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The Effect of Different Ratios of n-6/n-3 Fatty Acids in Broodstock Diets on Egg Quality of Catfish, *Clarias batrachus*

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Abstract

This experiment was conducted to acquire biochemical information on the quality of catfish eggs in relation to different n-6/n-3 ratios of fatty acids in the broodstock diet. Five experimental diets, which were isonitrogenous and isoenergy, but containing different levels of n-6 and n-3 fatty acids (diet A: essential fatty acid (EFA)-deficient diet containing 0.21%n-6 fatty acid, 0.03% n-3 fatty acid; diet B: 0.67%, 2.09%; diet C: 2.24%,0.07%; diet D: 1.85%, 0.56%; and diet E: 0.26%, 1.68%) were used in the experiment. Five-month-old fish were fed the experimental diets for 10 months. Samples of the eggs produced by the respective broodstock were fertilized by the sperms. Samples of eggs during embryogenesis were analyzed for total lipid (TL), polar lipid (PL) and nonpolar lipid (NL) content as well as fatty acid (FA) composition. The hatching rate of the eggs and the percentage of abnormal larvae were determined. Results showed that the lipid content of the eggs decreased from the first hour after fertilization. The lipid content of the EFA-deficient eggs (diet A) and the n-3 fatty acid deficient eggs (diet C) decreased significantly during embryogenesis compared to the lipid content of the eggs from the other diets. Also, in general, the NL of eggs was much lower than PL during embryogenesis. The NL of diet A and diet C eggs was also much lower during embryogenesis compared to the NL of the eggs from the other diets. The n-6 and n-3 fatty acid levels of unfertilized eggs were influenced by the fatty acid level in the broodstock diet. The n-6 and n-3 fatty acids of NL were much lower than those of PL, especially in the organogenesis stage of the larvae. The n-6 and n-3 fatty acids of NL from diets A, B and C were also much lower compared to those of diets D and E. Hatching rates of eggs produced by the broodstock fed diets and D and E were also higher than those fed diets A, B and C.

Introduction

Several experiments have shown that the quality of broodstock diet could affect the fecundity and the hatching rate of eggs (Takeuchi *et al.* 1981;

Watanabe *et al.* 1984a; Watanabe *et al.*1984b; Watanabe *et al.*1984c; Watanabe *et al.* 1984d; Watanabe *et al.* 1985). The hatching rate of eggs produced by the broodstock fed a linolenic acid-deficient diet was very low (Watanabe *et al.* 1984c; Watanabe *et al.* 1984d). Morphological studies of developing embryos showed some cleavage disorder at the 16 to 32 cell stage, a blocking effect before gastrulation and various alterations later on in the organogenesis phase (Leray *et al.* 1985). These symptoms showed that essential fatty acids (EFA) are extremely important for embryogenesis.

Catfish (*Clarias batrachus*) juveniles required 1.5% linoleic acid and 0.6% linolenic acid in its diet to support a high growth rate (Mokoginta 1986). The linoleic and linolenic acid requirements for broodstock development of this species have not been determined. This experiment was conducted to acquire biochemical information about the quality of ggs in relation to different n-6/n-3 ratios of the broodstock diet. This basic information is very important in preparing a nutritionally adequate diet for catfish broodstock.

Materials and Methods

Experimental Diets

Five experimental diets were used in this experiment. Table 1 shows the composition of the diets and Table 2 shows the proximate composition of the diets.

Broodstock Rearing

Five-month-old fish, ranging from 91.7-122.3g for females and 90.0-110.0g for males, were used in this experiment. The fish were acclimatized to laboratory conditions and trained to feed on the control diet (diet A) for two weeks prior to the beginning of the experiment. Six females and three males were randomly placed in each tank. During the experiment, the fishes were fed experimental diets to satiation once a day at 1700 h.

Each tank (80 x 80 x 100 cm) contained 320 liters of water, the water was aerated and the temperature was regulated at 25-27°C by using a thermostat. The water was completely changed once every two days and the tanks were scrubbed and cleaned thoroughly once a month. During tank cleaning, the fish were sexed macroscopically and judged for maturity. The mature female have a swelling urogenital with red color.

At the beginning of the experiment, the total ammonia ranged from 1.0-1.9 ppm, dissolved oxygen ranged from 7.2-7.4 ppm and pH ranged from 7.0-7.4 ppm. After six months of the rearing experiment, the total ammonia ranged from 0.32-3.23 ppm and the dissolved oxygen ranged from 2.9-5.8 ppm. Some of the fish died and several fish did not grow at all. Presumably, the stocking rate of the fish was too high, so some of the fish were removed on the seventh month after rearing time, and eventually four females were left in each tank. All males were collected and were placed in different tanks from the females.

Table 1.The composition of the experimental diets for broodstock *C. batrachus* (Mokoginta 1986).

