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高度不飽和脂肪酸對黑鯛種魚之產卵與卵質的影響

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Effects of Highly Unsaturated Fatty Acids in Broodstock Diets on Spawning and Egg Quality of Black Porgy (*Acanthopagrus schlegeli*)

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ABSTRACT

A feeding experiment was conducted to determine the effect of HUFA (highly unsaturated fatty acids) level in broodstock diet on spawning and egg quality of black porgy. After broodstocks were fed four test diets for about three and half months, they spawned for about two months continually.

In this study, the eggs produced/kg/fish and percentage of buoyant eggs of broodstocks fed diets with fish meal as main protein source and supplemented 0, 4 and 12 % fish oil were 67.9, 248.2 and 146.8 $\times$ 10^4 eggs, and 69.0, 73.8 and 66.9% respectively. The eggs produced/kg/fish and percentage of buoyant eggs of broodstocks fed diet with casein as main protein source and only supplemented soybean oil were the lowest among the four test diets, which were 45.2 $\times$ 10^4 eggs and 58.1%, respectively. It indicated if fish meal serve as main protein source for broodstock diet, supplement of 4% fish oil (or 2.79% HUFA in diet) is enough to improve spawning and egg quality for black porgy.

The results of analyzing lipid and fatty acids of buoyant eggs showed polar and nonpolar lipid content and fatty acid composition were not related to egg quality and spawning period. Fatty acid composition of diets reflected to that of buoyant eggs, and both 18:2n6 and 20:n5 were the two most distinguishing fatty acids among all fatty acids. The percentage of 22:6n3 tended to keep constant in polar lipids and did not reflect the amount in diet to buoyant eggs.

INTRODUCTION

Egg quality is one of the most important factors for the success of mass production fish larvae. Springate *et al.*, (1985) indicated that the nutritional condition of broodstock caused the differences of egg number, diameter, chemical composition and hatchability. It

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has also been known that egg quality was related to the survival of fish larvae especially at early stage. So, maintaining broodstock in good nutritional status can ensure high quality eggs for mass production fish larvae.

Highly unsaturated fatty acid (HUFA) of n-3 series was the essential fatty acids for the growth of seawater fish (Castell, 1979; Kanazawa,1985). Watanabe *et al.* (1984b) reported that the broodstock of red sea bream (*Pagrus major*) fed HUFA deficiency diet resulted low egg production and poor egg quality. However, Watanabe *et al.* (1985) also indicated that egg quality of red sea bream was improved by feeding broodstock with diets supplemented HUFA before spawning. Millamena *et al.* (1986) and Millamena (1989) reported that broodstock of tiger shrimp (*Penaeus monodon*) fed food containing high level of HUFA spawned high fertility and hatchability eggs. Although the above reports suggest that HUFA in broodstock diet affects egg quality, the quantitative effects of HUFA in broodstock diet on egg quality is not clear. Black porgy (*Acanthopagrus latus*) is a popular cultured seawater fish in Taiwan. There have been several studies on protein utilization for black porgy (Lee *et al.*, 1983; Chuang *et al.*, 1986), but the relationship between egg quality and broodstock diet for black porgy has not been studied.

The objective of this study was to determine the effects of HUFA level in broodstock diet on spawning and egg quality of black porgy.

MATERIALS AND METHODS

Preparation of the test diets

Four test diets were formulated to contain 3 level HUFA (diets 1-3) and one control diet (diet 4) by adding different amount of EPA fish oil and corn oil (purchased locally) to compare the effect of HUFA in broodstock diet on spawning and egg quality of black porgy. The composition of the feed ingredients for four test diets is presented in Table 1. The diets were prepared as powder form and stored frozen prior to their use. Each day, diets were prepared by mixing powder with water and fed as paste.

The feeding experiment

Wild broodstocks aged from two to four years old were obtained from fisherman of Than-Tou-Lang from June to October 1989 continually, and they were acclimated in an 100 ton cement circle tank indoor. During this period they were fed commercial eel powder feed. Since their sex characteristics were not distinguished, four groups and each group with 13 broodstocks were randomly assigned to four square cement tanks ($4 \times 2 \times 1.5$m) on November 1. Test diets were fed one time daily to satiation at 17.00 per day. The lighting schedule was according to natural. Water temperature ranged from 18 to 20 ℃ and

salinity from 30 to 32%.. Until sex could be distinguished from the difference of gonad, the broodstocks were sorted to four females and six males for each group on January 1, 1990. The body weight of the broodstocks is presented in Table 3.

