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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

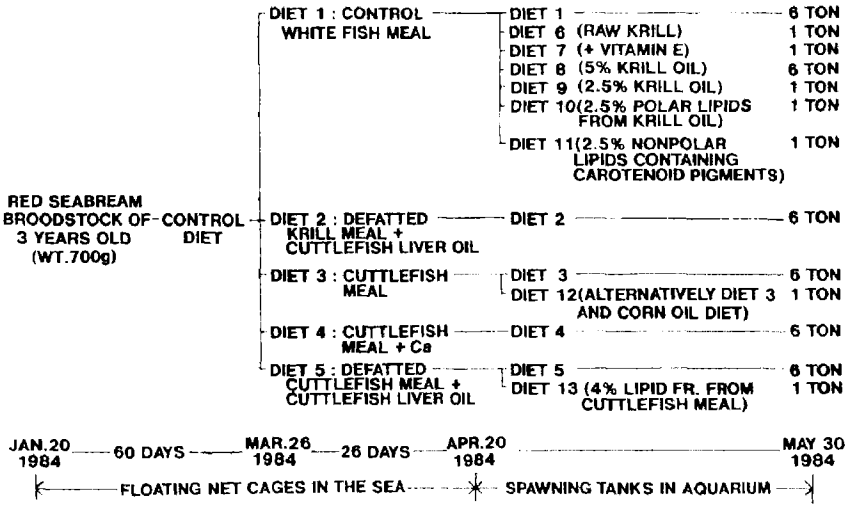

Fig. 1. The feeding schedule for red seabream broodstock.

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, phosphatidylcholine; 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

Table 6. Proximate and mineral compositions of the experimental diets for red seabream broodstock

Diet no.		1	2	3	4	5	6*	7	8	9	10	11	12	13
Moisture	(%)	7.1	8.6	10.5	10.7	7.8	4.0	11.8	12.0	10.6	7.6	8.0	8.1	7.1
Crude protein	(%)	44.2	46.2	44.9	44.7	46.6	54.1	43.2	44.8	44.8	43.5	44.2	44.6	44.3
Crude lipid	(%)	8.7	9.6	9.1	8.2	9.2	19.4	10.0	11.5	10.2	10.6	10.5	11.4	13.2
Crude ash	(%)	14.9	11.0	9.0	15.9	7.8	21.1	15.8	16.6	16.6	16.3	16.4	16.3	17.3
Ca	(mg/g)	30.8	14.3	5.4	30.4	6.1	20.2	37.9	40.1	39.1	31.5	32.1	31.7	31.9
P	(mg/g)	33.9	18.3	15.6	33.4	33.9	7.6	33.2	33.9	25.1	40.7	37.5	38.1	40.5
Mg	(mg/g)	2.3	4.9	3.0	3.1	2.9	4.4	2.2	2.5	2.4	2.5	2.4	2.4	2.5
K	(mg/g)	8.4	7.4	7.7	7.9	7.3	3.7	7.4	8.0	8.4	7.9	7.8	7.8	8.3
Na	(mg/g)	8.9	11.6	11.1	11.1	11.5	39.7	9.6	10.0	9.9	8.0	7.7	7.8	8.1
Fe	(μg/g)	122	143	127	133	191	tr	64	64	65	154	149	158	156
Zn	(μg/g)	46.2	45.9	49.3	52.6	45.7	43.1	40.2	41.3	36.1	46.9	46.4	55.0	46.7
Mn	(μg/g)	26.5	23.7	25.4	28.7	25.5	16.8	24.8	27.0	24.8	21.7	23.0	22.4	26.6
Cu	(μg/g)	7.9	52.3	35.7	36.1	30.9	17.4	7.4	10.5	10.2	7.1	8.3	6.8	8.2
Vitamin E (mg/100 g)		43.6	41.9	53.0	54.7	35.0	37.2	129.4	44.2	52.0	43.3	42.0	36.1	38.4

* The values of frozen raw krill are expressed on a dry basis.

Table 7. Lipid classes of the experimental diets for red seabream broodstock

Diet no.		1	2	3	4	5	6	7	8	9	10	11	12	13
Polar lipid	(%)	32.2	11.4	42.0	56.3	4.2	15.7	29.1	43.3	33.4	62.7	28.8	23.5	32.4
Nonpolar lipid	(%)	67.8	88.6	58.0	43.7	95.8	84.3	70.9	65.7	66.6	37.3	71.2	76.5	67.6
Cholesterol esters	(%)	1.3	0.8	0.9	0.6	1.1	1.0	0.8	0.6	0.7	0.7	0.8	0.7	1.1
Triglycerides	(%)	62.8	77.5	28.3	21.0	94.0	21.1	65.1	47.0	58.8	32.9	60.2	71.2	40.3
Free sterols	(%)	2.3	5.5	22.4	20.2	0.1	5.1	3.0	6.2	4.3	2.3	6.8	3.2	22.8