Component	Diet (*n*-6;*n*-3 fatty acid level)				
(g/100g)	A	B	C	D	E
	(0.21;0.03)	(0.67;2.09)	(2.24;0.07)	(1.85;0.56)	(0.26;1.68)
Casein	35.0	35.0	35.0	35.0	35.0
Gelatin	9.0	9.0	9.0	9.0	9.0
Dextrin	35.0	35.0	35.0	35.0	35.0
CMC[a)]	3.0	3.0	3.0	3.0	3.0
a-cellulose	5.0	5.0	5.0	5.0	5.0
Mineral mix[b)]	5.0	5.0	5.0	5.0	5.0
Vitamin mix[b)]	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Beef tallow	6.0	2.0	2.0	2.0	0.0
Corn oil	0.0	0.0	4.0	3.0	0.0
Linseed oil	0.0	4.0	0.0	1.0	0.0
Cuttle fish liver oil	0.0	0.0	0.0	0.0	6.0
(g/100 gdry basis)					
n-6 series[c)]	0.2	0.6	2.0	1.5	0.2
n-3 series[c)]	0.0	2.0	0.0	0.6	1.9

[a)] CMC = Carboxymethyl Cellulose
[b)] Takeuchi (1988).
[c)] *n*-6series consist of16:3*n*-6,18:2*n*-6,20:2*n*-6,20:3*n*-6,20:4*n*-6,22:5*n*-6.
n-3series consist of 18:3*n*-3,18:4*n*-3,20:4*n*-3,20:5*n*-3,22:5*n*-3,22:6*n*-3.

Table 2. Proximate composition and the *n*-6 and *n*-3 fatty acid contents of the experimental diets used for *C. batrachus* broodstock.

Proximate composition	Diet (*n*-6;*n*-3 fatty acid level)				
(g/100g dry basic diets)	A	B	C	D	E
	(0.21;0.03)	(0.67;2.09)	(2.24;0.07)	(1.85;0.56)	(0.26;1.68)
Crude protein	47.47	48.29	46.51	47.72	47.58
Crude lipid	5.83	5.89	5.96	5.85	5.81
Ash	5.02	5.69	5.16	6.08	6.15
Crude fiber	3.19	4.86	5.10	4.79	5.83
n-6series	0.21	0.67	2.24	1.85	0.26
n-3series	0.03	2.09	0.07	0.56	1.68
n-3HUFA*	0.00	0.00	0.00	0.00	1.45

* HUFA = Highly Unsaturated Fatty Acid.

Induced Ovulation and Egg Incubation

The mature females were removed to a fiberglass tank before the fish were injected with common carp pituitary suspension in order to stimulate ovulation. Female catfish received one dose (means the body weight of donor:body weight of recipient = 1:1, with the body weight of common carp being 400-500g per individual) of carp pituitary suspension for every injection. The females received two injections, one at 1600h, and the other at 2400h. At 0700 to 0800h, the fish were stripped. The eggs produced by the respective females were fertilized with the milt sperms of the males from the other tank. In order to get viable spermatozoa at the same time, the males were injected with one dose of carp pituitary suspension and were sacrificed for that purpose.

About 20% of unfertilized (UF) eggs from the respective treatment groups were collected for total lipid (TL), polar lipid (PL) and nonpolar lipid (NL) fraction analysis, and fatty acid composition analysis. The rest of the eggs were fertilized by spermatozoa and then spread on a glass surface (20 x 10 x 0.2cm) which was placed in a fiberglass tank containing freshwater. After two minutes, three or four of these glass plates were transferred to an aquarium (60 x 50 x 40 cm) containing freshwater for egg incubation. The number of eggs on the glass plates and the number of larvae produced were counted to determine the hatching rate of the eggs. The rest of the fertilized eggs were divided into three batches and were incubated in another aquarium.

Egg Sampling

One batch of eggs was collected one hour after fertilization (1-HAF); another batch at eleven hours after fertilization (11-HAF); and the rest after the eggs have hatched (larvae). The samples of eggs and larvae were used for total lipid content, PL and NL fraction analysis, and fatty acid composition analysis. The egg samples were collected at specific times after fertilization to determine the fatty acid contents of all eggs before gastrulation (1HAF) and organogenesis (11HAF), and in the larvae. The numbers of abnormal larvae were also counted for each treatment group.

Chemical Analysis

The crude lipid of the egg samples were extracted by using the method of Folch *et al.* (1957) and the NL was separated from the PL fraction by using a silicic acid column (Sep-Pak Cartridge; Water Associates, USA) (Takeuchi 1988). Furthermore, the fatty acid composition of the NL and PL fractions were determined by using gas liquid chromatography (ShimadzuGC-9AM, with Chromatopac C-R6A; Stationary phase used in this analysis was SP 2330 on 100/120 chromosorb, 68% cyanoprophyl, with maximum temperature 275°C, from Supelco, USA). Fatty acid standards were from Sigma Chemical Corp. USA; detector temperature was 260°C, the column temperature was 150-210°C (5°C min-1). Crude protein content of the diets was analyzed by using the micro Kjeldahl method and the crude lipid by the ether extraction method.

Statistical Analysis

This experiment used a completely randomized design, with five treatments and three replicates for each treatment. The hatching rate of eggs and the percentage of abnormal larvae were subjected to one-way analysis of variance and Tuckey test to determine significant differences among treatments (Steel and Torrie 1980). The homogeneity and normality test was done prior to analysis of variance.