Table 1. Compositions of the test diets for broodstork of black porgy.

Diets No.	1.	2.	3.	4.
White fish meal[1]	62	62	62	--
Casein	--	--	--	36
Gelatin	--	--	--	9
Corn oil	12	8	--	18
EPA oil	--	4	12	--
Cellulose	--	--	--	11
Fixed ingredient[2]	26	26	26	26
Crude protein[3]	40.3	40.3	40.3	41.0
lipid	17.4	17.4	17.2	18.2
Gross enery (kcal/100g)	385.8	385.8	384.9	395.8
HUFA[3](%)	1.36	2.97	5.87	0.00

1. Crude protein: 65%
2. Fixed ingredient: 17% α-starch , Mineral mix 5% (Sakamoto and Yone,1979) , Vitamin mix 3% (Halver, 1957), alanine 0.1%, glycine 0.1% and choline hloride 0.8% .
3. Analytical value.

Evaluation of spawning and egg quality

Spawning started from February 19 and end on April 20, 65 days totally. Fertilized eggs were collected with 50 mesh plankton net to count number of produced eggs and buoyant eggs from 8 to 12 clock every morning. Each time, 50 buoyant eggs were used to measure egg diameter and oil globules with profile projector (Nikon V-10). 100 buoyant eggs were put into a beaker filled with 1000 ml seawater incubated at 21 + 1 C. Hatchability and abnormality were determined by microscope. Collected buoyant eggs were freeze dried and stored in freezer.

Analytical methods

Lipid of diets and buoyant eggs were extracted by chloroform/methanol (Folch *et al.*,1957). The extracted lipid was separated into polar and nonpolar classes with a silica acid column chromatography technique (Bitman *et al.*, 1984). Sep-Pak silica acid column

(Waters Division of Millipore, Milford, MA) were used for seperarion. Nonpolar lipids were eluted with 40 ml of hexane/ethyl ester (1:1, v/v), and polar lipids were eluted with 20 ml each of methanol and chloroform/methanol (1:1, v/v).

The lipid were esterified with borontrifluoride in methanol (Metcalfe and Schmitz, 1961). The methyl esters were analyzed by Hitchi 163 Gas-Liquid Chromatograph, equipped with a flame ionization detector and glass column (3 × 2mm) packed with 5% schinchrom E70 coated on simalite (AW, 80-100 mesh, Shimadzu, Tokyo, Japan). Fatty acids were identified by comparison with retention time of a reference standard (GLC-68A, Nu-Chek Prep., Elysian, MN, U.S.A.) consisting of a mixture of saturated and unsaturated standard fatty acids. The magnitude of each peak of each chromatogram was quantified by a Hitachi D-2000 Chromatograms integrator-recorder. Cod liver oil were served as a secondary reference standard.

Statistical method

The percentage data were normalized with angle transformation (arcsin square root of frequency) (Snedecor and Cochron, 1967). All data were analyzed as a completely randomized design of one way analysis of ANOVA. Duncan's new multiple range test was used to resolve the differences among treatment means (Duncan, 1955).

RESULTS

Table 2 shows the fatty acid composition of four test diets, which indicated the amount of HUFA in diets were diet 3, 2, 1, and 4 in order.

Total feed intake of each test diet for broodstocks at each month during the experimental period is presented in Fig. 1. It indicates that diet 4 (HUFA deficient diet) was consumed inferior to the other diets because poor acceptability for casein serves as only protein source in diet 4.

The distribution of eggs spawned by broodstocks fed four different diets during spawning period is presented in Figure 2. In the figure, the interval was based on 7 days as a unit. Spawning of broodstocks was stopped on February 27 due to a cold front, and it recovered on March 11 for diet 2, 3 and 4 group and until March 30 for diet 1 group. The highest total egg production (1571.1 × 104) was obtained in broodstocks fed diet 2, followed by those fed diet 3 (622.8 × 104) and diet 1 (507.5 × 104), and the broodstocks fed diet 4 showed the lowest value (258.4 × 104) among the four diets.

