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Hatchery-Produced Milkfish (*Chanos chanos*) Fry should be Fed Docosahexaenoic Acid-Enriched Live Food: A Case of the Difficulty in the Transfer of Improved Aquaculture Technology in the Philippines

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Abstract

Levels of highly-unsaturated fatty acids, the most important nutritional factors in fry production of marine fish, were compared between hatchery-produced and wild-caught milkfish *Chanos chanos* fry. The most striking difference found between the fry was in docosahexaenoic acid (DHA: 22:6n-3) levels: DHA levels in hatchery-produced fry were only 37% and 18% of those in wild-caught fry in the polar lipids and neutral lipids, respectively. However, high DHA levels were detected in ovary and spawned eggs from hatchery-reared broodstock. Investigation on the time course change in DHA levels of hatchery-produced fry revealed that the DHA levels of polar lipids drastically declined from 25% at day 0 posthatching to 5% at day 14 posthatching. *Nannochloropsis* sp. and rotifers *Brachionus* sp., which were used as live food from day 2 to day 14, did not contain DHA with relatively high eicosapentaenoic acid (EPA: 20:5n-3) levels. DHA level was restored to 13% in 45-day old fry by feeding of formulated diets with a substantial amount of DHA from day 15. Thus, the lack of DHA in the live food appears to lead to the low DHA level in hatchery-produced fry. On the other hand, the cost of DHA enrichment for one milkfish fry was estimated to be 2.6 Philippines centavos, which is equivalent to about 10% of the market price of milkfish fry. The increase of the production cost might not be accepted in domestic hatcheries under competitive marketing with imported fry. Financial and marketing support by the government will be one of the measures to encourage the stable production of domestic milkfish fry with high quality in the Philippines. It is also necessary to conduct institutional campaigns to inform local fry producers and milkfish farmers of the importance of DHA-enrichment.

Discipline: Aquaculture
Additional key words: eicosapentaenoic acid, enrichment, production cost

Introduction

The most important finding in the establishment of fry production technology is that marine fish larvae and fry require dietary docosahexaenoic acid (DHA: 22:6n-3) and eicosapentaenoic acid (EPA: 20:5n-3) for normal growth and survival[17-19]. Marine fish, especially larvae and fry, are unable to biosynthesize DHA and EPA from linolenic acid (LN: 18:3n-3). DHA and EPA have critical functions as the main components of phospholipids of cell membranes. DHA, in particular, plays important roles not only in growth but also in the development of neural tissues and stress resistance during earlier larval stage. Moreover, DHA and EPA improve reproductive performance and egg quality[12]. Indeed, the introduction of EPA and DHA-enriched feeds has enabled hatcheries to carry out stable mass production of marine fish fry with high quality, and broodstock and larvae/fry are given EPA and DHA-enriched feeds in many hatcheries.

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Milkfish *Chanos chanos*, a low trophic level species, is the most important food fish cultured in the Philippines, Indonesia and Taiwan as a cheap protein source. In the Philippines, the annual production of cultured milkfish was over 230,000 metric tons in 2002. Milkfish made up 17.3% of the total cultured fish value and the milkfish industry is still expanding. The national demand for milkfish fry has been estimated to be in the range of 1 to 2.45 billion fry a year[1,3]. Fry availability is one of the major constraints in the development and expansion of any aquaculture industry. Although hatchery production of milkfish fry is now commercially feasible, there still remain some issues in hatchery production: the decline of natural fry resources, the balance between supply and demand, fry quality, marketing, high operational cost, etc[1]. Moreover, there still exists a misconception among milkfish farmers that the quality of hatchery-produced fry is inferior to that of wild fry[8].

In the present study, we first compared fatty acid composition between hatchery-produced and wild-caught fry of milkfish to reevaluate the nutritional quality of the hatchery-produced fry. Subsequently, we investigated the time course change of fatty acid levels, especially DHA, in hatchery-produced fry after hatching. We also estimated the production cost of DHA-enriched milkfish fry.

