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Effective Components in Cuttlefish Meal and Raw Krill for Improvement of Quality of Red Seabream *Pagrus major* Eggs*1

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Red seabream broodstock were fed various diets of different nutritional quality for either 26 days or shortly before spawning to clarify the effective components in cuttlefish meal and raw krill which aid in quality egg production.

The percentage of buoyant eggs was lowest in the control group receiving the white fish meal diet, and was elevated by the addition of 200 mg DL-α-tocopheryl acetate. The value was also effectively improved by replacement of white fish meal with defatted or intact cuttlefish meal as a protein source. Feeding broodstock with frozen raw krill after previously being fed control diet resulted in elevation of the percentage of buoyant eggs and normal larvae. Equally good results were obtained by substitution of cuttlefish liver oil in the control diet with 2.5% krill polar lipid or 2.5% krill nonpolar lipid. However, neither defatted krill meal nor fat-soluble fraction of cuttlefish meal showed the good effect on the egg quality.

Consequently, the superior quality of cuttlefish meal to the white fish meal as a protein source for red seabream broodstock diets was reconfirmed. And the effective components in raw krill, aiding the reproduction of red seabream, are suggested to be the polar and nonpolar lipid fractions. In addition, vitamin E was also found to have the same efficiency for improvement of the egg quality.

In the series of studies on red seabream *Pagrus major* broostock nutrition,[1-6] it was found that spawning and egg quality were always greatly improved by feeding the broodstock on a diet containing cuttlefish meal as protein source or on frozen raw krill shortly before spawning or during spawning. Supplementation of diets with β-carotene and canthaxanthin or with krill oil extract containing astaxanthin was also found to improve egg quality. This promoting role of raw krill on reproduction of red seabream may be due to the carotenoid pigments in krill. The productivity of viable larvae from the total eggs produced by one female ranged from 24 to 39% in the broodstock fed the control diet containing white fish meal as protein source in this series of experiments during 8 years. The viability increased to 70–90% by replacement of white fish meal with cuttlefish meal and to 68–80% by feeding frozen raw krill. One of the major chemical difference between white fish meal and cuttlefish meal is a high content of calcium and phosphorus in the former, derived mainly from tricalcium phosphate (hydroxyapatite) in the bones. This probably suggests the ill effect of a large amount of tricalcium phosphate on reproduction of red seabream. The supplementation of a high quantity of α-tocopherol was also found to be effective in improving spawning and egg quality.

These experiments were carried out to clarify the effective components in cuttlefish meal and raw krill which aid in aquality egg production by red seabream. For this purpose cuttlefish meal and raw krill were separated into lipid and nonlipid fractions. The lipid fraction of raw krill was further fractionated into polar and nonpolar, the latter containing astaxanthins. Effect of these fractions, together with vitamin E, on egg quality were compared by feeding diets to broodstock red seabream shortly before spawning. The effect of

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a supplement diet with tricalcium phosphate was also examined.

As already demonstrated in the previous experiments,[3,6] quality of eggs of the red seabream is quite easily influenced by nutritional quality of the diets given to broodstock even during the spawning period. Therefore, one group of broodstock was fed alternatively every three days, on a low and a high nutritive diets, to correlate the daily egg quality with the dietary composition.

Materials and Methods

Feeding of Red Seabream Broodstock

Broodstock were developed from juvenile red seabream by feeding them on a commercial diet and minced fish meat for about 3 years, according to the same procedures described previously,[1] at the Aquaculture Research Laboratory of Nagasaki Prefectural Institute of Fisheries. These broodstock weighing about 700 g were kept on the control diet (diet 1, Table 4) for 60 days from January 20 to March 26, in floating net cages ($4\times4\times2$ m) in the Nomo Inlet. Later about 50 fish were randomly selected and stocked in each of the 5 floating nets ($3\times3\times2$ m; diets 1 to 5) in the Inlet for 26 days from March 26 to April 20, 1984. Twelve to sixteen males and eleven to fourteen females from each lot were then transferred to 6 t concrete tanks in the aquarium of the laboratory for the investigation of spawning and egg quality (Fig. 1). Furthermore, the broodstock, which had been fed on the control diet from January 20 to April 20, were divided into 7 lots and fed on raw krill (diet 6) and diets containing 200 mg of α-tocopherol (diet 7), 5% krill oil extract (diet 8), 2.5% krill oil extract (diet 9), 2.5% polar lipid fraction of krill oil (diet 10) and 2.5% nonpolar lipid fraction (astaxanthin fraction; diet 11), respectively. The broodstock, which had been fed on the cuttlefish meal diet from March 26 to April 20, were divided into two groups, one continued to be fed the same diet (diet 3) and the other was fed on diet 3 and a diet containing corn oil in place of cuttlefish liver oil, alternatively every three days. The broodstock on diet 5 were also divided into two groups from April 20, and one of the groups fed on a diet containing the lipid fraction of cuttlefish meal. The broodstock on diets 1–5 and 8 were kept in 6 t tanks and those on diets 6, 7, and 9–13 in 1 t polycarbonate thanks for spawning (Fig. 1).