The effects of broodstock diet on spawning and egg quality are presented in Table 3. Diet 2 group had the highest values for total egg production and egg production/kg/fish. If the value of egg production/kg/fish for diet 1 group was considered as 1, the value for diet 2

Table 2. The fatty acid compositition of four test diets.

Fatty acid	Diet No. 1.	2.	3.	4.
14:0	1.93	3.01	7.07	0.24
16:0	12.97	12.89	19.04	9.75
16:1	1.68	3.07	6.67	--[1]
18:0	2.76	2.71	2.27	3.21
18:1n9	22.67	20.11	16.18	25.62
18:2n6	45.04	32.66	3.65	60.27
18:3n3	0.32	0.54	0.80	0.53
20:0	0.37	0.86	1.77	0.24
20:1n9	3.82	3.92	7.74	0.14
20:4n6	0.14	0.46	0.46	--
22:0	0.25	0.45	--	--
20:5n3	4.07	10.76	20.24	--
22:5n3	0.28	1.26	2.46	--
22:6n3	3.51	6.01	11.27	--
Σ n3-HUFA (%)	7.85	18.03	33.96	0.00
Σ n6 (%)	45.18	33.12	4.11	60.27
Σ n3 (%)	8.17	18.57	34.76	0.53

1. Undetectable.

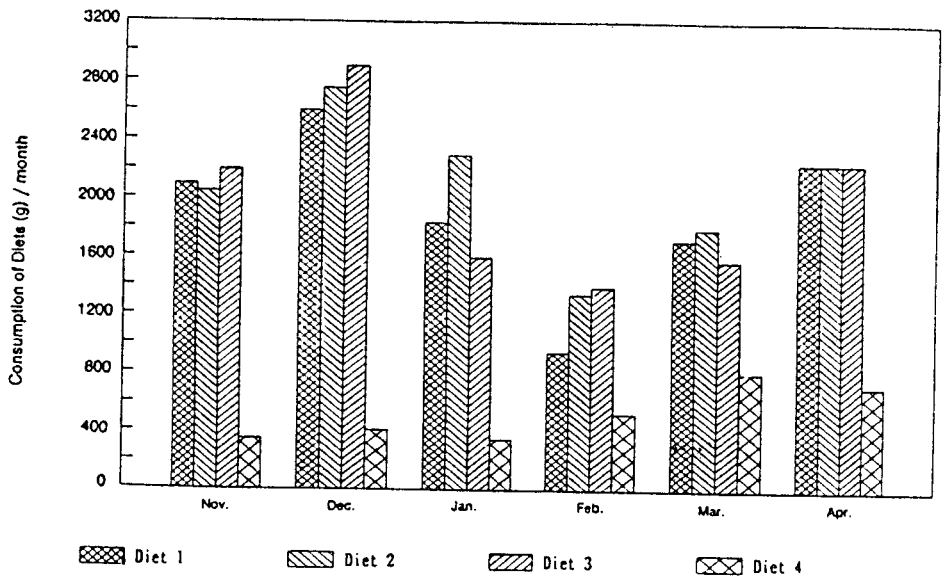

Fig. 1. Total feed intakes of each broodstock diets fed to black porgy during each month of experimental period (g/Groud).

