マダイ卵の化学組成に及ぼす飼料のタンパク質含量および産卵前給餌期間の影響

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Effect of Dietary Protein Levels and Feeding Period before Spawning on Chemical Components of Eggs Produced by Red Sea Bream Broodstock*1

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Both buoyant (normal eggs floating on water surface) and deposited (abnormal eggs that sink to the bottom of tanks) eggs produced by broodstock of different dietary histories were analysed for lipid class, fatty acids, vitamins A and E, cholesterol, minerals, and proximate composition. The dietary treatments were diets containing different levels of protein which were fed for 6 months, or broodstock fed diet supplemented with pigments and fat-soluble vitamins or frozen raw krill shortly before spawning.

There were no marked differences in proximate and mineral composition due to the different broodstock diets, although the concentration of protein in the buoyant eggs was proportional to dietary protein levels used, while moisture content varied inversely. The fatty acids in eggs were greatly affected by dietary fatty acids supplied to broodstock either shortly before their spawning or even during spawning. In the eggs from the broodstock fed on corn oil diet immediately, and prior to spawning, the percentage of 18:2ω6 was as high as 26% in comparison with the original value. Vitamin E was also found to be easily incorporated in the eggs together with lipids via the diet. The level of cholesterol in eggs was almost constant irrespective of the cholesterol levels in the diets.

Thus no marked differences that might related to egg quality were observed in chemical components in either buoyant or deposited eggs among the experimental groups, although significant differences occurred between the buoyant and deposited eggs.

In the previous studies[1,2] of this series[1-4] a relatively long-term feeding experiment was conducted in order to investigate the effect of protein level in diet of red sea bream broodstock on reproduction, and a relatively short-term experiment was carried out to examine the effect of nutritional quality of diets given to broodstock shortly before spawning. The results obtained have demonstrated that an optimal protein level in diet for broodstock is about 45% and that the percentage of buoyant eggs was improved by supplementing with pigments such as β-carotene and canthaxanthin in the diets given to broodstock on the eve of spawning. Feeding frozen raw krill shortly before spawning also resulted in marked improvement in both the total eggs produced and percentage of buoyant eggs. On the other hand, in the group given the corn oil diet on the verge of or during spawning the proportion of buoyant eggs was markedly reduced from the original value by replacement of cuttlefish liver oil with corn oil. Thus the reproduction and egg quality of red sea bream were found to be deeply affected by the nutritional quality of diet given to broodstock even shortly before or during spawning. These results also suggested that not only quality of eggs, but also their chemical components are influenced by the nutritional composition of diets given to broodstock for a short period before spawning and during spawning. It was also demonstrated in the previous experiment[2] that the nutritional composition of diets exerts effects on the chemical components of eggs of red sea bream for 6 months before their spawning in the same

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manner as rainbow trout.[5,6] Among chemical components of eggs most remarkably affected by diets are fat-soluble materials.

Following the previous experiment[2] this study was conducted to obtain some basic data necessary for biochemical evaluation of egg quality by investigating a relationship between composition of diets given to broodstock and chemical components of eggs produced. For this purpose eggs, both buoyant and deposited, produced by broodstock, which had been fed the diets containing different levels of protein for 6 months and those produced by broodstock fed on various other diets shortly before spawning, as described in the preceding paper,[4] were analysed for proximate composition, fatty acids, minerals, and fat-soluble vitamins.

This paper deals with these results.

Materials and Methods

Eggs, both buoyant and deposited, were collected from broodstock which had been kept on experimental diets containing different levels of protein (Table 1) for 6 months from October 8, 1980 to April 14, 1981 in floating net cages according to the food schedule shown in Fig. 1, as described in the preceeding paper[4] (Experiment I). The broodstock which had been fed the high-protein diet (diet 4) were divided into 3 lots each of 3 males and 3 females and the broodstock which had been fed on the diet containing both white fish meal and cuttlefish meal as protein source (diet 5) were divided into 1 lot of 3 males and 3 females and transported to the spawning tanks inland. These broodstock were kept in a 1 t tank and then fed on other experimental diets as shown in Table 2 for 2–3 months just before their spawning and as long as spawning continued (Experiment II). Eggs spawned by these broodstock in both experiments I and II on April 25, May 16, 19, 20, 27 and 28, and buoyant eggs on June 3 and 4 were used for analyses of proximate, mineral and fatty acid compositions together with phospholipids, cholesterol, and vitamins A and E.