Materials and methods

1. Fish

Tissue samples (ovary, white muscle and liver) and naturally spawned eggs were obtained from cage-reared broodstock (body weight: 3,000 g to 4,500 g), which were fed formulated broodstock diets[2], at the Igang Marine Substation, Southeast Asian Fisheries Development Center's Aquaculture Department (SEAFDEC AQD) (Guimaras, Philippines).

Wild-caught milkfish fry were obtained from private fry-collectors at Tigbauan (body weight 3.8–6.9 mg, body length 1.2–1.4 mm) (Iloilo), Guimbal (Iloilo) and Hamtic (average body weight 8.5 mg) (Antique). Hatchery-produced larvae and fry were obtained from the Tigbauan Main Station of SEAFDEC AQD. Egg collection, hatching and larval/fry culture were performed according to standard practice at SEAFDEC AQD[4]. Briefly, hatched-larvae were fed *Brachionus* rotifers cultured on *Nannochloropsis* sp. from day 2 to day 14 and then formulated starter diets developed by SEAFDEC AQD[2] from day 15. Larvae and fry at day 0, 7, 10, 14, 21, and 45 as well as juveniles with the body weights of 13.1 g and 22.8 g were sampled.

2. Analyses

All the samples were freeze-dried and pulverized. The dried samples were stored at –80ºC until lipid extraction. The extraction of lipid from the dried samples (about 0.4 g) was carried out with a mixture of chloroform and methanol (2:1, v/v)[9] containing 0.01% butylhydroxytoluene (BHT). Total lipids were separated into polar (PL) and neutral lipids (NL) with a silica cartridge (Sep-pak plus, Waters, Milford, MA, USA) as described by Juaneda and Rocquelin[13]. Fatty acid methyl esters (FAME) were prepared by transesterification with boron-trifluoride in methanol according to the procedure of Miyashita et al.[15] and were purified with thin-layer chromatography (Silicagel 70 Plate, Wako, Osaka, Japan; solvent system: petroleum ether/diethyl ether/acetic acid = 90/10/1, v/v). FAME were analyzed on a gas liquid chromatography (GC-17A; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector (FID) and an Omegawax 320 fused silica capillary column (30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA, USA). The column temperature was initially held at 160ºC for 5 min, followed by an increase at a rate of 4ºC/min to a final temperature of 210ºC. The carrier gas was helium and the pressure was 80 kPa. Individual FAME was identified using a reference standard (Funakoshi, Tokyo, Japan) and known fish meal FAME, and was quantified with an integrator (C-R7A plus; Shimadzu).

3. Statistics

Data were statistically compared between the wild-caught and hatchery-produced fry using Student's *t*-test ($P < 0.05$). One-way ANOVA was used to examine the effect of the time posthatching on each fatty acid level and Fisher's protected least significant difference (PLSD) method was used for mean comparison. Differences were considered significant if $P < 0.05$. All statistical analyses were done by Statview 5.0 Jpn version (SAS, 1998).

Results

Total, neutral and polar lipids in hatchery and wild fry and broodstock tissues are shown in Table1. There were no significant differences in total, neutral and polar lipid levels between hatchery-produced and wild-caught fry. Table 2 shows the whole-body fatty acid composition of wild-caught and hatchery-produced fry (day 21). DHA level in polar lipids of hatchery-produced fry (7.3%) was lower than that of wild-caught fry (19.6%), with a similar trend in neutral lipids. DHA levels of polar and neutral lipids in hatchery-produced fry were only 37% and 18% of wild-caught fry, respectively. By con-

Table 1. Composition of total lipids (TL, % of dry matter), neutral lipid (NL, % of total lipid) and polar lipid (PL, % of total lipid)