Results and Discussion

Total Lipid, Polar and Nonpolar Lipid Analysis

Table 3 shows the total lipid content of UF eggs, 1-HAF eggs and 11-HAF eggs, and the larvae of treatments A, B, C, D and E. The lipid content of UF eggs varies between treatments. In general, the lipid content of eggs decreases after 1-HAF until the eggs hatch and become larvae. The ratio of 1-HAF/UF, 11-HAF/UF and larvae/UF lipid content of diet A (n-6 0.21; n-3 0.03) and diet C (n-6 2.24; n-3 0.07) were much lower compared to diets B (n-6 0.67; n-3 2.09) and E (n-6 0.26; n-3 1.68). Larvae of the EFA-deficient diet and diet C had a lower lipid level compared to the larvae of the other diets.

Table 4 shows the PL and NL of UF eggs, 1-HAF eggs, 11-HAF eggs, and the larvae. The PL content of UF eggs was lower than the content of NL. This pattern was similar to that in red sea bream eggs (Watanabe *et al.* 1984b) and yellowtail eggs (Verakunpiriya *et al.* 1996). However, the PL content of UF eggs from this experiment was higher (36-40% of the total lipids) than that of red sea bream and yellowtail eggs (about 20% of the total lipids).

In general, the NL of eggs was much higher than that of PL. The NL of diet A (n-6 and n-3-deficient diet) and diet C (n-3 deficient diet) were also

Table 3.The total lipid content of UF eggs, 1-HAF eggs, 11-HAF eggs and the larvae of *C. batrachus* produced by broodstock fed on experimental diets (%db[1]).

Diets (*n*-6;*n*-3)	UF eggs	1-HAF eggs	11-HAF eggs	Larvae
A (0.21;0.03)	24.55(1.00)[2]	20.83(0.85)	17.50(0.71)	15.14(0.62)
B (0.67;2.09)	24.32(1.00)	22.76(0.94)	20.78(0.85)	18.29(0.75)
C (2.24;0.07)	26.74(1.00)	24.70(0.92)	22.44(0.84)	16.07(0.60)
D (1.85;0.56)	25.44(1.00)	24.89(0.97)	22.89(0.90)	19.89(0.78)
E (0.26;1.68)	25.32(1.00)	23.68(0.94)	22.52(0.89)	20.96(0.83)

[1]db =dry weight basis (g/100 g).
[2]In parentheses are the ratio of UF/UF, 1-HAF/UF, 11-HAF/UF,and larvae/UFlipidcontent.
UF: unfertilized eggs.
1-HAF: one hour after fertilization.
11-HAF: eleven hours after fertilization.

Table 4. The phospholipid and neutral lipid NL of UF, 1-HAF, 11-HAF eggs, and the larvae* of *C. batrachus*.

Diets	PL				NL			
(*n*-6;*n*-3)	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
A	7.82	7.80	7.00	9.32	14.73	13.02	10.5	5.82
(0.21;0.03)	(1.00)	(1.00)	(0.90)	(1.19)	(1.00)	(0.88)	(0.71)	(0.40)
B	9.50	9.10	8.58	8.58	14.82	13.66	12.20	9.71
(0.67;2.09)	(1.00)	(0.96)	(0.90)	(0.90)	(1.00)	0.92)	(0.82)	(0.66)
C	9.72	10.81	11.22	8.04	17.02	13.89	11.22	8.04
(2.24;0.07)	(1.00)	(1.11)	(1.15)	(0.83)	(1.00)	(0.82)	(0.66)	(0.47)
D	9.88	11.90	10.17	8.16	15.66	12.99	11.72	11.73
(1.85;0.56)	(1.00)	(1.20)	(1.03)	(0.83)	(1.00)	(0.83)	(0.75)	(0.75)
E	9.74	11.84	10.24	9.67	15.58	11.84	12.28	11.29
(0.26;1.68)	(1.00)	(1.22)	(1.05)	(0.99)	(1.00)	(0.76)	(0.79)	(0.73)

*% dry weight basis of eggs and larvae.
Figures in parentheses are the ratio of UF/UF; 1-HAF/UF; 11-HAF/UF; and larvae/UF of the PL and NL.
UF; 1-HAF; 11-HAF: see page 12.

much lower than that of diets B, D and E. These conditions of diets A and C give the ratio of PL/NL of the larvae of 1.6 for diet A and 1.0 for diet C. These data indicate that lipids, especially NL, are the most important energy sources during embryogenesis, as was reported for other species (Blaxter 1969; Sargent *et al.* 1989; Verakunpiriya *et al.* 1996).

Fatty Acid Composition During Embryogenesis

The effect of n-6 and n-3 fatty acid levels of the broodstock diets on the n-6 and n-3 fatty acid levels of the eggs, which may be conjectured to control the quality of the eggs, can be clearly seen from the fatty acid composition of the eggs. Tables 5, 6, 7, 8 and 9 show the fatty acid distribution of eggs during embryogenesis for diets A, B, C, D and E, respectively.

Generally, fish synthesize a high level of fatty acids if the fish receive a low level of n-6 and n-3 fatty acids in their diet. As such, those fatty acids are the focus of the fatty acid evaluation during embryogenesis.