Eggs were washed with distilled water, stored at −20°C, and water on the surface of eggs was wiped out with filter paper before analyses. The analytical procedures such as lipid extraction, separation of polar and nonpolar lipids with gel column, preparation of methyl esters, GLC operating conditions and mineral composition were all as reported in the previous papers.[7,8] Polar lipid classes were resolved and quantified by an Iatroscan (Iatron TH-10). A solvent mixture of chloroform: acetone: methanol: acetic acid:

Table 1. Compositions of the experimental diets for red sea bream broodstock

(%)

Ingredient	Diet no.						
	1	2	3	4	5	6	7
Fish meal*1	52	67	67	84	34	—	
Cuttlefish meal*2	—	—	—	—	31	61	
α-Starch	15	15	15	7	15	15	Frozen
Mineral mix.*3	5	5	5*4	5	5	5	
Vitamin mix.	2	2	2	2	2	2	sardine
Choline chloride	1	1	1	1	1	1	
Cuttlefish oil*5	5.5	4	4	3	3	2	
Cellulose	19.5	6	6	0	9	14	
Nutrient content calculated							
Crude protein	35	45	45	55	45	45	—
Crude lipid	10	10	10	10	10	10	
Nutrient content determined							
Crude protein	33.4	43.1	43.9	52.1	43.6	43.0	—
Crude lipid	9.3	9.4	9.5	9.5	9.0	9.1	—
Gross energy (kcal/100 g)	416	414	437	419	420	421	—
P % in diet	2.4	2.7	2.1	3.1	2.0	1.4	

*1 Crude protein: 66.9%, Crude lipid: 8.8%, Σω3HUFA: 24.1%.
*2 Crude protein: 73.6%, Crude lipid: 13.6%, Σω3HUFA: 33.6%.
*3 Ogino salt mixture (Table 3).
*4 Phosphorus-deficient salt mixture.
*5 VE 1%.

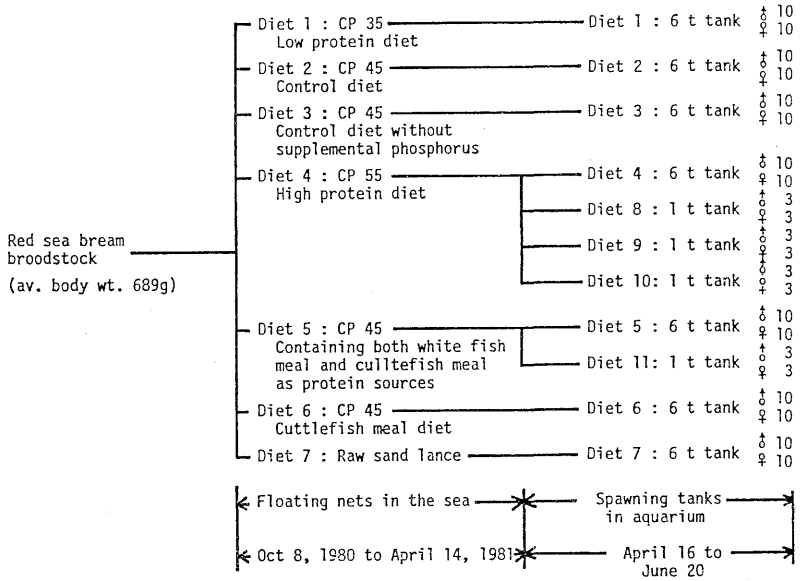

Fig. 1. Food schedule for red sea bream broodstock before their spawning and during spawning.

Table 2. Compositions of the experimental diets given to red sea bream broodstock just before the spawning

(%)

Ingredient	Diet no. 8*1	9*1	10*1	11*2
Fish meal*3	82	74.5		67
α-Starch	7	9		15
Mineral mix.*3	5	4.5		5
Vitamin mix.	2	1.8*5		2
Choline chloride	1	0.9		1
Cuttlefish oil*3	3	—		—
Oil extracts from krill*4	—	9		—
Corn oil	—	—		10
β-Carotene*6	0.1	—		—
Canthaxanthin*7	0.3	—		—
Nutrient content calculated				
Crude protein	55	51	—	45
Crude lipid	10	17	—	16
Nutrient content determined				
Crude protein	53.0	50.1	—	45.7
Crude lipid	9.9	14.9	—	16.6

Frozen raw krill

*1 Previously given diet 4.
*2 Previously given diet 5.
*3 See the footnote of Table 1.
*4 See table 3.
*5 VA and VD_3: 10^4 IU/100 g diet, VE: 200 mg/100 g diet.
*6 30% oil.
*7 10% purity.