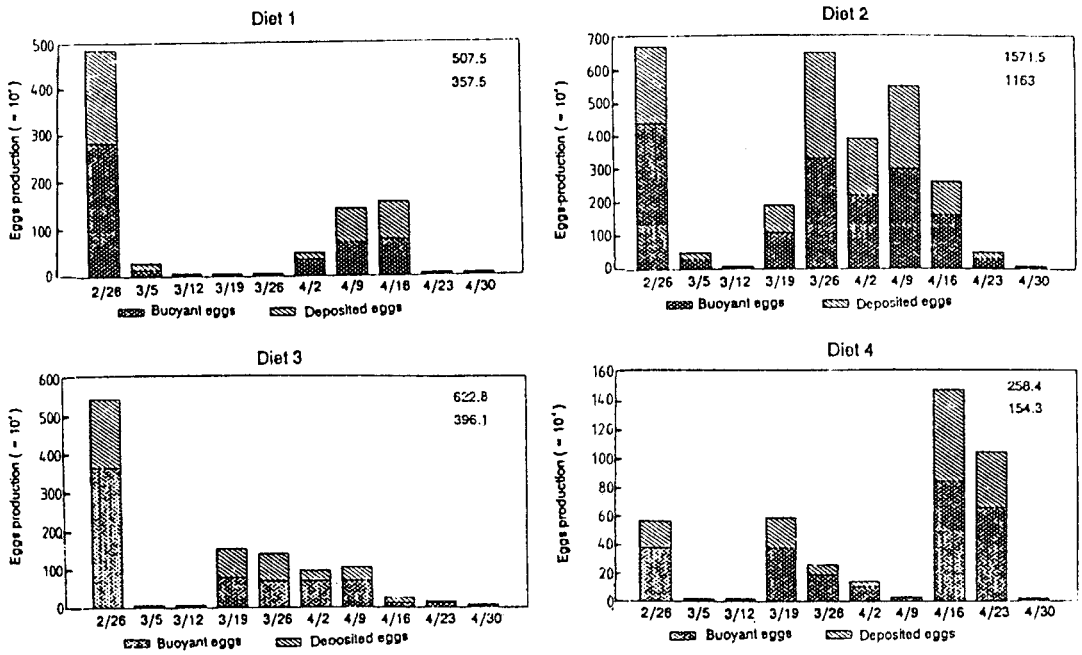

Fig. 2. Result of spawning by parent black porgy brodstock fed the different diets. The values in the figure indicate the total of eggs production ($\fallingdotseq 10^4$) during the spawning period (upper) and the amount of buoyant eggs ($\fallingdotseq 10$) (lower).

Tabe 3. Effect of broodstock diets on the spawning and egg quality of black porgy[1].

Diet No.	1.	2.	3.	4.
Average weight of Broodstock				
Male (g)	794±184	600±74	925±510	653±193
Female (g)	1870±502	1582±420	1062±773	1425±319
Egg				
Eggs produced/kg/fish (*10^4)	67.9	248.2	146.8	45.2
Total egg produced	507.5	1571.1	622.8	258.4
Buoyant egg (%)	69.0±17.4^{ab}	173.8±14.8^{a}	66.9±20.2^{ab}	58.1±20.3b
Egg diameter (mm)	0.87±0.03	0.89±0.04	0.86±0.04	0.87±0.09
Av. number of oil globules	1.01±0.02	1.08±0.34	1.09±0.19	1.00±0.01
Hatched larvae (%)				
Hatching rate of buoyant	92.3±12^{a}	94.2±5.33^{a}	93.5±5.7^{a}	84.1±15.23^{b}
egg Deformity	7.7±12	8.8±6.4	8.1±5.5	13.1±10.4
Normal larvae obtained from buoyant egg	84.6±7.8	84.87±9.33	85.3±7.5	71.1±17.9
Normal larvae obtained from total eggs (%)	58.4	62.6	57.1	41.3

[1] Means not sharings a common superscript letter in the same column or row are significantly different ($P<0.05$).

group was 3.66, for diet 3 group was 2.16, and for diet 4 group was 0.67. The order of eggs produced/kg/fish was similar to that of the total egg production.

The largest and the smallest egg diameter among test diet groups were obtained in the broodstock fed diet 2 and diet 3, respectively. In general, egg diameter of the four test diet groups was similar. The average number of oil globule for all test diets was close to 1.0. The percentage of buoyant eggs in total produced eggs for diet 2 group was significantly ($P<0.05$) higher than that for diet 4 group, and no significant difference ($P>0.05$) was observed among others. The hatchability for diet 1, 2 and 3 were close to each other, and they were significantly higher than that for diet 4 ($P<0.05$). The values of deformity for diet 1, 2 and 3 were similar, and they were lower than that for diet 4, but due to large variation, they were not significantly ($P>0.05$) different from that for diet 4. The percentage of normal larvae hatched from total eggs for diet 2 was the highest among all test diets, and it was close to those for diet 1 and diet 3. However, all of them were higher than that for diet 4.