Table 8. Fatty acid compositions of the experimental diets for red seabream broodstock (area %)

Fatty acid	Diet no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
14:0	5	7.3	3.9	4.6	5.2	11.5	5.0	10.0	7.2	5.0	7.9	1.6	3.8
16:0	17.8	16.1	21.8	24.2	16.6	21.3	17.2	20.0	18.9	19.8	15.3	13.4	19.0
16:1	7.6	10.6	4.3	4.5	6.3	7.8	7.3	8.5	7.7	7.1	7.8	2.8	6.0
18:0	3.2	3.2	7.6	6.9	3.2	2.2	3.1	2.7	2.8	2.7	2.7	2.9	5.0
18:1	18.1	15.4	12.3	11.7	16.3	17.0	18.6	21.2	19.7	17.9	19.2	31.3	16.3
18:2n-6	1.7	6.2	2.8	1.9	2.0	2.5	1.2	1.7	1.4	1.4	1.8	29.5	1.8
18:3n-3	1.0	2.4	2.0	1.3	1.6	0.6	1.2	0.8	2.5	1.3	0.9	1.5	0.9
18:4n-3	1.6	3.5	1.6	1.2	2.4	2.5	1.7	2.4	2.3	2.0	4.4	0.4	1.0
20:1n-9	8.5	4.7	6.2	5.2	7.1	0.8	8.9	5.8	7.5	7.3	6.8	3.8	8.8
20:2n-6	0.2	—	0.3	0.3	0.2	0.1	0.2	0.1	0.3	--	0.1	0.1	0.2
20:3n-6	—	—	—	—	0.1	0.4	0.1	—	—	0.1	0.3	--	0.1
20:3n-3 + 20:4n-6	0.8	0.6	2.8	3.1	1.2	0.7	0.9	0.5	0.8	0.8	0.7	0.2	1.9
20:4n-3	0.7	—	0.4	0.5	0.9	0.6	0.7	0.3	0.4	0.6	0.6	0.1	0.4
20:5n-3	10.3	8.9	9.4	11.2	11.9	17.5	10.8	9.3	9.4	12.5	10.5	3.6	11.5
22:1	5.1	3.8	2.6	2.3	4.0	0.8	5.4	3.6	4.2	4.5	3.9	2.2	4.9
22:4n-6	0.1	—	0.2	0.2	0.1	0.1	0.1	—	—	0.1	--	--	0.1
22:5n-6	0.2	—	0.3	0.4	0.3	0.1	0.2	0.1	--	0.2	0.1	--	0.2
22:5n-3	0.9	—	0.7	0.8	0.9	0.4	0.9	0.4	0.7	0.7	0.8	0.3	0.9
22:6n-3	11.9	8.9	12.6	14.1	13.2	8.7	11.5	7.2	12.6	10.6	11.2	4.2	13.0
Sum of n-3HUFA	23.8	17.8	23.1	26.6	26.9	27.2	23.9	17.2	23.1	24.4	23.1	8.0	25.8
Lipid (%)	8.3	9.5	9.8	8.3	9.2	19.4*	10.0	11.5	10.2	10.6	10.5	11.4	13.2

* The value of frozen raw krill is expressed on a dry basis.

egg collection and quality evaluation by percentage of buoyant eggs (normal eggs floating on water surface), deposited eggs (abnormal eggs going down to the bottom of the tank), number of oil globules in an egg, rate of hatching and percentage of normal larvae hatched from buoyant eggs have all been described earlier.[1]

Analytical Methods

Both buoyant and deposited eggs produced by each experimental broodstock were washed with distilled water, stored at −20°C, water on the surface of eggs was wiped off with filter paper before analysis. Analytical procedures such as lipid extraction, separation of polar and nonpolar lipids, preparation of methyl esters and GLC operating conditions were all the same as reported in the previous papers.[9,10] Mineral distribution was determined by atomic absorption spectrometry,[11] and that of phosphorus by the method of Lowery and Lopez.[12] Lipids extracted from eggs and the test diets were separated into lipid classes and quantified by using an Iatroscan (Iatron TH-10).[2] Cholesterol and vitamin E (α-tocopherol) were measured using GLC,[13] and high speed liquid chromatography,[14] respectively.