water (30: 10: 7: 2: 1) or chloroform: methanol: acetic acid: water (20: 20: 4: 2) was used as a developer for chromatorods. Cholesterol was determined by the method of KANEDA *et al.*[9]

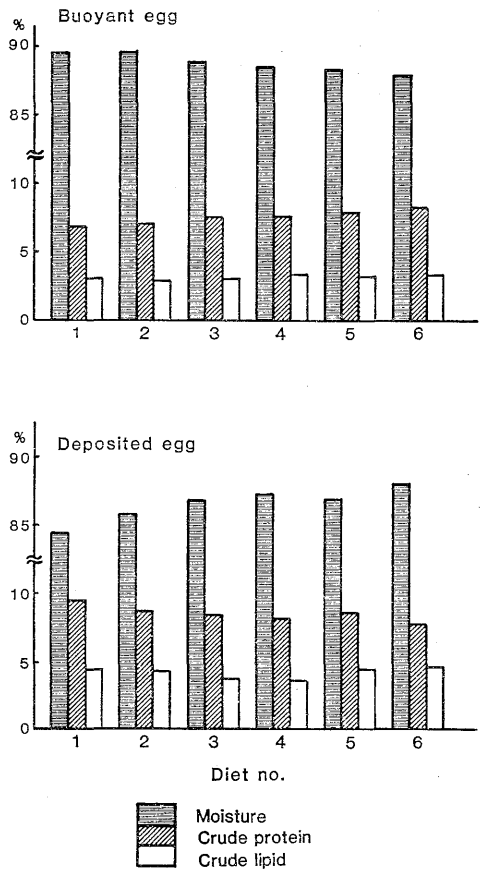

Fig. 2. Proximate compositions of eggs produced by each experimental group.

Table 3. Proximate and mineral compositions of eggs from

Diet no.		1	2	3	4	5
Bouyant egg						
Moisture	(%)	89.6±1.47*1	89.7±1.77*1	88.9±2.12*1	88.6±2.01*1	88.3±2.29*1
Crude protein	(%)	6.8±0.78	7.1±0.75	7.5±1.29	7.7±1.36	7.9±1.39
Crude lipid	(%)	3.0±0.53	2.9±0.43	3.1±0.70	3.3±0.84	3.2±0.55
Crude ash	(%)	0.6±0.06	0.6±0.12	0.6±0.17	0.5±0.09	0.6±0.23
K	(mg/g)	2.09±0.29	2.21±0.47	1.98±0.54	1.67±0.31	2.02±0.46
Na	(mg/g)	0.42±0.16	0.38±0.11	0.30±0.09	0.32±0.08	0.39±0.10
Ca	(mg/g)	0.05±0.02	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01
Mg	(mg/g)	0.09±0.02	0.09±0.02	0.07±0.02	0.09±0.01	0.09±0.02
P	(mg/g)	1.21±0.14	1.09±0.18	1.18±0.14	1.19±0.25	1.35±0.15
Deposited egg						
Moisture	(%)	84.6±3.06*2	85.8±2.66*2	86.9±0.74*2	87.3±1.90*2	86.9±1.06*4
Crude protein	(%)	9.6±1.82	8.8±1.69	8.4±0.33	8.2±0.84	8.7±0.62
Crude lipid	(%)	4.5±0.71	4.4±0.75	3.8±0.59	3.7±0.49	4.5±1.29**2
Crude ash	(%)	0.7±0.15	0.6±0.15	0.6±0.10	0.6±0.12	0.5±0.02
K	(mg/g)	0.67±0.27	0.51±0.33	0.78±0.38	1.14±0.33	0.75±0.38
Na	(mg/g)	1.04±0.81	0.86±0.86	0.83±0.35	0.75±0.25	0.43±0.25
Ca	(mg/g)	0.25±0.04	0.27±0.06	0.16±0.06	0.21±0.10	0.13±0.07
Mg	(mg/g)	0.41±0.08	0.31±0.09	0.31±0.06	0.22±0.05	0.18±0.07
P	(mg/g)	1.37±0.33	1.15±0.18	1.20±0.07	1.20±0.17	1.02±0.25

*1 Mean±SD, n=5. *2n=4. *3 n=3. *4 n=2. ** Only lipid.

using GLC. Quantitative determination of α-tocopherol and vitamin A was carried out by using high-speed liquid chromatography (HSLC). HSLC operating conditions are described in the previous paper.[10]

Results and Discussion

Proximate and Mineral Compositions of Eggs Produced

1. Experiment I Both buoyant and deposited eggs produced by each group of experimental broodstock fed on the diets containing different levels of protein or the diet containing white fish meal or cuttlefish meal as a protein source for 6 months before spawning were analysed for proximate and mineral composition separately and the average values of each determination are summarized in Table 3 and Fig. 2.