Three stages, early, middle and later, were divided evenly from the beginning to the end of spawning. It was observed that the total lipid of buoyant eggs for middle and later stages were significantly higher than that for early stage ($P<0.05$), and the highest value was obtained for diet 4 (Table 4). Since the percentage of polar and nonpolar lipid in total lipid and the fatty acid composition of polar or nonpolar lipid were similar in the three spawning stages, the values for different stages were pool together to compare the dietary effects. The order for the percentage of polar lipid in total lipid was as follow diet: 2, 4, 3 and 1 (Table 5).

Table 4. Total lipids in the buoyant eggs produced by black porgy fed four different diets at three spawning stages[1].

Spawning stages	Diet No. 1	2	3	4	AVG ± SD[2]
Early stage	0.193	0.189	0.186	0.203	0.193 ± 0.014^{b}
Middle stage	0.220	0.205	0.221	0.261	0.227 ± 0.028^{a}
Later stage	0.202	0.202	0.212	0.227	0.211 ± 0.019^{ab}
AVG±SD[3]	0.205 ± 0.023	0.199 ± 0.014	0.206 ± 0.026	0.230 ± 0.024	Overall AVG: 0.210 ± 0.025

1 % in dry weight of buoyant eggs.

2 Means not sharing a common superscript letter in the same column are significantly different($P<0.05$).

3 Means not sharing a common superscript letter in the same row are significantly different ($P<0.05$).

Table 5. Polar lipid content of the total lipid in buoyant eggs produced by black progy fed four different diets at three spawning stages[12].

Spawning stages	Diet No. 1	2	3	4	AVG ± SD
Early stage	0.290	0.335	0.327	0.341	0.323±0.026
Middle stage	0.302	0.348	0.299	0.327	0.319±0.028
Later stage	0.301	0.350	0.310	0.314	0.319±0.022
					Overall AVG:
AVG±SD	0.298^{b}±0.016	0.344^{a}±0.020	0.312^{b}±0.019	0.327^{ab}±0.020	0.320±0.026

1 % in total lipid.
2 Means not sharing a common superscript letter in the same column or row are significantly different.($P<0.05$)

Table 6. The fatty acid composition in polar lipids of buoyant eggs produced by black porgy broodstock[1].

Fatty	Diet No. 1	2	3	4
14:0	1.09 ± 0.13^{b}	1.48 ± 0.09^{a}	1.40 ± 0.11^{a}	0.75 ± 0.11^{c}
16:0	17.82±2.60	20.39±1.59	19.79±0.76	20.55±2.38
16:1	3.28±1.48	3.22±1.06	2.90±1.56	3.18±0.89
18:0	10.86±0.71	10.24±0.78	10.94±0.32	10.58±0.72
18:1n9	13.38±1.04	13.80±0.43	12.17±0.90	13.76±1.61
18:2n6	10.52 ± 0.60^{a}	7.75 ± 0.81^{a}	4.24 ± 1.20^{b}	9.88 ± 1.12^{a}
18:3n3	1.29±0.34	0.91±0.07	0.92±0.23	1.40[2]
20:1n9	1.34±0.57	0.92±0.12	1.21± 0.47	1.35±0.75
20:4n6	1.89±0.46	1.47±0.31	1.68±0.28	2.21±0.60
20:5n3	8.97 ± 0.28^{b}	10.39 ± 0.76^{bc}	2.64 ± 0.62^{a}	6.40 ± 1.51^{c}
22:5n3	2.30±0.61	1.59±0.45	1.71± 0.34	2.16±1.05
total n3	36.28 ± 0.92^{bc}	37.94 ± 1.51^{b}	41.82 ± 0.74^{a}	34.82 ± 1.72^{c}
total n6	12.59 ± 0.92^{a}	9.56 ± 0.02^{a}	6.10 ± 1.61^{b}	12.38±1.66a

1. Means not sharings a common superscript letter in the same column or row are significantly different ($P<0.05$).
2. one detectable data only.

Table 7. The fatty acid composition in nonpolar lipids of buoyant eggs produced by black porgy broodstock(n=3).