Sample	TL	PL	NL	No. of samples
Comparison of hatchery and wild fry				
Hatchery-produced fry[a)]	14.7	66.0	34.0	2 pooled samples
Wild-caught fry[b)]	12.7	62.6	37.4	3 pooled samples
Tissues of broodstock				
White muscle	18.0	12.5	87.5	4
Liver	43.4	13.4	86.6	4
Ovary[c)]	7.1	39.8	60.2	4
Eggs	23.4	70.6	29.4	2 pooled samples
Feeds				
Formulated diets for broodstock	10.6			2 pooled samples
Formulated diets for fry	14.5			2 pooled samples
Nannochloropsis sp.	12.5			2 pooled samples
Rotifers[d)]	13.8			2 pooled sample
Time course changes posthatching				
Day 0	26.7	57.3	42.7	2 pooled samples
Day 7	15.6	46.1	53.9	4 pooled samples
Day 10	16.8	52.4	47.6	2 pooled samples
Day 14	16.1	59.3	40.7	3 pooled samples
Day 21	13.3	62.1	37.9	4 pooled samples
Day 45	10.4	48.9	51.1	3 pooled samples
13.1 g[e)]	15.1	17.0	83.0	2 pooled samples
22.8 g[e)]	16.8	18.0	82.0	2 pooled samples

a): Day 23 fry, body weight 2.6–23.3 mg, body length 1.11–1.68 cm.
The fry were produced according to a standard practice developed by SEAFDEC AQD.
b): Wild fry collected at three different places, at Tigbauan (body weight 3.8–6.9 mg, body length 1.2–1.4 mm) (Iloilo), Guimbal (Iloilo) and Hamtic (average body weight 8.5 mg) (Antique).
c): Average gonadosomatic index 0.6%.
d): *Nannochloropsis* sp.-fed rotifers.
e): Average body weight of fingerlings.

trast, docosapentaenoic acid (DPA: 22:5n-3) levels of polar and neutral lipids in hatchery-produced fry (5.9% and 2.9%) were higher than those in wild-caught fry (0.7% and 0.3%). Arachidonic acid (ArA) levels of hatchery-produced fry were higher than those of wild-caught fry, and there was no difference in EPA level between them. The DHA/EPA and DHA/ArA ratios in polar lipids were 1.2 and 1.1 for hatchery-produced fry and 2.8 and 6.7 for wild-caught fry, respectively. Thus, the hatchery-produced fry had lower DHA/EPA and DHA/ArA ratios than the wild-caught fry.

Table 3 shows fatty acid composition of tissues sampled from SEAFDEC AQD's broodstock which were fed the formulated broodstock diet. Table 4 lists fatty acid composition of live food and formulated diets which are used for fry production and broodstocking[2]. White muscle (18.6%), liver (14.6%), ovaries (14.2%), and especially eggs (29.6%) of the hatchery broodstock had high DHA levels in polar lipids. Although the dietary DHA level in total lipids was 3.1% with the DHA/EPA ratio of 1.0, the DHA/EPA ratios of polar lipids in ovaries and eggs were 6.3 and 15.7, respectively. Thus, the dietary DHA was selectively concentrated in the polar lipid fraction of the ovaries and eggs. Arachidonic acid levels were always higher than EPA levels in white muscle, liver, ovaries, and eggs, although arachidonic acid level (0.5%) was lower than EPA level (3.2%) in the formulated diet. DHA was not detected in total lipids of *Nannochloropsis* sp. and rotifers, which had high EPA levels of 14.0% and 8.3%, respectively. DHA and EPA levels of the artificial starter diet for fry were 2.8% and 2.8%, respectively, with the DHA/EPA ratio of 1.0.