The concentration of crude protein in the buoyant eggs was found to be proportional to dietary protein levels, being highest in the eggs produced by the broodstock receiving the high-protein diet (diet 4, CP 55%); moisture content, on the other hand varied inversely with dietary protein level, as already observed in the previous experiment.[2] When compared at the same dietary protein level (CP 45%), the protein content of eggs correlated with the proportion of cuttlefish meal in the diet, being highest in the eggs from the broodstock fed on the diet containing cuttlefish meal as a sole protein source (diet 6).

The analytical levels of macroelements in the buoyant eggs showed no marked differences in distribution, even the phosphorus content of the eggs produced by the given phosphorus deficient diets broodstock as shown in the previous experiment.[2]

On the other hand, in the deposited eggs, abnormal eggs which sank to the bottom of tanks, from each experimental group a reverse trend was observed in the proximate composition. The concentration of protein and lipid in the eggs was higher and that of moisture lower in the low-protein groups. When the mineral distribution of buoyant and deposited eggs is compared, the concentration of K was characteristically higher in the former eggs and that of Na, Ca and Mg was higher in the latter eggs from each experimental broodstock, suggesting a reduction of ability to control permeability of egg membrane in the deposited eggs. There was also a difference in the content of P between both eggs.

Thus some marked differences were observed in the proximate and mineral compositions between the buoyant and deposited eggs, although no significant difference was detected among the buoyant eggs produced by different groups of

red sea bream fed respectively different diets

6	7	8	9	10	11
87.9±2.08*1	88.9±1.19*1	91.5±1.31*2	89.9±2.43*2	89.9±1.08*2	90.4
8.2±1.24	7.5±0.62	5.9±0.62	6.5±1.89	6.8±0.75	5.8
3.3±0.52	3.0±0.27	2.7±0.62	2.7±0.34	3.0±0.23	3.2
0.6±0.09	0.6±0.13	0.6±0.05	0.5±0.18	0.5±0.12	0.1
1.87±0.39	1.79±0.46	1.50±0.21	1.32±0.37	1.41±0.27	0.40
0.37±0.09	0.40±0.11	0.74±0.39	0.42±0.11	0.39±0.17	0.87
0.04±0.01	0.04±0.01	0.04±0.00	0.04±0.01	0.04±0.01	0.05
0.11±0.02	0.10±0.01	0.14±0.05	0.10±0.02	0.11±0.02	0.14
1.40±0.31	1.14±0.12	0.94±0.10	0.95±0.18	1.10±0.16	1.00
88.1	87.7±0.83*3	85.7±5.52*4	—	87.7±0.64*4	—
7.8	7.7±0.78	9.5±4.38	—	6.9±0.40	—
4.7±1.61**2	3.2±0.58**2	3.4±0.21**3	5.1±1.72**3	3.4±0.25**3	—
0.6	0.7±0.12	0.7±0.07	—	0.7±0.14	—
1.31	0.85±0.20	0.86±0.54	—	0.94±0.43	—
0.94	0.83±0.34	1.47±0.03	—	1.41±0.06	—
0.08	0.51±0.42	0.20±0.04	—	0.41±0.34	—
0.28	0.46±0.37	0.38±0.07	—	0.41±0.11	—
1.14	1.13±0.09	0.89±0.00	—	0.95±0.08	—

Table 4.*1 Lipid class in egg lipids from parent red sea bream fed respectively different diets