Results and Discussion

Results of Spawning and Egg Quality

The size and number of broodstock used for spawning is shown in Table 9 and the results of natural spawning by these female broodstock fed different diets containing various fractions of cuttlefish meal and krill meal are shown in Table 10 and Fig. 2. As indicated therein the average number of eggs produced by one female during the experimental period ranged from 22.7×10^4 to 110.9×10^4, the lowest in the broodstock fed diet 10 containing 2.5% krill polar lipid and the higherst in those on diet 3 with cuttlefish meal as a protein source. These numbers of eggs produced were quite lower than those obtained in

Table 9. Average body length (mm), body weight (g) and number of red seabream broodstock used for spawning

Diet no.	Sex	Initial		Final		Number
		Body length	Body weight	Body length	Body weight	
1	Male	282.1±24.8*	771.4±243.5	296.3±52.2	884.3±443.3	14
	Female	285.1±21.4	794.3±162.6	278.7±13.6	769.7± 93.6	14
2	Male	286.8±14.7	817.1±132.6	275.7± 6.5	687.7± 19.9	14
	Female	286.5±18.5	802.7±151.9	290.0±36.4	869.0±311.0	13
3	Male	282.1±19.4	810.0±119.1	285.7±18.1	802.0±183.8	12
	Female	287.9±21.2	820.9±173.1	276.7±15.5	721.3±147.6	11
4	Male	284.7±23.8	791.3±202.3	227.3± 4.6	657.0± 67.6	16
	Female	296.6±18.7	857.9±114.0	295.0±24.3	794.0±115.9	14
5	Male	289.3±18.1	816.2±166.4	300.3±22.9	855.0±194.1	13
	Female	282.9±20.5	780.0±153.9	284.5± 9.2	795.0±183.8	12
6	Male	299.7±10.3	956.7±156.3	302.3±16.6	926.7±184.3	3
	Female	292.0± 7.8	783.3± 9.4	293.7±11.7	832.3± 21.1	3
7	Male	290.3±22.9	816.7±167.8	268.0±32.0	755.0±226.3	3
	Female	284.0±11.2	753.3± 62.4	276.7±14.0	699.7± 72.1	3
8	Male	274.4±22.0	711.4±173.4	272.7±22.9	678.0±223.1	14
	Female	283.9±23.1	763.6±175.4	287.0±13.1	740.3±147.8	14
9	Male	283.0±29.4	776.7±197.5	280.3±35.5	679.0±217.7	3
	Female	282.0± 7.8	773.3± 45.0	279.7± 9.0	724.0± 57.8	3
10	Male	286.7±18.9	726.7±140.6	298.0± 3.5	783.3± 66.6	3
	Female	298.3±19.9	853.3±134.7	280.0±46.7	805.0±360.6	3
11	Male	273.3±18.4	703.3±110.9	277.7±20.6	638.3±100.6	3
	Female	287.3±11.5	763.3±110.9	292.0±10.8	775.3±135.1	3
12	Male	309.7±25.9	963.3±295.8	339.3±32.9	878.3±341.0	3
	Female	295.7±15.4	870.0± 58.9	295.0±19.7	808.3±103.2	3
13	Male	310.3±17.2	960.0±129.3	312.0±22.5	898.0±175.1	3
	Female	287.5± 3.5	740.0± 20.0	306.7±34.4	982.2±366.1	3

* Mean±S.D.

Table 10. Effect of broodstock diets on the spawning and egg quality of red sea bream

	Diet no.												
	1	2	3	4	5	6*1	7*1	8*1	9*1	10*1	11*1	12*2	13*3
	Control	Defatted Krill meal	Cuttle-fish meal	Cuttle-fish meal +Ca	Defatted cuttle-fish meal	Frozen raw krill	VE 200 mg /100 g diet	5.0% Krill oil	2.5% Krill oil	2.5% Krill PL	2.5% Krill NL	Diet 3 or Corn oil	Cuttle-fish meal oil
Egg produced/fish ($\times 10^{-4}$)	63.1	42.9	110.9	66.0	30.2	71.3	79.0	32.9	63.5	22.7	31.1	79.0	105.5
Buoyant eggs/fish ($\times 10^{-4}$)	46.6	36.0	109.1	65.5	30.1	67.6	50.9	31.3	50.9	20.7	26.1	62.1	54.1
Rate of buoyant egg (%)	65.6	69.2	99.3	99.5	99.8	96.5	95.1	97.1	95.3	96.7	98.0	72.1	51.3
Egg diameter (mm)	0.88	0.87	0.86	0.86	0.85	0.89	0.86	0.89	0.87	0.89	0.88	0.86	0.85
Abnormal egg*4 (%)	46.7	48.5	14.5	18.5	8.2	14.2	37.7	59.2	54.9	29.6	42.9	31.4	19.7
Av. number of oil globules	1.62	1.67	1.21	1.26	1.13	1.22	1.50	1.80	1.66	1.45	1.66	1.43	1.24
Hatched larvae													
Rate of hatching (%)	57.1	59.7	84.5	82.5	82.0	83.1	77.8	83.4	78.0	80.8	91.9	70.6	87.3
Normal larvae obtained from buoyant egg (%)	89.5	92.3	97.8	98.8	98.8	98.0	97.1	92.8	98.1	92.3	89.8	94.7	87.2
Abnormality in Position*5 (%)	3.4	3.6	1.5	0.5	0.5	2.6	2.4	4.2	1.5	3.7	9.0	2.9	2.7
oil globule Number*4 (%)	7.5	5.5	0.8	0.9	1.0	0.3	1.7	3.7	0.3	0.4	2.1	3.0	8.8
Rate of normal larvae obtained (%)	33.4	41.4	80.7	79.4	79.1	75.0	72.4	78.4	74.2	76.4	83.0	48.2	41.2

*1 Separated from diet 1 just before spawning.
*2 Separated from diet 3 just before spawning.
*3 Separated from diet 5 just before spawning.
*4 Eggs or larvae with more than two oil globules.
*5 Larvae with oil globules at an abnormal position.