The whole-body DHA levels at day 0, day 14 and day 45 were 24.5%, 4.7% and 13.2% in polar lipids and

Table 2. Major fatty acid composition (mean% ± S.E.) of hatchery-produced and wild-caught milkfish fry

Fatty acids	PL		NL	
	Hatchery	Wild	Hatchery	Wild
14:0	1.2 ± 0.1	1.5 ± 0.1	2.2 ± 0.1*	5.9 ± 0.4
16:0	22.7 ± 0.1	26.8 ± 1.0	26.6 ± 1.3	29.3 ± 3.4
16:1n-7	3.7 ± 0.1*	5.1 ± 0.2	10.2 ± 0.3	13.4 ± 1.4
18:0	10.7 ± 0.1	9.4 ± 0.4	9.6 ± 0.2	6.7 ± 1.0
18:1n-9	9.4 ± 0.3*	7.7 ± 0.2	7.3 ± 0.2	4.7 ± 1.0
18:1n-7	3.7 ± 0.1	3.2 ± 0.1	5.1 ± 0.2	4.6 ± 0.5
18:2n-6	2.3 ± 0.1	1.9 ± 0.1	2.5 ± 0.0	2.2 ± 0.4
18:3n-6	0.21 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.2 ± 0.1
18:3n-3	0.1 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	1.0 ± 0.3
20:1	0.8 ± 0.1	0.4 ± 0.3	1.8 ± 0.1*	0.2 ± 0.1
20:2n-6	0.1 ± 0.0	0.7 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
20:3n-6	2.1 ± 0.1*	0.1 ± 0.0	1.3 ± 0.0*	0.1 ± 0.0
20:4n-6	8.2 ± 0.3*	3.0 ± 0.2	4.5 ± 0.1*	1.4 ± 0.2
20:5n-3	6.0 ± 0.0	7.1 ± 0.3	4.4 ± 0.1	5.1 ± 1.0
22:4n-6	1.3 ± 0.0	0.5 ± 0.3	0.7 ± 0.0	0.3 ± 0.2
22:5n-6	0.5 ± 0.0*	2.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.3
22:5n-3	5.9 ± 1.2*	0.7 ± 0.3	2.9 ± 0.1*	0.3 ± 0.1
22:6n-3	7.3 ± 0.1*	19.6 ± 0.8	1.4 ± 0.0*	7.8 ± 1.3
Others	13.8 ± 1.4	9.2 ± 1.5	18.5 ± 1.9	16.1 ± 3.1
Σsaturate	35.8 ± 0.2	40.2 ± 1.5	40.6 ± 1.8	46.3 ± 5.1
Σmonoene	18.5 ± 0.5	16.6 ± 0.3	25.3 ± 0.7	18.8 ± 0.3
Σn-6	13.6 ± 0.4	7.4 ± 0.4	9.9 ± 0.1	10.6 ± 1.7
Σn-3	20.0 ± 1.2	29.4 ± 1.1	9.8 ± 0.3	21.0 ± 0.8
Σn-3HUFA	19.5 ± 1.2	28.0 ± 1.0	8.9 ± 0.3	18.8 ± 0.8
DHA/EPA	1.2 ± 0.0*	2.8 ± 0.0	0.3 ± 0.0*	1.4 ± 0.1
DHA/ArA	1.1 ± 0.1*	6.7 ± 0.6	0.3 ± 5.4*	5.4 ± 0.3
ArA/EPA	1.4 ± 0.0*	0.4 ± 0.0	1.0 ± 0.0	0.8 ± 0.3

*: Data were significantly different when compared between the wild-caught and the hatchery-produced fry using Student's *t*-test ($P < 0.05$). The wild-caught fry were collected at three different places (see Table 1) and the hatchery-produced fry were reared according to a standard practice developed by SEAFDEC AQD.
PL: Polar lipids, NL: Neutral lipids, HUFA: Highly unsaturated fatty acids, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, ArA: Arachidonic acid, Others: Minor fatty acids + unidentified fatty acids.