Diet no.	Crude lipid	In egg (%)					In egg lipid (%)				
		Polar lipid	Non-polar	Polar lipid			Polar lipid	Non-polar	Polar lipid		
				PE*2	PC*2	Others			PE*2	PC*2	Others
Bouyant egg											
1	3.3	0.84	2.74	0.06	0.56	0.23	25.38	74.62	1.81	16.90	6.95
2	3.1	0.70	2.40	0.05	0.46	0.20	22.58	77.42	1.61	14.84	6.45
3	3.0	0.77	2.24	0.06	0.45	0.26	25.58	74.42	1.99	14.95	8.64
4	2.6	0.69	1.91	0.05	0.43	0.20	26.54	73.46	1.92	16.54	7.69
5	3.4	0.90	2.55	0.06	0.52	0.27	26.09	72.17	1.74	18.55	8.99
6	3.5	1.01	2.49	0.06	0.64	0.31	28.86	71.14	1.71	18.29	8.86
7	3.1	0.94	2.16	0.10	0.57	0.27	30.32	69.68	3.23	18.39	8.71
9	2.8	0.78	2.02	0.04	0.46	0.28	27.86	72.14	1.43	16.43	10.00
10	2.8	0.73	2.07	0.11	0.41	0.29	26.07	73.93	3.93	14.64	10.36
Deposited egg											
1	4.6	1.47	3.12	0.09	1.25	0.14	32.03	67.97	1.96	27.23	3.05
2	4.8	1.51	3.29	0.07	1.36	0.08	31.46	68.54	1.46	28.33	1.67
3	4.2	1.29	2.91	0.07	1.14	0.08	30.71	69.29	1.67	27.14	1.90
4	3.7	1.14	2.56	0.10	0.98	0.05	30.81	69.19	2.70	26.49	1.35
5	4.0	1.18	2.82	0.09	1.04	0.06	29.50	70.50	2.25	26.00	1.50
6	5.4	1.61	3.79	0.13	1.37	0.11	29.81	70.19	2.41	25.37	2.07
7	3.0	0.97	2.03	0.07	0.77	0.13	32.33	67.67	2.33	25.67	4.33
8	3.4	1.11	2.30	0.08	0.98	0.04	32.55	67.45	2.35	28.74	1.17
9	4.4	1.16	3.24	0.04	1.02	0.10	26.36	73.64	0.91	23.18	2.27
10	3.4	1.19	2.21	0.06	1.12	0.02	35.00	65.00	1.76	32.94	0.59

*1 Average values of five determinations on the eggs obtained in different days.
*2 PE: Phosphatidyl ethanolamine, PC: Phosphatidyl choline.

broodstock due to the difference of egg quality as described previously.[2] A difference between buoyant and deposited eggs was also reported by TAGUCHI *et al.*[*1] that ATPase activity was higher in the deposited eggs than the buoyant eggs, resulting in a higher concentration of inorganic phosphorus in the former. Furthermore, a marked difference in the concentration of free amino acids was observed between both the eggs.[*2]

2. Experiment II In the eggs of the broodstock fed diets 8, 9 and 10 (Table 2) shortly before spawning and after being separated from the original broodstock which had been fed diet 4 the content of protein was reduced and that of moisture elevated from the original values (Table 3), although quality of eggs was found to be improved by these treatments on the verge of spawning.[2] The concentration of protein together with ash was lowest in the eggs from the broodstock fed on the diet containing corn oil shortly before spawning or during spawning; they were previously part of the diet 5 group. The same tendency as in Experiment I was observed in proximate and mineral composition between buoyant and deposited eggs, except for a slightly lower concentration of phosphorus in the deposited eggs.

Lipid Class in Eggs Produced

The proportion of polar to nonpolar lipids and the percentage of phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) in both the buoyant and deposited eggs obtained from each experimental group, are summarized in Table 4. No data are available for diet 11 as insufficient eggs were produced.

The concentration of polar lipids in both the buoyant and deposited eggs was almost constant, being little affected by dietary components, and fluctuation of the content of total lipids in eggs was mainly due to nonpolar lipids, as observed in the eggs of red sea bream[2] and rainbow trout[6] and other tissues of rainbow trout.[11] Polar lipid level ranged from 0.7 to 1.0% in the buoyant eggs, slightly lower than those determined before,[2]

Table 5.[*1] Certain fatty acids of total lipid in buoyant eggs obtained from red sea bream broodstock fed respectively different diet