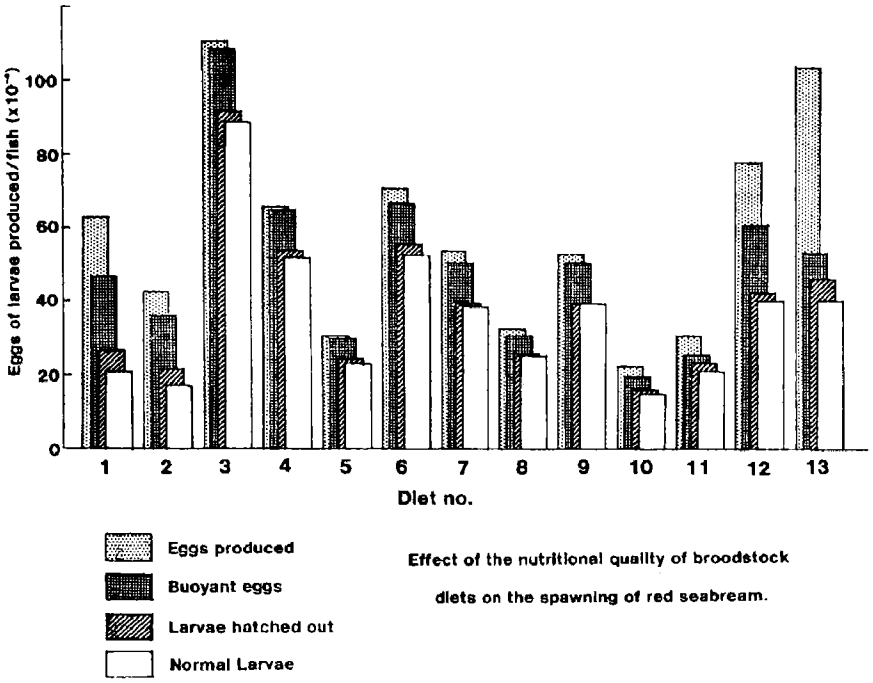

Fig. 2. Effect of the nutritional quality of broodstock diets on the spawning of red seabream.

the previous experiments due to a shorter spawning period of 21–26 days. However, these values are doubtful because number of female broodstock in each test lot which spawned could not be detected. The percentage of buoyant eggs is very important for evaluation of egg quality as described previously.[1] When the results were compared, among the broodstock fed on diets 1 to 5 for the same period from March 26 to May 30 in 6 t tanks, the percentage of buoyant eggs was lowest in the group on the control diet (diet 1). The replacement of white fish meal by defatted krill meal was not effective in improving egg quality, although the value of 65.6% in the control group was slightly elevated to 69.2%, suggesting that the non-fat-soluble fraction is not an effective component of raw krill on reproduction of red seabream. On the other hand, the value was almost 100% for the eggs produced by the broodstock fed on the diets containing defatted or in tact cuttlefish meal as a protein source. Supplementation of the cuttlefish meal diet with Ca at a level equivalent to the white fish meal diet exerted no ill effect on the percentage of buoyant eggs, suggesting that the difference of nutritional quality between white fish meal and cuttlefish meal is not due to a high Ca content in the former meal. The rate of hatching was also high in these eggs, the value being over 80% in comparison to less than 60% in the control group and those on the defatted krill meal diet. More than 97% of the hatched larvae were normal, resulting in high production of healthy seedlings. Thus the superior quality of cuttlefish meal to white fish meal as a protein source for red seabream broodstock diets was reconfirmed by this experiment.

The addition of the fat-soluble fraction of cuttlefish meal to the control diet (diet 13) resulted in no marked improvement in final productivity of normal larvae, nevertheless an increase from 33.4 to 41.2% was brought about by the treatment. This suggests that the high dietary value of cuttlefish meal is mainly due to the non-fat-soluble fraction in the meal. The high egg quality obtained in the group fed the cuttlefish meal deteriorated on feeding them the corn oil diet alternatively every three days (diet 12). The percentage of buoyant eggs was reduced from 99.3% to 72.1%, and the percentage of abnormal eggs with more than 2 oil globules especially increased when fed the corn oil diet. The productivity of normal larvae was better than that on the corn oil diet alone,[1,3,5] but lower than that on the cuttlefish meal diet. Thus quality of red seabream eggs was found to be greatly influenced by the quality of diets given to broodstock even during

spawning. The effectiveness of vitamin E on the reproduction of red seabream was confirmed again by this experiment. The addition of 200 mg of DL-α-tocopheryl acetate to the control diet elevated the rate of normal larvae production from 33.4% to 72.4%, although only about 130 mg of the vitamin was detected in the diet.