From Tables 5, 6, 7, 8 and 9, the n-6 and n-3 fatty acid contents of the UF eggs were affected by the fatty acid level in the broodstock diet. Broodstock fed the n-6 and n-3 fatty acid-deficient diet produced eggs which contained a very low level of these fatty acids and a high amount of n-9 fatty acids, especially in the NL. Diet C also produced eggs that contained a high amount of n-9 fatty acids. This diet contained a very low level of n-3 fatty acids (0.07%), even though it contained a high level of n-6 fatty acids (2.24%). This

Table 5. Fatty acid composition of UF eggs, 1-HAF and 11-HAF eggs, and the larvae of *C. batrachus* broodstock fed diet A (% area).

Fatty acids	PL				NL			
	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
12:0	0.04	0.03	0.23	0.38	0.13	-	0.67	0.13
14:0	0.27	0.72	0.13	0.17	1.76	-	0.44	0.27
14:1	0.02	-	0.53	0.56	-	-	-	-
16:0	12.88	14.40	12.80	15.19	10.13	14.09	18.61	16.92
16:1	2.75	2.95	2.71	2.78	2.88	2.39	4.85	4.94
17:0	-	0.47	0.90	1.01	0.30	-	0.25	0.49
18:0	13.14	13.44	12.32	14.77	3.67	6.79	6.53	6.35
18:1*n*-9	11.59	35.84	29.58	34.66	18.71	23.19	30.36	35.43
18:2*n*-6	4.23	3.97	4.71	5.38	1.73	2.07	2.91	4.97
18:3*n*-3	0.11	0.07	0.58	0.41	0.12	0.27	0.54	0.55
18:4*n*-3	0.28	0.31	0.35	0.33	0.27	0.49	-	0.63
20:1*n*-9	-	1.34	1.07	1.13	0.94	0.61	0.80	1.07
20:2*n*-9	-	0.82	0.45	0.37	-	-	-	0.74
20:2*n*-6	0.14	4.54	3.36	2.67	0.36	0.90	2.86	1.76
20:3*n*-6	-	3.70	3.14	3.12	2.03	4.53	2.64	2.55
20:4*n*-6	1.08	3.33	2.99	2.20	2.45	5.95	2.53	1.89
20:4*n*-3	0.15	0.67	0.30	-	0.47	-	-	-
20:5*n*-3	0.42	0.12	0.81	0.81	0.60	0.29	0.34	0.76
22:1*n*-9	3.19	0.49	0.63	0.47	0.23	-	0.77	-
22:5*n*-3	-	0.45	0.33	0.49	0.16	-	-	0.28
22:6*n*-3	2.29	5.37	3.34	5.70	2.00	3.62	4.33	3.49
24:1*n*-9	3.58	0.22	1.65	0.09	8.27	6.29	2.03	-
n-6 series	5.45	15.54	14.20	13.37	6.57	13.45	10.94	11.17
n-3 series	3.25	6.99	5.71	7.74	3.62	4.67	5.21	5.71
n-9 series	18.26	38.71	33.38	36.72	28.15	30.81	33.96	37.64
n-3 HUF	2.86	6.61	4.78	7.00	3.23	3.91	4.67	4.53

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

Table 6. Fatty acid composition of UF eggs, 1-HAF and 11-HAF eggs, and the larvae of *C. batrachus* broodstock fed diet B (% area).

Fatty acids	PL				NL			
	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
12:0	-	-	0.75	0.32	1.75	0.45	0.11	0.73
14:0	-	-	0.64	0.76	0.26	1.71	0.98	1.12
14:1	-	-	-	-	-	-	-	-
16:0	-	17.62	11.32	14.72	16.60	12.92	11.14	11.21
16:1	-	-	1.15	1.01	3.40	2.20	2.11	2.14
17:0	-	-	0.72	-	0.35	-	0.34	-
18:0	-	17.66	13.01	14.89	5.08	3.81	4.68	4.60
18:1*n*-9	-	27.02	16.69	17.31	21.47	26.70	16.75	16.24
18:2*n*-6	-	7.95	4.71	5.85	5.89	4.61	5.09	4.87
18:3*n*-3	-	5.86	3.89	3.97	8.28	5.72	6.80	6.79
18:4*n*-3	-	0.74	0.48	1.38	0.69	0.78	-	2.70
20:1*n*-9	-	-	-	-	-	-	-	1.49
20:2*n*-9	-	-	0.70	-	-	-	-	-
20:2*n*-6	-	1.56	1.10	1.38	1.73	2.38	1.86	1.87
20:3*n*-6	-	0.20	-	0.85	2.05	0.93	0.69	1.97
20:4*n*-6	-	2.02	2.19	2.08	1.99	0.90	1.18	1.46
20:4*n*-3	-	-	0.61	0.88	0.95	0.66	0.64	-
20:5*n*-3	-	4.77	3.08	4.41	1.11	1.25	1.37	2.84
22:1*n*-9	-	-	-	-	-	-	-	-
22:5*n*-3	-	-	1.01	1.37	0.91	0.12	0.53	0.67
22:6*n*-3	-	2.83	6.75	3.96	3.41	5.08	2.77	1.56
24:1*n*-9	-	-	0.42	-	2.24	3.81	-	0.33
n-6 series	-	11.73	8.00	10.16	11.57	8.82	8.82	10.57
n-3 series	-	14.20	15.82	15.97	15.35	13.61	12.11	14.56
n-9 series	-	27.02	17.88	17.31	23.71	30.51	16.75	18.16
n-3 HUF	-	7.60	11.45	10.62	6.35	7.11	5.31	5.07

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

Table 7. Fatty acid composition of HF eggs, 1-HAF and 11-HAF eggs, and the larvae of *C. batrachus* broodstock fed diet C (% area).