Fatty acid	Diet no.										
	1	2	3	4	5	6	7	8	9	10	11
16:0	19.8	20.9	20.6	20.7	22.1	22.5	21.4	23.3	23.8	22.6	17.4
16:1	9.3	9.0	9.3	9.8	9.0	9.8	10.6	8.9	9.8	10.8	5.9
18:0	5.4	5.3	5.1	5.1	5.2	5.7	5.7	4.9	4.8	4.9	3.8
18:1ω9	23.6	23.9	24.2	24.7	22.2	22.8	18.0	22.7	20.7	22.8	25.5
18:2ω6	3.4	3.4	2.6	2.8	2.6	3.1	2.8	3.4	2.7	2.2	22.1
18:3ω3	0.7	0.7	0.6	0.7	0.7	0.7	0.8	0.6	0.5	0.4	0.5
18:4ω3	0.5	0.6	0.7	0.7	0.5	0.2	0.6	0.4	0.5	0.4	0.3
20:1	2.6	2.8	2.9	2.9	2.1	1.6	0.8	1.8	1.6	1.0	1.0
20:3ω3 20:4ω6	1.6	1.4	1.4	1.2	0.7	2.5	1.9	1.6	1.2	1.3	1.1
20:4ω3	0.7	0.8	0.7	0.8	0.7	0.5	0.7	0.6	0.8	0.8	0.4
20:5ω3	6.1	6.4	6.7	6.4	6.7	5.5	6.4	4.6	9.9	7.9	3.7
22:1	0.5	0.5	0.6	0.7	0.5	0.4	0.2	0.3	0.4	0.4	0.3
22:5ω3	2.1	2.1	2.2	2.2	2.1	2.0	2.1	2.0	2.2	1.9	1.1
22:6ω3	16.1	15.6	15.5	13.5	16.2	14.3	17.1	14.4	12.4	14.2	11.9
$\Sigma\omega$3 HUFA[*2]	25.0	24.9	25.1	22.9	25.7	22.3	28.1	25.7	25.6	24.6	17.0
Saturated	28.5	29.7	29.4	29.8	31.1	32.4	30.9	31.6	33.9	33.2	22.8
Monoene	36.9	37.0	37.8	38.9	34.5	35.4	30.5	34.7	33.3	35.8	32.7
Polyene	32.6	32.3	31.8	29.8	31.8	30.8	34.4	30.0	30.8	30.1	41.1

[*1] Average values of four determinations on the eggs obtained in different days.
[*2] $C_{20:3}<\omega3$ fatty acids.

[*1] T. TAGUCHI, S. SATOH, and T. WATANABE: Oral presentation at the annual meeting of Japan. Soc. Sci. Fish., on October 3, 1982.
[*2] T. SUZUKI, T. YANAI, and M. SUYAMA: Oral presentation at the annual meeting of Japan. Soc. Sci. Fish., on April 3, 1981.

Table 6.[*1] Certain fatty acids[*1] of total lipids in deposited eggs obtained from red sea bream broodstock fed respectively different diets

Fatty acid	Diet no.									
	1	2	3	4	5	6	7	8	9	10
16:0	19.9	20.5	21.0	20.6	22.6	21.5	22.1	24.3	23.4	23.4
16:1	8.5	8.5	9.1	9.7	9.2	9.7	11.3	9.8	9.4	11.1
18:0	5.2	5.4	5.3	4.8	5.4	5.1	5.4	4.6	4.0	4.5
18:1ω9	23.5	25.1	25.1	24.9	23.6	22.9	18.5	23.2	21.5	22.6
18:2ω6	3.3	3.3	3.1	2.8	2.6	2.9	2.6	2.6	2.3	1.8
18:3ω3	0.7	0.6	0.7	0.7	0.7	0.7	0.8	0.6	0.5	0.4
18:4ω3	0.6	0.7	0.7	0.7	0.5	0.3	0.7	0.5	0.6	0.5
20:1	2.9	3.2	3.1	2.9	2.4	1.8	0.9	2.7	3.4	1.0
20:3ω3 } 20:4ω6 }	1.6	1.4	1.3	1.3	1.9	2.5	1.8	1.4	1.3	1.2
20:4ω3	0.7	0.7	0.7	0.8	0.7	0.5	0.8	0.6	0.5	0.5
20:5ω3	6.3	6.2	6.0	6.4	6.3	5.9	6.7	6.5	7.5	8.0
22:1	0.5	0.6	0.4	0.5	0.4	0.4	0.2	0.6	1.4	tr
22:5ω3	2.2	2.1	2.0	2.2	2.0	2.0	2.0	1.9	1.6	1.8
22:6ω3	17.0	14.5	13.1	14.1	13.8	16.2	17.7	13.0	13.4	15.6
$\Sigma\omega$3 HUFA[*2]	26.2	23.5	21.8	23.5	22.7	24.6	27.0	21.7	23.5	25.9
Saturated	28.3	29.2	30.0	29.2	31.9	30.5	32.6	34.0	32.7	33.6
Monoene	36.6	38.5	38.9	39.2	36.6	35.6	31.7	36.7	36.5	35.2
Polyene	33.9	31.0	29.2	30.4	30.0	32.5	34.9	28.2	28.7	30.6