The results obtained in the broodstock fed diet 6 (raw krill) and diets 8–11, containing each fraction of raw krill clearly indicate effective components in krill for reproduction of red seabream. Feeding broodstock with frozen raw krill (diet 6) after previously being fed the control diet resulted in elevation of the percentage of buoyant eggs and their hatchability together with the rate of normal larvae, lead to a high productivity of seed available for mass propagation of juvenile fish. The productivity was elevated to 75.0% from 33.4% by feeding frozen raw krill. Equally good results, at times even better than forzen raw krill, were obtained by substitution of cuttlefish liver oil in the control diet with 5.0% (diet 8) or 2.5% (diet 9) of krill oil extract. These results, together with those obtained in the broodstock fed diet 2 containing the non-fat-soluble fraction of krill, indicate that the fat-soluble fractions are effective for reproduction of red seabream. The addition of 2.5% krill polar lipid or 2.5% nonpolar lipid containing astaxanthins to the control diet effectively improved egg quality in terms of percentage of buoyant eggs and hatchability.

The productivity of seed increased from the original value of 33.4% to 76.4% with polar lipid and 83.0% with nonpolar lipid. Thus, the effective components in raw krill, aiding the reproduction of red seabream, are suggested to be the polar and nonpolar lipid fractions. As shown in Table 1, the specific component is phosphatidyl choline in the polar lipid fraction and astaxanthins in the nonpolar lipid fraction. In addition, vitamin E was also found to have the same efficiency for imporvement of egg quality. These facts suggest that the seabream are common factors between phospholipids and astaxanthins or vitamin E such as free radical scavengers.

Further experiments will be necessary to reconfirm the effectiveness of these components on the reproduction of red seabream.

Chemical Components of the Eggs

Buoyant eggs produced on May 3, 5, 6, 8 and 10 by all the groups of broodstock, and the deposited eggs obtained on May 10, 13 and 15 from the broodstock fed on diets 1 and 2, those obtained on May 18, 20 and 22 from the fish fed on diet 12, and those on May 13, 15 and 17 from the broodstock fed on diet 13 were analysed for proximate, mineral and fatty acid compositions, lipid classes and vitamin E. The values are indicated by the average of five determinations.

Proximate composition of buoyant eggs and deposited eggs is shown in Table 11. There was no marked difference in proximate composition of buoyant eggs produced by each experimental broodstock as observed previously,[3] except for a slight decrease of crude ash content in the groups fed diets 6 (raw krill) and 7 (vitamin E). The crude ash content was higher in deposited eggs than buoyant eggs as noted in the previous experiment.[3] This may be caused by permeation of seawater into eggs due to the loss of membrane potential of the deposited eggs. This is also supported by mineral composition of deposited eggs which were high in Na, Mg, and Mn, rich in seawater (Table 12). The higher ash content reflected a lower protein content in the deposited

Table 11. Proximate composition of both the buoyant and deposited eggs from each experimental broodstock (%)

Diet no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Buoyant egg*1													
Moisture	90.3	90.3	90.4	90.5	90.3	90.6	90.2	90.3	90.4	90.3	90.8	90.7	90.4
Crude protein	5.2	5.2	5.1	5.2	5.2	5.1	5.4	5.1	5.3	5.2	5.1	5.0	5.1
Crude lipid	2.1	2.3	2.2	2.2	2.3	2.2	2.1	2.2	2.1	2.0	2.0	2.0	2.3
Crude ash	2.0	1.9	2.0	1.9	1.7	1.7	1.6	1.8	1.9	1.8	1.8	1.8	1.9
Deposited egg*2													
Moisture	91.0	90.9										91.1	90.4
Crude protein	3.9	3.7										3.4	4.5
Crude lipid	2.1	2.2										2.2	2.3
Crude ash	2.7	2.8										2.8	2.4

*1 The average values of five determinations on the eggs produced by broodstock in different days.
*2 The average values of three determinations on the eggs produced by broods tock in different days.