Fatty acids	PL				NL			
	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
12:0	-	-	-	-	0.41	-	0.09	-
14:0	0.61	0.67	0.35	0.71	1.57	1.50	0.49	-
14:1	-	-	-	-	-	-	-	-
16:0	13.55	14.68	17.47	14.67	16.59	18.98	14.31	14.09
16:1	1.28	-	1.93	-	3.08	-	1.97	2.39
17:0	0.23	1.57	0.37	1.42	0.20	2.54	0.23	-
18:0	17.27	16.15	14.61	15.69	6.77	7.47	13.86	6.79
18:1*n*-9	21.20	22.40	26.22	20.20	23.36	27.50	23.14	23.91
18:2*n*-6	10.51	10.06	12.01	9.27	9.75	12.83	10.43	11.07
18:3*n*-3	1.54	1.38	1.71	1.24	0.17	0.96	1.71	2.70
18:4*n*-3	-	0.19	0.33	0.24	0.32	0.43	0.52	0.49
20:1*n*-9	0.50	0.61	0.64	0.74	-	0.56	0.57	0.61
20:2*n*-9	-	-	-	-	0.41	-	-	-
20:2*n*-6	1.40	1.54	1.70	1.96	-	1.15	1.15	0.90
20:3*n*-6	6.12	6.04	5.60	6.28	2.61	4.92	4.37	4.53
20:4*n*-6	8.87	9.60	5.74	9.20	3.04	5.95	9.33	5.92
20:4*n*-3	-	-	-	-	-	-	-	-
20:5*n*-3	-	-	0.75	0.26	-	-	0.80	0.29
22:1*n*-9	-	2.35	1.37	2.20	-	1.17	2.54	-
22:5*n*-3	0.44	1.33	0.75	1.30	1.17	0.63	0.81	-
22:6*n*-3	0.80	0.44	0.53	0.58	-	0.53	0.33	0.81
24:1*n*-9	4.15	3.25	3.04	3.73	7.98	-	3.63	3.26
n-6series	26.90	27.24	25.05	26.71	15.40	24.85	25.28	22.42
n-3series	3.78	3.34	3.32	3.62	1.66	1.92	3.37	4.29
n-9series	2.50	28.61	31.27	26.87	31.75	29.76	29.88	28.14
n-3HUFA	2.24	1.77	2.03	2.14	1.17	1.16	1.94	1.09

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

Table 8. Fatty acid composition of UF eggs, 1-HAF and 11-HAF eggs, and the larvae of *C. batrachus* broodstock fed diet D (% area).

Fatty acids	PL				NL			
	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
12:0	0.03	0.17	0.04	-	0.20	0.20	0.44	0.04
14:0	0.22	0.74	0.08	0.42	0.98	1.74	0.69	0.91
14:1	0.09	-	56.00	0.23	-	0.39	1.83	0.15
16:0	12.00	15.95	12.53	18.62	10.83	16.65	14.54	8.11
16:1	1.38	-	1.26	1.77	1.71	3.20	2.84	2.29
17:0	0.25	1.76	0.48	1.48	0.18	2.29	0.50	0.53
18:0	9.54	17.26	13.68	15.93	8.06	5.79	4.50	3.29
18:1*n*-9	17.27	24.39	17.88	21.64	16.05	24.41	18.51	18.23
18:2*n*-6	9.05	11.62	9.23	11.13	8.49	11.37	9.24	9.16
18:3*n*-3	0.73	1.01	-	0.62	1.03	2.63	1.59	1.62
18:4*n*-3	1.26	1.53	0.90	1.09	1.71	-	2.11	2.32
20:1*n*-9	0.51	0.29	1.41	-	0.58	-	-	0.80
20:2*n*-9	0.42	-	0.62	-	0.69	1.99	1.66	1.16
20:2*n*-6	1.36	1.63	1.60	1.05	1.40	1.96	1.26	-
20:3*n*-6	3.46	5.34	3.43	2.04	3.34	4.30	2.81	3.84
20:4*n*-6	2.60	6.05	4.62	5.64	4.66	4.62	2.96	4.53
20:4*n*-3	0.30	0.27	0.43	5.93	0.47	0.89	0.63	0.61
20:5*n*-3	1.10	1.98	2.21	1.91	1.62	1.59	1.07	1.73
22:1*n*-9	0.36	0.87	0.34	1.05	0.46	0.88	0.54	1.21
22:5*n*-3	0.64	1.19	0.66	-	1.04	0.75	0.48	0.62
22:6*n*-3	2.10	6.16	5.60	6.00	3.76	2.48	2.23	2.19
24:1*n*-9	-	-	0.30	-	-	0.30	0.99	-
n-6 series	16.47	24.64	18.88	19.86	17.89	22.25	16.27	17.53
n-3 series	6.13	12.14	9.80	15.55	9.63	8.34	8.11	9.09
n-9 series	18.56	25.55	20.55	22.69	17.78	27.55	21.70	21.40
n-3 HUF	4.14	9.60	8.90	13.84	6.89	5.71	4.41	5.15

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

Table 9. Fatty acid composition of UF eggs, 1-HAF and 11-HAF eggs, and the larvae of *C. batrachus* broodstock fed diet E (% area).