Each test diet was given twice daily and there was no marked difference in the total amount consumed by fish during the feeding experiment, ranging from 250 to 300 g per broodstock except for raw krill 900–1200 g of which was accepted by one broodstock shortly before and during spawning. Water temperature increased gradually from 13°C in March to 21°C in May during the experimental period. Other experimental conditions were as described in a previous paper.[1]

Fractionation of Cuttlefish Meal and Krill Meal

Cuttlefish meal was extracted with 20 folds of a hexane-ethanol mixture (71 : 29) and separated into lipid and nonlipid fractions. Krill meal was also fractionated into lipid and non-lipid fractions by hexane and the lipid fraction was then separated into polar and nonpolar components by

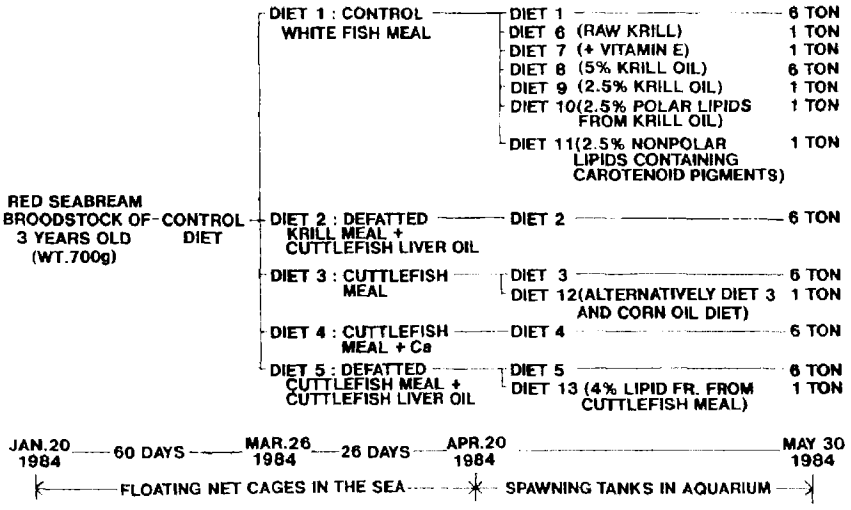

Fig. 1. The feeding schedule for red seabream broodstock.

Table 1. Lipid classes of four kinds of oils used in the experimental diets for red seabream broodstock* (%)

	Krill oil			Oil extracted from cuttlefish meal
	Total lipid	Polar lipid	Nonpolar lipid	
Nonpolar lipid				
SE	—	—	—	0.5
TG	48.0	0.5	80.9	3.0
FFA	1.5	0.1	1.9	4.5
FS	1.8	tr	9.6	26.9
DG	2.1	tr	2.3	—
MG	1.4	—	3.8	—
Polar lipid				
PE	3.2	4.6	—	4.1
PC	37.4	84.9	1.1	40.7
SPM	2.6	8.0	0.1	—
PLC	—	—	—	16.0

* Values expressed as percentage in total lipid.
Abbreviation: SE, sterol esters; TG, triglycerides; FFA, free fatty acids; FS, free sterols; DG, diglycerides; MG, monoglycerides; PE, phosphatidylethanolamine; PC, phospbatidylcholine; SPM, sphigomyelin; LPC, lysophosphatidylcholine.