Table 12. Mineral compositions and the vitamin E content in both the buoyant and deposited eggs from each experimental broodstock

		Diet no.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Buoyant eggs*1														
Ca	(mg/g)	0.19	0.19	0.18	0.19	0.17	0.16	0.15	0.19	0.19	0.19	0.17	0.18	0.19
P	(mg/g)	0.93	0.99	0.94	0.90	1.03	0.93	0.93	0.93	0.97	0.95	0.86	0.97	0.96
Mg	(mg/g)	0.61	0.58	0.59	0.60	0.55	0.51	0.46	0.63	0.60	0.58	0.53	0.60	0.61
K	(mg/g)	1.38	1.53	1.45	1.48	1.66	1.78	1.60	1.45	1.60	1.57	1.45	1.71	1.50
Na	(mg/g)	3.48	3.87	3.46	4.09	1.80	3.17	2.77	4.26	3.63	3.65	3.33	3.36	3.80
Fe	(μg/g)	1.37	2.82	2.05	2.19	2.85	1.94	2.02	2.05	1.78	1.54	2.54	2.45	1.87
Zn	(μg/g)	8.17	8.54	8.02	7.70	8.31	8.12	7.50	7.68	7.57	7.31	7.27	7.68	7.91
Mn	(μg/g)	0.10	0.11	0.11	0.10	0.12	0.11	0.10	0.11	0.11	0.10	0.11	0.12	0.14
Cu	(μg/g)	0.45	0.32	0.36	0.31	0.31	0.37	0.30	0.32	0.35	0.26	0.32	0.35	0.41
Vitamin E	(μg/g)	13.3	15.1	12.8	8.5	11.4	12.0	15.9	10.5	8.5	8.6	12.4	8.3	13.3
Deposited egg*2														
Ca	(mg/g)	0.17	0.17										0.16	0.13
P	(mg/g)	0.60	0.55										0.47	0.83
Mg	(mg/g)	1.13	1.16										1.13	0.88
K	(mg/g)	0.65	0.54										0.46	1.25
Na	(mg/g)	9.18	9.73										9.33	7.74
Fe	(μg/g)	1.84	1.66										1.69	1.78
Zn	(μg/g)	7.63	7.25										6.29	7.37
Mn	(μg/g)	0.09	0.10										0.08	0.11
Cu	(μg/g)	0.37	0.33										0.22	0.29
Vitamin E	(μg/g)	10.1	13.8										8.2	9.4

*1 The average values of five determinations on the eggs produced by broodstock in different days.
*2 The average values of three determinations on the eggs produced by broodstock in different days.

eggs.

The mineral distribution of both buoyant and deposited eggs was little influenced by the mineral composition of diets as shown in Table 12; for the reasons described earlier. Supplementation of a high level of clacium phosphate to the cuttlefish meal diet resulted in no difference in the Ca and P contents compared to those on the same diet without the supplement.

The concentration of α-tocopherol in eggs is also shown in Table 12. Supplementation of the control diet with 200 mg of DL-α-tocopheryl acetate (determined value was 129.4 mg) greatly improved egg quality, and the amount was highest in the eggs produced by the broodstock fed the vitamin E fortified diet. However, the incorporation of the vitamin in the eggs through the broodstock diet was not so much enhanced by the supplement as that observed previously.[3] Further experiments will be needed to identify the function of vitamin E in reproduction and egg quality.

Proportion of lipid classes in eggs are presented in Table 13. The content of polar lipid ranged from 0.5 to 0.7% for both buoyant and deposited eggs, slightly lower in eggs from the broodstock receiving diets 6 (raw krill) and 10 (2.5% krill polar lipid). The cholesterol level was slightly higher in eggs from the broodstock fed diets containing intact cuttlefish meal and each a fraction of krill lipids.

The fatty acid composition of both buoyant and deposited eggs is shown in Table 14. The eggs produced by all dietary groups had almost the same fatty acid profiles except for diet 12 fed group. It was however noted that the concentration of n-3 HUFA was higher in the deposited eggs. In the eggs produced by the broodstock fed on the cuttlefish meal diet (diet 3) and the corn oil diet alternatively, the percentage of 18: 2n-6 was the highest and that of n-3 HUFA the lowest. The percentage was especially higher in the deposited eggs. Tables 15 and 16 show the changes in fatty acid distribution of both buoyant and deposited eggs obtained from the broodstock fed on both the diets during the period of spawning from May 5 to 25. The percentage of 18: 2n-6 was kept in low values of 4.4 to 5.1% for the first week after the initiation of feeding, and that of n-3 HUFA increased from the initial value of

22.1 to 26.0%. The former acid gradually accumulated and reached a plateau at 22.3% after 20 days feeding. The n-3 HUFA level was inversely proportional to the 18: 2n-6 level. These facts indicate that 18: 2n-6 is not utilized in red seabream eggs, and that accumulation of this fatty acid resulted in decrease in the n-3 HUFA level and increase of 18: 1 which is one of the characteristics for red seabream receiving an EFA-deficient diet. The broodstock had accepted almost the same amount of both the diets, however, corn oil rich in 18: 2n-6 seems to have a greater effect on the fatty acid composition than cuttlefish liver oil, leading to the production of

Table 13. Lipid classes of both the buoyant and deposited eggs from each experimental broodstock