Fatty acids	PL				NL			
	UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
12:0	0.32	0.44	0.04	0.05	0.04	0.04	0.08	0.09
14:0	2.21	0.22	0.44	0.25	0.30	1.23	2.35	1.92
14:1	1.52	0.87	0.20	0.83	0.57	0.10	0.35	0.11
16:0	3.71	14.48	14.5	15.64	10.54	10.37	18.88	16.36
16:1	1.59	2.21	0.32	2.23	1.38	2.61	3.95	4.14
17:0	0.58	0.73	1.02	1.21	0.42	0.55	0.46	0.79
18:0	10.1	16.67	16.34	16.21	11.46	5.55	8.36	7.32
18:1*n*-9	15.91	19.85	19.46	19.94	13.61	13.01	20.71	20.20
18:2*n*-6	1.07	1.61	1.40	1.76	0.83	2.56	3.86	3.51
18:3*n*-3	0.28	0.08	0.11	0.19	0.15	1.61	2.65	0.18
18:4*n*-3	-	1.00	-	-	0.64	1.11	-	2.08
20:1*n*-9	-	1.77	0.91	0.85	1.17	0.72	1.45	1.47
20:2*n*-9	-	-	1.83	1.82	-	-	0.95	0.85
20:2*n*-6	0.44	0.62	0.69	0.75	0.35	1.01	0.82	0.95
20:3*n*-6	1.59	1.40	2.33	2.54	0.22	1.32	1.00	1.22
20:4*n*-6	2.86	2.42	0.61	0.48	1.47	1.93	1.93	2.08
20:4*n*-3	-	0.45	-	-	0.22	0.99	1.17	1.43
20:5*n*-3	5.59	8.35	8.45	8.40	5.18	5.07	6.54	8.35
22:1*n*-9	1.20	0.51	0.51	0.57	0.37	0.63	-	0.40
22:5*n*-3	2.40	2.51	2.51	2.53	1.79	1.52	1.94	1.91
22:6*n*-3	23.31	16.69	17.22	16.69	10.68	8.53	10.33	10.04
24:1*n*-9	3.45	0.21	-	0.59	-	4.25	-	0.20
n-6 series	6.32	6.05	5.03	5.53	2.87	6.82	7.61	7.76
n-3 series	31.58	29.08	28.29	27.81	18.66	18.83	22.63	23.99
n-9 series	19.84	22.34	22.71	23.77	15.15	18.61	23.11	23.12
n-3 HUF	31.30	28.00	28.18	27.62	17.87	16.11	19.98	21.73

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

might indicate that both fatty acids are important for the growth of the embryo.

During embryogenesis, the n-6, n-3 and n-9 fatty acids of PL were not catabolyzed for only a small amount of these fatty acids were catabolyzed or changed to another compound (see Table 10).

Table 10 also showed that the n-6, n-3,and n-9 fatty acids of NL were considerably reduced compared to those of PL, especially in the organogenesis stage of the larvae (11-HAF through to larvae); the n-6, n-3 and n-9 fatty acids of NL from diets A, B and C were also considerably reduced compared to those of diets D and E.

The data indicated the necessity of these fatty acids for the developing embryo; and a certain level of the fatty acids has to be supplied in the

Table 10. The *n*-6, *n*-3 and *n*-9 contents of the UF, 1-HAF and 11-HAF eggs, and larvae of *C. batrachus* based on dry weight basis of eggs and larvae of diets A, B, C, D, and E.