Fatty	Diet No. 1	2	3	4
14:0	2.97±0.42^{b1}	3.95±0.70^{ab}	5.35±0.99^{a}	2.83±0.24^{b}
16:0	16.26±0.96^{b}	16.83±0.76^{ab}	16.71±0.68^{ab}	18.36±0.88^{a}
16:1	9.49±1.13	9.62±0.82	11.59±1.24	9.65±0.54
18:0	3.53±0.27	3.33±0.45	0.33±0.10	0.96±0.43
18:1n9	28.82±1.33^{a}	26.41±1.04^{ab}	25.30±1.79^{b}	27.35±1.18^{ab}
18:2n6	21.26±0.41	15.91±1.62	12.05±2.61	14.98±1.73
18:3n6	0.53±0.30	0.54±0.14	0.55±0.11	0.80±0.29
18:3n3	0.54±0.38	1.08±0.19	0.96±0.03	0.97±0.22
20:0	0.11±0.01^{b}	1.87±1.16^{a}	0.76±0.29^{a}	1.12±0.32^{a}
20:1n9	0.42±0.05^{b}	1.15±0.06^{a}	0.85±0.29^{a}	0.70±0.23^{ab}
20:3	0.19±0.02	0.46±0.07	0.18±0.03	0.42±0.09
20:4n6	0.45±0.02^{b}	0.58±0.01^{ab}	0.52±0.09^{ab}	0.67±0.12^{a}
20:5n3	3.05±0.37^{b}	4.00±0.08^{b}	6.16±1.30^{a}	2.81±0.10^{b}
22:0	0.23±0.03^{b}	0.56±0.07^{a}	0.54±0.08^{a}	0.34±0.10^{ab}
22:5n3	2.04±0.75	2.12±0.16	1.99±0.61	2.49±0.49
22:6n3	8.57±0.34^{c}	7.92±0.27^{c}	9.88±0.52^{b}	11.28±0.39^{a}
total n3	13.54±0.86^{c}	15.18±0.36^{bc}	19.47±0.97^{a}	16.09±1.43^{b}
total n6	22.27±0.25^{a}	17.02±1.74^{b}	13.11±2.64^{b}	16.45±1.65^{b}

1 Means not sharings a common superscript letter in the same column or row are significantly different ($P<0.05$).

Dietary fatty acid effects (Table 2) on the fatty acid composition for buoyant eggs are presented in Table 6 and Table 7. However, the dietary effect on fatty acid composition for polar lipid was less than that for nonpolar lipid. If the main fatty acids in polar lipid (Table 6) and nonpolar lipid (Table 7) were considered, the percentage of 18-2n6, 20-5n3 and 22-6n3 reflected dietary effect significantly ($P<0.05$). It was also observed that the percentage of EPA (20-5n3) and DHA (22-6n3) in polar lipid were higher than those in nonpolar. It was obvious that polar lipid had higher percentage of n-3 fatty acids and nonpolar lipid had higher percentage of n-6 fatty acids when the fatty acids of both lipids were compared to each other.

DISSCUSSION

The lowest total egg production among four test diets was obtained from diet 4 group, and it was only 51% of that for diet 1 group. However, the lowest n-3 HUFA in diet 4 might not be the only reason because the low feed intake caused from the poor acceptability of broodstock for diet 4 might be another reason. Hislop *et al.* (1978) reported that egg production for cod (*elanogrammus aeglefinus*) had a positive relationship with their feeding level; Scott (1962) indicated that egg production for rainbow trout (*Salmo gairdneri*) decreased with the reduction of feeding level, but the egg diameter was not affected; Begenal (1969) and Springate *et al.* (1985) suggested that the short of food before spawning caused a reduction of egg production and egg diameter; however, Kato (1969) and Ridelman *et al.* (1984) reported that rainbow trout was starved for 40 days or four months before spawning, and their egg production and egg diameter were not affected. In this study, the results indicate that feed intake affected the egg production but egg diameter were not affected (Fig. 1 and Table 3). The fertilized egg diameter for diet 3 group was smaller than that for others group, which probably could be attributed to the smallest size of female broodstocks for diet 3 group among the four test diet groups (Table 3).