Diet no.		1	2	3	4	5	6	7	8	9	10	11	12	13
Buoyant eggs*														
Polar lipid	(%)	33.7	29.9	25.1	31.4	30.2	23.9	25.7	28.9	30.5	26.9	27.8	29.3	29.2
Nonpolar lipid	(%)	66.3	70.1	79.4	68.6	69.8	76.1	74.3	71.1	69.5	73.1	72.2	70.7	70.8
Sterol esters	(%)	29.9	29.4	34.6	30.1	31.9	34.3	38.0	32.9	31.9	30.6	32.9	30.8	32.6
Triglycerides	(%)	30.3	31.8	31.5	30.4	33.3	31.3	29.9	29.9	27.1	29.2	27.2	29.5	29.1
Free sterols	(%)	2.8	3.3	3.7	3.1	2.2	2.4	3.9	3.0	3.5	3.9	3.7	3.2	2.8
Deposited eggs*														
Polar lipid	(%)	30.5	33.0										33.5	34.8
Nonpolar lipid	(%)	69.5	67.0										66.5	65.2
Sterol esters	(%)	30.5	33.0										27.0	30.7
Triglycerides	(%)	28.0	27.4										35.4	30.4
Free sterols	(%)	2.4	2.8										2.4	2.6

* See the footnote of Table 11.

Table 14. Fatty acid composition of both buoyant and deposited eggs from each experimental broodstock (area %)

Fatty acid	Buoyant egg*													Deposited egg*			
	Diet no.													Diet no.			
	1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	12	13
14: 0	4.0	3.8	3.9	4.1	3.8	5.3	3.7	3.9	3.5	3.4	3.7	3.0	3.9	3.8	3.8	2.7	3.6
16: 0	22.4	21.4	23.0	23.5	21.4	21.4	21.4	20.0	22.7	20.8	21.0	18.6	21.8	19.1	19.5	16.4	18.7
16: 1	11.1	9.8	9.8	10.0	9.1	9.9	10.5	9.5	9.9	8.8	9.9	6.9	8.6	8.7	8.2	5.8	8.6
18: 0	4.8	4.7	5.1	5.1	4.6	4.1	4.7	4.7	4.9	4.8	4.6	4.8	4.8	4.2	4.4	3.9	4.7
18: 1	22.5	21.9	22.1	21.5	21.0	19.9	22.3	21.7	22.7	20.8	22.1	26.8	21.3	23.5	20.5	27.9	22.2
18: 2n-6	4.1	3.9	5.0	4.1	4.2	2.5	4.0	4.2	4.8	4.1	4.0	12.8	4.0	2.7	2.6	20.2	3.2
18: 3n-3	0.6	0.9	0.8	0.7	0.8	0.7	0.6	0.7	0.6	0.6	0.7	0.8	0.7	0.9	1.0	0.9	0.7
18: 4n-3	0.4	1.0	0.6	0.6	0.7	0.7	0.6	0.8	0.6	0.6	0.8	0.5	0.8	0.8	1.3	0.4	0.6
20: 1	1.5	1.7	1.1	1.2	1.4	1.0	1.3	1.5	1.3	1.3	1.4	2.0	1.3	2.9	1.9	2.0	2.7
20: 2n-6	0.4	0.5	0.5	0.9	0.7	0.6	0.7	0.4	0.4	0.3	0.8	0.6	0.4	0.3	0.3	0.5	0.2
20: 3n-6	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
20: 3n-3 / 20: 4n-6	1.0	1.0	1.2	1.2	1.2	1.1	1.1	1.1	0.9	0.9	1.1	1.1	1.3	1.0	1.1	0.9	1.8
20: 4n-3	0.8	0.9	0.7	0.7	0.8	1.1	0.7	0.8	0.8	0.9	0.9	0.6	0.8	1.2	1.3	0.5	0.9
20: 5n-3	5.9	7.2	5.4	5.4	6.3	9.7	6.2	6.8	5.8	6.5	6.2	4.8	6.5	7.6	9.4	4.0	7.8
22: 4n-6	0.1	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.3	0.2	0.4	0.2	0.2	0.2	0.2	0.1	0.2
22: 5n-6	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.3	0.3	0.1	0.3
22: 5n-3	1.9	1.7	1.9	2.0	2.1	2.5	2.1	2.2	1.8	2.3	2.3	1.9	2.4	2.7	2.4	1.5	2.6
22: 6n-3	14.2	14.4	14.6	14.8	17.3	17.2	15.7	18.2	16.2	20.3	17.9	12.8	17.6	16.9	17.5	9.4	17.1
Sum of n-3 HUFA	22.0	25.1	22.6	22.9	26.5	30.5	24.7	28.0	24.4	29.9	26.2	20.1	27.3	28.4	30.7	15.4	28.7
Lipid (%)	2.1	2.3	2.2	2.3	2.3	2.2	2.1	2.2	2.1	2.0	2.0	2.0	2.3	2.2	2.2	2.3	2.3

* See the footnote of Table 11.