Diets (*n*-6;*n*-3)	Fatty acids	PL				NL			
		UF	1-HAF	11-HAF	Larvae	UF	1-HAF	11-HAF	Larvae
A	*n*-6 series	0.43	1.21	0.99	1.25	0.97	1.75	1.15	0.65
(0.21;0.03)		(1.00)	(2.81)	(2.30)	(2.91)	(1.00)	(1.80)	(1.19)	(0.67)
	n-3 series	0.25	0.55	0.40	0.72	0.53	0.61	0.55	0.33
		(1.00)	(2.22)	(1.60)	(2.88)	(1.00)	(1.15)	(1.04)	(0.63)
	n-9 series	1.43	3.02	2.34	3.42	4.15	4.21	3.57	2.19
		(1.00)	(2.22)	(1.60)	(2.88)	(1.00)	(0.97)	(0.86)	(0.53)
	n-3 HUFA	0.22	0.52	0.33	0.65	0.48	0.51	0.49	0.26
		(1.00)	(2.36)	(1.50)	(2.95)	(1.00)	(1.06)	(1.02)	(0.54)
B	*n*-6 series	-*	1.07	0.69	0.87	1.71	1.20	1.08	1.03
(0.67;2.09)						(1.00)	(0.70)	(0.63)	(0.60)
	n-3 series	-*	1.29	1.36	1.37	2.27	1.86	1.48	1.41
						(1.00)	(0.82)	(0.65)	(1.41)
	n-9 series	-*	2.46	1.53	1.49	3.51	4.17	2.04	1.76
						(1.00)	(1.19)	(0.58)	(0.50)
	n-3 HUFA	-*	0.69	0.98	0.91	0.94	0.97	0.65	0.49
						(1.00)	(1.03)	(0.69)	(0.52)
C	*n*-6 series	2.61	2.94	2.81	2.15	2.62	3.45	2.84	1.80
(2.24;0.07)		(1.00)	(1.13)	(1.08)	(0.82)	(1.00)	(1.32)	(1.08)	(0.69)
	n-3 series	0.37	0.36	0.37	0.29	0.28	0.27	0.38	0.34
		(1.00)	(0.97)	(1.00)	(0.78)	(1.20)	(0.96)	(1.36)	(1.21)
	n-9 series	2.19	3.09	3.51	2.16	5.40	4.13	3.35	2.26
		(1.00)	(1.41)	(1.60)	(0.99)	(1.00)	(0.76)	(0.62)	(0.41)
	n-3 HUFA	0.22	0.19	0.23	0.17	0.20	0.16	0.22	0.09
		(1.00)	(0.86)	(1.05)	(0.77)	(1.00)	(0.80)	(1.10)	(0.45)
D	*n*-6 series	1.63	2.93	1.92	1.62	2.80	2.89	1.91	2.06
(1.85;0.56)		(1.00)	(1.80)	(1.18)	(0.99)	(1.00)	(1.03)	(0.68)	(0.74)
	n-3 series	0.61	1.44	1.00	1.27	1.51	1.08	0.95	1.07
		(1.00)	(2.36)	(1.64)	(2.08)	(1.00)	(0.72)	(0.63)	(0.71)
	n-9 series	1.83	3.04	2.09	1.85	2.78	3.58	2.54	2.51
		(1.00)	(1.66)	(1.14)	(1.01)	(1.00)	(1.29)	(0.91)	(0.90)
	n-3 HUFA	0.41	1.14	0.91	1.12	1.08	0.74	0.52	0.60
		(1.00)	(2.78)	(2.22)	(2.73)	(1.00)	(0.69)	(0.48)	(0.56)
E	*n*-6 series	0.62	0.72	0.52	0.53	0.45	0.81	0.93	0.88
(0.26;1.68)		(1.00)	(1.16)	(0.84)	(0.85)	(1.00)	(1.80)	(2.07)	(1.96)
	n-3 series	3.08	3.44	2.90	2.69	2.91	2.23	2.78	2.71
		(1.00)	(1.12)	(0.94)	(0.87)	(1.00)	(0.77)	(0.96)	(0.93)
	n-9 series	1.93	2.65	2.33	2.30	2.36	2.20	2.84	2.61
		(1.00)	(1.37)	(1.21)	(1.19)	(1.00)	(0.93)	(1.20)	(1.11)
	n-3 HUFA	3.05	3.32	2.89	2.67	2.78	1.91	2.45	2.45
		(1.00)	(1.09)	(0.95)	(0.88)	(1.00)	(0.69)	(0.88)	(0.88)

*Samples of eggs lost by accident.

UF = unfertilized; 1-HAF = one hour after fertilization; 11-HAF = 11 hours after fertilization.

broodstock diet. The n-6 and n-3 fatty acid content of the UF eggs appears to be the determining factor for normal development of the embryo.

The fatty acid composition, especially the EFA (n-6 and n-3 fattty acids) of the cell membrane can influence membrane fluidity (Robblee and Clandinin 1984). Furthermore, membrane fluidity can influence the activity of enzymes in the membrane. Changes in the physiological properties of biological membranes have been shown to influence the behavior of certain membrane-associated enzymes and, in so doing, alter physiological processes. This experiment showed that a significant amount of the EFA were used as structural components of the cell membrane of the embryos, since the EFA level did not significantly decline during embryogenesis, especially.

Another function of EFA in the reproductive processes may also be related to the synthesis of oxygenated cyclic and non-cyclic derivatives, such as prostaglandins (Leray *et al.* 1985). Prostaglandin-related compounds are formed from 20:3n-6, 20:4n-6 20:5n-3 and 20:6n-3. The compounds might act as a modulator or mediator in various physiological events, especially in the processes of cellular recognition during embryonic development (Leray *et al.* 1985). Thus, embryogenesis could be influenced by the EFA composition of the eggs, through both or either one of the functions stated above. The EFA level of the eggs was much lower during the organogenesis stage (11-HAF) larvae. The results demonstrated that during this stage, part of the EFA might have been catabolyzed or converted to prostaglandin-related compounds. The embryos of diet A (EFA deficient) would certainly not develop well during this stage, and would eventually result in low hatching rates.

Table 11 shows the hatching rates and the percentage of abnormal larvae for the various treatment diets. The data in Table 11 showed that 1.85% n-6 and 0.56% n-3 fatty acid levels in the broodstock diet resulted in high hatching rates and low percentages of abnormal larvae. The levels of the fatty acids of the broodstock diet were almost the same as those for the growth of young catfish (n-6 1.5% and n-3 0.5%). It was interesting to note that broodstock fed diet E which contained a high amount of n-3 highly unsaturated fatty acids but very low levels of n-6 fatty acid also produced the same hatching rates as in broodstock fed diet D. Diets B and C contained both fatty acids, but the n-6 (diet C) n-3 (diet B) fatty acid levels may have been too high and as such, resulted in lower hatching rates when compared to those for diets D and E. This observation may be similar to that in young catfish. The growth of young catfish declined when the EFA level of the diet was too high (Mokoginta 1986).