[*1] Average values of four determinations on the eggs obtained in different days.
[*2] $C_{20:3}<\omega3$ fatty acids.

and from 1.0 to 1.5% in the deposited eggs. The lipids from buoyant eggs consisted of 22–30% polar lipids and 70–78% nonpolar lipids as observed previously.[2] The percentage of PE was higher in the buoyant eggs produced by the broodstock fed on frozen raw krill (diet 10) and sand lance (diet 7), and in the deposited eggs from the cuttlefish meal diet group. The percentage of PC was higher in the deposited eggs than the buoyant eggs. However, there seems to be no relationship between quality of eggs and relative proportion of lipid classes in the buoyant eggs, although a marked difference in lipid class was lipid classes in the buoyant eggs, although a marked difference in lipid class was recognized between the buoyant and deposited eggs. Lipid class seems to be not much affected by the duration of fedding, long- or short-term before spawning.

Fatty Acid Distribution of Eggs Produced

Fatty acid composition measured on total lipids from both buoyant and deposited eggs obtained in Experiments I and II is summarized in Tables 5 and 6, respectively. The values are the average of four or five measurements on eggs produced separately on different days.

1. Experiment I The fatty acids in eggs were greatly affected by dietary fatty acids supplied to broodstock as already reported in the previous paper.[3] The proportion of ω3 HUFA (20:3ω3, 20:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3) was higher, in general, in the buoyant eggs from each experimental broodstock, affected by the dietary fatty acids shown in Table 7, and was highest in the eggs from broodstock fed on raw sand lance (diet 7). In the eggs from the latter broodstock the percentage of 18:1ω9 and 20:1ω9 was low. Also there was no marked difference in fatty acid distribution in the deposited eggs among the experimental groups and between the buoyant and deposited eggs.

2. Experiment II The fatty acids in eggs of red sea bream were also found to be greatly affected by the lipids in diets given to broodstock shortly before their spawning (Table 8). In the eggs from the broodstock fed on the corn oil diet before and during spawning having previously been given diet 4, the percentage of 18:2ω6 was as high as about 26% in comparison with 2.8% of the original value, being a reflection of the dietary corn oil used. But feeding corn oil rich in 18:2ω6 (diet 11) did not result in again an increase of 20:4ω6 and 22:5ω6, suggesting no conversion of the former acid to the latter fatty acids unlike

Table 7. Fatty acid compositions of dietary lipids red sea bream broodstock

Fatty acid	Diet no.					
	1	2	3	4	5	6
14:0	4.0	4.2	4.3	4.5	4.0	3.6
16:0	15.8	16.5	16.7	17.0	18.4	21.9
16:1*1	5.7	6.1	6.2	6.3	5.5	4.1
18:0	2.3	2.6	2.5	2.5	3.8	6.8
18:1ω9*1	16.6	16.7	17.1	16.6	14.8	10.7
18:2ω6	1.7	1.8	1.8	1.4	1.8	2.1
18:3ω3	0.8	0.8	0.9	0.8	0.9	1.0
18:4ω3	1.3	1.7	1.9	1.9	1.4	0.7
20:1	9.0	9.6	10.1	10.4	9.3	6.0
20:4ω6	2.9	1.1	1.0	0.9	1.8	3.8
20:4ω3	0.7	0.5	0.5	0.4	0.4	0.4
20:5ω3	11.7	12.1	11.9	12.3	12.1	11.9
22:1	6.0	6.4	5.8	6.2	4.8	2.7
22:5ω3	0.9	0.9	0.9	0.8	0.9	0.9
22:6ω3	13.4	12.3	11.6	11.7	13.4	16.8
24:1	1.5	1.5	1.3	1.3	1.2	1.0
Σ ω3HUFA*2	26.7	25.8	24.9	25.2	26.8	30.0

*1 Small amounts of other monoenes were included.
*2 $C_{20:3}<\omega 3$ fatty acids.