Table 15. Changes in fatty acid composition of total lipids from buoyant eggs produced by the red seabream broodstock fed the cuttlefish meal diet and the corn oil diet alternatively every three days (area %)

Fatty acid	May											
	3	5	6	8	10	16	17	18	20	21	22	25
14:0	4.6	4.1	3.8	3.8	3.6	3.1	2.8	2.2	2.2	2.0	2.1	2.1
16:0	23.2	22.8	22.1	20.3	20.8	18.7	19.8	16.9	15.3	14.5	15.1	15.2
16:1	10.6	9.2	10.7	9.0	9.0	6.4	6.5	5.5	5.2	5.2	5.1	5.0
18:0	5.0	5.0	5.1	4.7	4.9	5.3	5.7	4.9	4.2	4.4	3.9	4.5
18:1	22.1	21.1	21.8	21.4	22.5	23.9	27.4	26.0	27.9	28.8	29.1	27.9
18:2n-6	4.4	4.5	5.0	4.4	5.1	9.2	13.0	14.9	19.1	21.4	22.3	21.8
18:3n-3	0.7	0.6	0.7	0.7	0.7	1.0	0.9	0.9	0.8	0.8	1.0	0.8
18:4n-3	0.6	0.6	0.5	0.7	0.6	0.5	0.5	0.4	0.3	0.3	0.4	0.3
20:1n-9	1.3	1.2	1.2	1.4	1.6	2.3	2.5	2.2	2.3	2.4	2.3	2.3
20:2n-6	0.6	1.2	0.7	0.1	0.2	0.9	0.5	0.5	0.6	0.7	0.7	0.7
20:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
20:3n-3 / 20:4n-6	1.1	1.3	1.3	1.5	1.4	1.4	1.1	1.1	0.9	0.8	0.7	0.8
20:4n-3	0.8	0.7	0.8	0.8	0.8	0.7	0.6	0.6	0.5	0.5	0.5	0.5
20:5n-3	5.5	5.9	5.5	6.6	6.1	5.5	3.9	4.6	4.0	3.8	4.0	4.0
22:4n-6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1
22:5n-6	0.2	0.1	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.1
22:5n-3	2.4	2.0	2.0	2.2	2.1	2.2	1.7	1.9	1.7	1.7	1.5	1.6
22:6n-3	13.9	15.6	15.4	18.8	17.0	15.3	8.7	14.1	12.1	10.1	9.1	9.7
Sum of n-3 HUFA	22.1	24.2	23.7	28.4	26.0	23.7	14.9	21.2	18.3	16.1	15.1	15.8
Lipid (%)	2.4	1.9	1.8	2.1	2.0	2.1	2.0	2.1	2.0	1.9	2.1	2.1

Table 16. Changes in fatty acid composition of total lipid from depositted eggs produced by the red seabream broodstock fed the cuttlefish meal diet and the corn oil diet alternatively every three days (area %)

Fatty acid	May						
	16	18	20	21	22	24	25
14:0	4.2	3.2	2.6	2.1	2.4	2.2	2.4
16:0	20.9	17.0	15.6	14.8	15.1	14.9	16.2
16:1	8.0	6.0	5.4	5.0	5.5	5.1	5.4
18:0	5.1	3.4	3.8	4.2	3.2	4.2	3.5
18:1	24.5	26.0	28.0	29.6	28.8	29.3	29.0
18:2n-6	11.4	17.9	22.8	22.4	23.6	22.7	20.4
18:3n-3	0.9	0.8	0.9	0.9	1.0	0.9	1.0
18:3n-3	0.5	0.4	0.5	0.4	0.4	0.4	0.4
20:1n-9	1.9	1.6	1.8	2.4	1.8	2.4	2.3
20:2n-6	0.3	0.4	0.5	0.7	0.5	0.6	0.6
20:3n-6	0.3	—	—	0.1	—	0.1	0.1
20:3n-6 / 20:4n-6	1.3	1.1	0.9	0.8	0.7	0.7	0.8
20:4n-3	0.6	0.6	0.5	0.5	0.5	0.5	0.5
20:5n-6	4.8	4.9	3.8	3.5	3.9	3.5	3.7
22:4n-6	0.2	0.2	0.1	0.1	0.1	0.1	0.1
22:5n-6	0.2	0.2	0.2	0.1	0.1	0.1	0.1
22:5n-6	1.7	1.7	1.4	1.5	1.4	1.5	1.6
22:6n-3	10.2	11.9	8.2	8.4	8.7	8.6	9.5
Sum of n-3 HUFA	17.3	19.1	13.9	13.9	14.5	14.1	15.3
Lipid (%)	2.1	2.1	2.5	2.2	2.3	2.1	1.9

medium quality eggs. No elevation of 20: 4n-6 and 22: 5n-6 was observed in these eggs, suggesting no conversion of 18: 2n-6 to these n-6 fatty acids in red seabream. The same tendency was observed in the deposited eggs.

These results briefly outline the dietary role in egg quality, an aspect warranting further research.

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