Table 11. Hatching rates (HR) and percentage of abnormal larvae (AL) of *C. batrachus* broodstock fed the different experimental diets.

Parameters	Diets (*n*-6; *n*-3 fatty acids)				
	A (0.21;0.03)	B (0.67;2.09)	C (2.24;0.07)	D (1.85;0.56)	E (0.26;1.68)
HR (%)	49.3±21.7	74.0±4.5b	74.4±10.8	82.3±7.9c	80.7±10.6c
AL (%)	6.8±1.6b	2.3±0.9a	1.7±0.9a	2.4±0.8a	2.5±1.2a

[1]Values in rows followed by different superscripts are significantly different. ($P<0.05$).

Most of the abnormal catfish larvae had a curled and helicoidal body. A similar effect was observed in trout (Watanabe *et al.* 1984d) and grass carp fingerlings fed the EFA deficient diet, and a high percentage of vertebral column curvature was reported (Takeuchi *et al.* 1991). The body deformity symptoms observed in this experiment were probably similar to the symptoms observed for grass carp. Additionally, EFA-deficient larvae in this experiment also showed low swimming activity, a behavior also reported for young catfish (Mokoginta 1986).

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References

Blaxter, J.H.S. 1969. Development: eggs and larvae.In: Fish physiology, vol. 3 (eds. W.S. Hoar and D.J. Randall), pp. 177-252. Academic Press, New York.

Folch, J., M. Lee and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497-507.

Leray, C., G. Nonnotte, P. Roubaud and C. Leger. 1985. Incidence of n-3 essential fatty acid eficiency on trout reproductive processes. Reproduction Nutrition Development 25:567-581.

Mokoginta, I. 1986. Kebutuhan ikan lele (*Clarias batrachus* Linn.) akan asam„asam lemak linoleat an linolenat. Master Thesis. Fakultas Perikanan, Institut Pertanian Bogor, Indonesia. 66 pp. (In Indonesian).

Robblee, G.L. and M.T. Clandinin. 1984. Effect of dietary fat level and polyunsaturated fatty acid content on the phospholipid composition of rat cardiac mitochondrial membranes and mitochondrial ATPase activity. Journal of Nutrition 101:1703-1710.

Sargent, J.R., R.J. Henderson and D.R. Tocher. 1989. The lipid. In: Fish nutrition (ed. J. Halver), pp. 153-217. Academic Press, New York.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. Biometrical Approach. McGraw-Hill Book Company. 633 pp.

Takeuchi, T., T. Watanabe, C. Ogino, M. Saito, K. Nishimura and T. Nose. 1981. Effect of low protein-high calorie diets and deletion of trace element from a fish meal diet on reproduction of rainbow trout. Nippon Suisan Gakkaishi 47:645-654.

Takeuchi, T. 1988. Laboratory work. Chemical evaluation of dietary nutrients. In: Fish Nutrition and Mariculture. JICA texbook, the general aquaculture course (ed. T. Watanabe), pp.179-233. Japan International Cooperation Agency, Tokyo, Japan.

Takeuchi, T., K. Watanabe, W-Y. Yong and T. Watanabe. 1991. Essential fatty acids of grass carp(Ctenopharingodon idella). Nippon Suisan Gakkaishi 53:467-473.

Verakunpiriya, V., T. Watanabe, K. Mushiake, V. Kiron, S. Satoh, T. Takeuchi. 1996. Effect of broodstock diet on the chemical components of milt and eggs produced by yellowtail. Fisheries Science 62:610-619.

Watanabe, T., T. Arakawa, C. Kitajima and S. Fujita. 1984a. Effect of nutritional quality of broodstock diets on reproduction of red sea bream. Nippon Suisan Gakkaishi 50:495.

Watanabe, T., A. Itoh, W. Murakami, Y. Tsukashima, C. Kitajima and S. Fujita. 1984b. Effect of nutritional quality of diets given to broodstock on the verge of spawning on reproduction of red sea bream. Nippon Suisan Gakkaishi 50:1023-1028.

Watanabe, T., S. Ohashi, A. Itoh, C. Kitajima and S. Fujita. 1984c. Effect of nutritional composition of diets on chemical components of red sea bream broodstock and eggs produced. Nippon Suisan Gakkaishi 50:503-515.

Watanabe, T., T. Takeuchi, M. Saito and K. Nishimura. 1984d. Effect of low protein - high calorie or essential fatty acid deficiency diet on reproduction of rainbow trout. Nippon Suisan Gakkaishi 50:1207-1215.

Watanabe,T., C. Itoh, S. Satoh, C. Kitajima and S. Fujita. 1985. Effect of dietary protein levels and feeding period before spawning on chemical components of eggs produced by red seabream broodstock. Nippon Suisan Gakkaishi 51:1501-1509.

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