Table 2. Fatty acid compositions of six kinds of oils used for red seabream broodstock diets (area %)

Fatty acid	Krill oil			Oil extracted from cuttlefish meal	Cuttlefish liver oil	Corn oil
	Total lipid	Polar lipid	Nonpolar lipid			
14:0	14.9	5.7	21.1	4.3	6.6	—
15:0	0.2	0.3	0.1	0.9	0.7	—
16:0	20.6	30.4	15.1	29.3	17.3	10.1
16:1	8.1	2.7	9.9	3.8	6.7	0.2
16:2	0.6	0.4	0.8	0.7	1.2	0.1
17:0	—	—	—	1.6	0.6	—
16:3	—	0.4	0.2	0.3	0.7	—
16:4	1.6	0.2	2.3	0.2	1.3	—
18:0	1.8	0.9	1.0	9.9	2.0	2.0
18:1	19.6	13.6	21.8	7.0	16.7	28.9
18:2n-6	2.2	2.3	2.9	2.4	1.6	55.6
18:3n-6	0.1	0.1	0.1	—	—	0.5
18:3n-3	0.9	1.4	1.0	1.3	1.2	1.7
18:4n-3	4.1	4.7	9.8	0.4	2.6	—
20:0	—	—	0.1	—	—	0.5
20:1	1.0	0.9	1.7	4.7	7.4	0.5
20:2n-6	0.1	0.1	—	0.4	0.6	—
20:3n-6	0.3	0.3	0.8	—	0.1	—
20:4n-6	0.5	0.6	0.2	4.1	1.0	—
20:4n-3	0.5	0.5	0.2	—	1.0	—
20:5n-3	12.4	20.1	5.1	12.7	11.4	—
22:0	—	—	—	0.4	—	—
22:1	0.8	1.0	0.4	1.0	5.0	—
22:4n-6	0.1	0.3	—	0.3	0.1	—
22:5n-6	0.1	0.7	0.1	0.4	0.3	—
22:5n-3	0.2	0.5	0.1	0.6	0.9	—
22:6n-3	8.0	7.5	4.0	11.1	10.6	—
Total n-6	3.4	4.4	4.3	7.6	3.7	56.1
Total n-3	26.1	34.7	20.2	26.1	27.7	1.7
Sum of n-3HUFA	21.1	28.6	9.8	24.4	23.9	—

Table 3. Proximate and mineral compositions of four kinds of meals for red seabream broodstock diets

		White fish meal	Defatted krill meal	Defatted cuttlefish meal	Cuttlefish meal
Moisture	(%)	10.9	9.8	7.0	10.0
Crude protein	(%)	63.2	70.6	83.4	74.7
Crude lipid	(%)	9.7	5.0	1.8	13.1
Crude ash	(%)	15.9	12.7	9.1	5.6
Ca	(mg/g)	49.12	24.31	7.63	3.15
P	(mg/g)	25.15	13.05	7.80	7.22
Mg	(mg/g)	2.02	7.32	3.80	3.02
K	(mg/g)	4.92	3.84	5.40	2.59
Na	(mg/g)	8.60	14.15	15.81	10.21
Fe	(μg/g)	129.0	184.0	246.2	201.4
Zn	(μg/g)	74.7	71.2	125.9	90.6
Mn	(μg/g)	10.1	5.64	5.74	3.59
Cu	(μg/g)	4.3	61.7	40.8	25.8

Table 4. Composition of the experimental diets for red seabream broodstock (%)

Ingredient	Diet no.				
	1	2	3	4	5
White fish meal	67	—	—	—	—
Defatted krill meal	—	64	—	—	—
Cuttlefish meal	—	—	61	61	—
Defatted cuttlefish meal	—	—	—	—	55
Alpha-starch	15	15	15	15	15
Mineral mixture	5	5	5	5	5
Vitamin mixture	2	2	2	2	2
Choline chloride	1	1	1	1	1
Cuttlefish liver oil	4*	7*	2*	2*	9*
Celluose	6	6	14	6	13
Tri-calcium phosphate	—	—	—	8	—

* All the diets contain about 50 mg VE/100 g diet.

Table 5. Composition of the experimental diets for red seabream broodstock just before spawning (%)

Ingredient	Diet no.							
		6	7	8	9	10	11	12	13
White fish meal	Frozen raw krill		67	67	67	67	67	67	67
Alpha-starch			15	15	15	15	15	15	15
Mineral mixture			5	5	5	5	5	5	5
Vitamin mixture			2	2	2	2	2	2	2
Choline chloride			1	1	1	1	1	1	1
Cuttlefish liver oil			4*	—	2.5*	2.5*	2.0*	—	1*
Cellulose			6	5	5	6	5	4	5
Krill oil			—	5*	2.5	—	—	—	—
Krill polar lipid			—	—	—	2.5	—	—	—
Krill nonpolar lipid			—	—	—	—	2.5	—	—
n-3 HUFA			—	—	—	—	0.5	—	—
Corn oil			—	—	—	—	—	6*	—
Oil extracted from cuttlefish meal			—	—	—	—	—	—	4

* All the diets except diet 7 contain 50 mg VE/100 g diet. The diet 7 contains 200 mg VE/100 g diet.