Watanabe *et al.* (1984c) indicated that egg quality of red sea bream was improved when HUFA was supplemented to broodstock diet before spawning; Millamena *et al* (1989) also reported that tiger shrimp eggs had better fertilized rate and hatchability for the broodstock food supplemented with high level of HUFA. However,in this study, the egg quality of the highest HUFA diet (diet 3) was poorer than that of diet 1 group, which either because the level of HUFA (5.87% in diet) in diet 3 was too high to have an opposite effect, or because the size of female broodstocks for diet 3 group was too small to produce poor quality eggs (Table 3).

Diet 4 which did not contain HUFA, had poor egg quality. Watanabe *et al.* (1984a, d) also reported that red sea bream or rainbow trout fed essential fatty acid deficiency diet spawned poor quality egg. In this study, the egg quality of diet 1 group or diet 2 group was similar, which indicates that adding 1.36% HUFA in diet was enough to improve the egg quality for black porgy. However, if the egg production was considered, the value of egg produced/kg female broodstock for diet 2 and diet 3 group was obviously higher than that for diet 1 group. It suggested that the level of HUFA in diet for black porgy broodstock had better be about 2.97% in diet.

Diet 4 group, which egg quality and egg production was the poorest among the four diets, had the highest lipid content, but its lipid content was not significantly ($P>0.05$) different from that for diet 1 and diet 3, and the values for diet 1, 2 and 3 were close to each other. The middle stage had the highest total lipid content during spawning period, but there was no difference among the percentage of ploar and nonpolar lipid at the three stages.

It suggested that the percentage of polar or nonpolar lipid in total lipid was kept a constant in buoyant egg during spawning period, and it did not have a co-relationship with egg quality in this study. Watanabe *et al.* (1984b) also reported that there was no obvious relationship between egg quality and egg lipid content for red sea bream, egg lipid content fluctuated with spawning period, and the effect of spawning period on egg lipid content was not obvious. The ratio between polar lipid and nonpolar lipid in buoyant egg for black porgy was 1:2 - 1:2.6, and that was in the range of 1:1.5 - 1:2.8 for red sea bream (Watanabe *et al.*, 1984b).

The percentage of 18:1n9, 18:2n6, 20:5n3 and 22:n6 in polar and nonpolar lipid for the buoyant eggs of black porgy in this study was similar to those for red sea bream (Watanabe *et al.*, 1984b). The percentage of 18:2n6 in diets was reflected to that in eggs, which was also reported by Watanabe *et al.* (1984b and 1985) on red sea bream. This study indicates, except 18:2n6, 20:5n3 was another one which could reflect its amount in diet to egg. On the contrary, 22:6n3 was kept a constant high value in buoyant egg despite its amount in broodstock diets. If the percentage of 20:5n3 and 22:6n3 in broodstock diets and these in eggs were considered, the reason might be that either 20:5n3 could be converted to 22:6n3 through a chain enlongation or 22:6n3 could be transported from body to eggs selectively. Although the the broodstocks were fed diet 4 with a deficiency of 22:n6 for 5 months, the eggs still had a high percentage of 22:6n3. It was possible to be the low feed intake , low egg production and transportation of 22:6n3 from storage of broodstock to eggs.

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高度不飽和脂肪酸對黑鯛種種魚之產卵與卵質的影響

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摘　要

本試驗以高度不飽和脂肪酸不同量的飼料飼養黑鯛種魚，以探討高度不飽和脂肪酸對產卵及卵質的影響。本試驗在種魚餵飼4種飼料約3個半月後，連續產卵了約2個月。

種魚餵以魚粉為主蛋白源分別添加0、4、12％魚油的飼料，其平均每公斤產卵量分別為67.9、248.2及146.8×10粒卵，而浮卵率則為69、73.8及66.9％。餵以酪蛋白為主蛋白源且僅添加黃豆油的種魚之產卵量及浮卵率為四組試料中最低的，其值分別為45.37×10粒卵及58.1%。此顯示黑鯛種魚飼料若以魚粉為主蛋白源添加4%魚油(或含2.79% HUFA於飼料中)即可達到良好的效果。

分析浮性卵之脂質結果顯示，浮性卵中的極性與非極性脂質含量及其脂肪酸組成與產卵期及卵質無關，其浮性卵脂質的脂肪酸組成可反應飼料中脂肪酸組成，尤以18:2n6及20:5n3最顯著，22:6n3之百分組成在極性脂中較穩定，且與飼料中含量的關係亦不明顯。