Table 8. Fatty acid compositions of dietary lipids for red sea bream broodstock

Fatty acid	Diet no.		
	8	9	11
14:0	5.2	8.8	1.5
16:0	18.7	21.3	14.1
16:1*1	6.5	8.3	2.3
18:0	2.9	2.0	2.2
18:1ω9*1	19.6	20.1	30.6
18:2ω6	1.7	3.0	34.0
18:3ω3	0.8	0.5	0.6
18:4ω3	1.6	1.0	1.2
20:1	9.9	5.1	3.7
20:4ω6	1.0	0.6	0.2
20:4ω3	0.6	0.5	0.1
20:5ω3	10.2	10.4	2.7
22:1	5.2	4.6	1.8
22:5ω3	0.8	0.4	0.2
22:6ω3	10.1	5.8	2.2
24:1	1.2	1.5	0.5
Σ ω3HUFA*2	21.7	17.1	5.2

*1 Small amounts of other monenes were included.
*2 $C_{20:3}<\omega 3$ fatty acids.

Table 9.* The contents of cholesterol and vitamins A and E in eggs obtained from red sea bream broodstock fed different diets

	Diet no.										
	1	2	3	4	5	6	7	8	9	10	11
Buoyant egg											
Cholesterol (mg/g egg)	2.43	1.96	2.12	3.53	2.23	2.63	1.57	1.03	1.37	1.93	1.45
Vitamin A (IU/g lipid)	0.8	tr	tr	0.7	1.3	tr	tr	tr	tr	tr	—
α-Tocopherol (μg/g lipid)	345.6	322.5	284.3	356.5	298.2	350.0	84.9	680.8	640.8	268.4	—
Deposited egg											
Cholesterol (mg/g egg)	4.25	2.85	2.24	2.86	1.78	3.33	2.08	2.53	5.20	3.42	—
Vitamin A (IU/g lipid)	1.8	1.2	0.6	0.5	tr	tr	tr	6.4	26.0	1.3	—
α-Tocopherol (μg/g lipid)	384.2	275.2	246.0	381.3	225.2	224.1	108.1	613.3	482.9	249.6	—
Cholesterol (mg/g diet)	3.46	4.40	4.40	5.34	7.76	10.89	—	5.33	6.66	—	3.66

* Average values of five determinations on the eggs produced in different days except for the experimental diets.

freshwater fish.[12]

Cholesterol and Fat-soluble Vitamins

The concentrations of cholesterol and vitamins A and E in both buoyant and deposited eggs are shown in Table 9.

1. Experiment I The concentration of cholesterol was almost the same among the experimental groups ranging from 2.0 to 2.6%, except for the eggs from the broodstock receiving frozen sand lance. Although dietary cholesterol levels were varied from lot to lot, highest values occurred in

the diet containing cuttlefish meal as a protein source. The content of vitamin A was generally much lower than that of vitamin E in each group of eggs and the level of α-tocopherol ranged from 280 to 360 mg per g egg lipid when the diets containing about 30 mg of DL-α-tocopheryl acetate were supplied to broodstock. This was true for all diets except diet 7 the lowest value of 85 mg occurring in the group fed on frozen raw sand lance a result similar to that for cholesterol. This may indicate an originally low level of this vitamin in frozen sand lance due to destruction during storage. The deposited eggs also showed a similar results as the buoyant eggs, although the vitamin A level was slightly higher in the former.

2. Experiment II The concentration of cholesterol in the eggs was reduced to about a half the original value when the broodstock were given different diets shortly before spawning. In the eggs from the fish fed diet 9 fortified with 10^4 IU of vitamin A and D and 200 mg of vitamin E the concentration of vitamin E was elevated to about twice the original value, although that of vitamin A was not increased in the buoyant eggs it did increase in the deposited eggs. The same level of α-tocopherol was detected in the eggs of the diet 8 group. This was due to the supplement of 3% cuttlefish oil containing 1% vitamin E. Thus fat-soluble substances not only fatty acids, but also vitamins were found to be very easily incorporated into eggs through the diets given to broodstock on the eve of spawning, as observed in the case of diets supplemented with pigments such as astaxanthin and canthaxanthin which are rapidly incorporated in the eggs a matter of hours later.[13,14]

Thus chemical analyses for eggs produced by the broodstock fed on various diets with different nutritional quality either for 6 months or shortly before spawning has revealed that there was no marked difference in their chemical composition, although noticeable differences were recognized between the buoyant and deposited eggs. One of the big differences in chemical components between white fish meal and cuttlefish meal is a higher concentration of cholesterol and of phospholipids in the latter. However, the level of cholesterol in the eggs of broodstock given diets containing either white fish meal or cuttlefish meal was not very different, suggesting no relationship exists between quality of eggs and their content of cholesterol. Further experiments will be necessary to identify effective components such as phospholipids and pigments in cuttlefish meal and raw krill for improvement of egg quality.

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