acetone.[7] Astaxanthins in the krill meal were transferred to the nonpolar lipid fraction. Lipid class and fatty acid compositions of each lipid fraction from cuttlefish meal and krill meal are shown in Tables 1 and 2. The main component of the total lipid from krill meal was triglycerides, whereas that of residual oil from cuttlefish meal was free sterol (cholesterol). Phosphatidyl choline was the main component in the polar lipid fraction of both cuttlefish meal and krill meal. Lysophosphatidyl choline was also high in the latter. Each lipid fraction from both the meals was high in the concentration of n-3 highly unsaturated fatty acids (n-3 HUFA) such as 20: 5n-3 and 22: 6n-3, except for the nonpolar lipid of krill oil. The lipid from the cuttlefish meal showed a similar fatty acid distribution to that of the cuttlefish liver oil.

Proximate and mineral compositions of defatted cuttlefish meal and krill meal are shown in Table 3, together with white fish meal and intact cuttlefish meal for comparison. White fish meal was characteristically high in the content of calcium and phosphorus due to tricalcium phosphate in fish bones and low in the copper content.

Experimental Diets

The composition of the experimental diets is shown in Tables 4 and 5. Diet 1 was a control diet containing white fish meal as a protein source and the composition was the same as that used in the previous experiments.[1,3,6] White fish meal was replaced by defatted krill meal indiet 2 to examine the effect of the nonlipid fraction of krill on egg quality and by cuttlefish meal in diets 3 and 4; diet 4 being supplemented with tricalcium phosphate at a level equivalent to the calcium level of white fish meal diet, to compare the dietary value of the two protein sources and examine supplemental effect of calcium on egg quality. In diet 5 white fish meal was also substituted by defatted cuttlefish meal to verify effective com ponents in the meal for imporvement of egg quality. Diet 6 was frozen raw Antarctic krill *Euphausia superba*. Diets 7 to 13 were all modification of the control diet. Diet 7 was essentially the same as diet 1 except for a supplement of 200 mg of DL-α-tocopheryl acetate, which has already proved to be effective for improvement of egg quality. Diets 8 to 12 were arranged to clarify the effective fraction of raw krill for reproduction of red seabream, containing respectively 5% krill oil (diet 8), 2.5% krill oil (diet 9), 2.5% polar lipids (diet 10) and 2.5% nonpolar lipids (diet 11). In diets 12 and 13, cuttlefish liver oil was substituted by 4% of lipid fraction from cuttliefish meal, the level being comparable to that contained in the cuttlefish meal diet and corn oil. The broodstock on diet 12 were fed on both a high quality diet (diet 3) and a low quality diet (the essential fatty acid (EFA)-deficient corn oil diet) alternatively every three days; to examine how the quality of eggs produced changes every day.

The protein and lipid levels were adjusted to approximately 45 and 10%, respectively, the same levels as those used in the previous experiments.[1,3,6]

The analytical data on the test diets and raw krill are shown in Tables 6–8. There was no marked difference in proximate composition among the test diets except for slightly low contents of crude lipid in diets 1 and 4, and of crude ash in diets 3 and 5, both containing cuttlefish meal as a protein source. The level of crude ash together with calcium and phosphorus of diet 4 was elevated to almost the same level as the control diet by a supplement of tricalcium phosphate in the diet. The mineral composition of white fish meal based diets indicated higher content of calcium and phosphorus, and lower level of copper. The values for diets and raw krill are all expressed on a dry basis. All the diets contained 40–50 mg of vitamin E except for diet 7 which had about 130 mg of the vitamin, the value being lower than that actually added to the diet (200 mg).

Among the lipid classes, the proportion of polar lipids was lower in diets 2 and 5, containing defatted krill meal and cuttlefish meal respectively and was highest in diet 10 supplemented with 2.5% krill polar lipid. Cholesterol was high in diets 3 and 4 containing cuttlefish meal and diet 13 containing the lipid fraction of krill meal.

As shown in Table 8, all the diets contained sufficient amount of n-3 HUFA, the EFA for red seabream, derived from cuttlefish liver oil, krill oil and white fish meal or cuttlefish meal to satisfy its requirement[8]; except for the corn oil diet (diet 12) which was rich in 18: 2n-6 and deficient in n-3 HUFA.

Investigation of Spawning and Evaluation of Egg Quality

The eggs produced naturally by female broodstock, given each test diet, were collected every day from 16: 00 to 09: 00 next morning during the experimental period until May 30 in both 6 t and 1 t tanks in the aquarium. The method of

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