を示し，これまでに報告された藻類に匹敵するか，もしくはそれ以上の効果を示した。

キーワード：飼料添加物，成長，飼料利用率，*Pagrus major,* マダイ，*Ascophyllum, Spirulina*