6.5%, 0.6% and 1.9% in neutral lipids, respectively (Tables 5 & 6). Thus, during the period when the larvae were fed the rotifers without DHA enrichment, the whole-body DHA level declined with time elapsed after hatching and reached a minimum level at day 14. After the initiation of feeding the artificial diets from day 15, the DHA level was restored to a constant level at day 45 (Tables 5 & 6). The whole-body EPA levels in polar lipids were 2.1% at day 0, 10.8% at day 14 and 3.9% at day 45, and the DPA levels were 0.9% at day 0, 8.9% at day 14 and 2.8% at day 45. Thus, unlike the DHA levels, the EPA and DPA levels increased gradually during the time when the larvae were fed the rotifers and then decreased when feeding the artificial diets. ArA level (6.3% to 7.9%) in polar lipids was relatively stable between day 0 and day 21 and then decreased gradually with time. At day 21, harvesting day for fry selling, DHA/EPA and DHA/ArA ratios were 1.0 and 1.0 in polar lipids and 0.3 and 0.5 in neutral lipids, respectively. Again, the hatchery-produced fry for marketing (day 21) had low DHA/EPA and DHA/ArA ratios compared to the wild-caught fry (Table 2). Although the hatchery-produced fry were fed artificial diets with a substantial amount of DHA from 15 days posthatching, their DHA level on the harvesting day did not reach those of wild-caught fry.

Table 3. Major fatty acid composition of white muscle, liver, ovaries, and eggs of milkfish broodstock

Fatty acids	PL				NL			
	Muscle[a)]	Liver[a)]	Ovary[a)]	Eggs[b)]	Muscle[a)]	Liver[a)]	Ovary[a)]	Eggs[b)]
14:0	0.3	0.4	0.4	0.1	1.6	0.8	1.2	0.3
16:0	15.4	25.4	27.1	22.7	22.7	30.3	26.0	34.2
16:1n-7	0.3	0.9	0.6	0.5	1.9	2.8	1.9	2.4
18:0	8.5	11.9	10.7	12.1	5.4	5.0	7.6	7.7
18:1n-9	18.8	13.0	11.3	13.8	25.1	29.2	22.8	33.2
18:1n-7	1.4	2.0	3.6	3.1	3.4	3.7	3.7	5.4
18:2n-6	9.3	8.0	6.1	3.9	13.1	11.1	10.8	3.5
18:3n-6	0.1	0.6	0.2	0.2	0.2	0.3	0.2	0.2
18:3n-3	0.2	0.5	0.2	0.1	0.7	0.6	0.6	0.1
20:1	0.5	1.2	0.7	0.7	7.2	3.0	3.8	3.3
20:2n-6	1.1	1.1	1.1	0.7	3.7	1.3	2.1	0.6
20:3n-6	2.1	2.1	1.7	2.6	1.1	1.3	1.3	0.9
20:4n-6	8.1	4.1	7.2	4.4	0.4	0.6	2.6	0.6
20:5n-3	6.0	2.3	2.3	1.9	0.4	0.7	1.2	0.3
22:4n-6	0.4	0.2	1.0	0.4	0.4	0.1	0.4	trace
22:5n-6	0.6	0.3	0.4	0.5	0.1	0.1	0.2	0.1
22:5n-3	1.4	0.8	1.1	0.6	1.5	0.7	1.3	0.2
22:6n-3	18.6	14.6	14.2	29.6	3.9	3.2	5.8	3.0
Others	6.9	10.6	10.1	2.1	7.2	5.2	6.5	4.0
Σsaturate	25.6	39.2	39.3	35.1	30.8	36.8	35.7	42.8
Σmonoene	21.9	17.8	17.5	18.2	39.1	39.0	33.1	44.4
Σn-6	21.8	16.4	17.8	12.7	19.2	15.0	17.8	6.3
Σn-3	26.9	18.8	18.6	32.3	7.6	6.0	9.7	3.8
Σn-3HUFA	26.1	17.9	17.8	32.2	6.1	9.0	8.6	3.6
DHA/EPA	3.1	6.4	6.3	15.7	9.4	5.3	2.2	8.8
DHA/ArA	2.3	3.6	2.0	6.7	9.8	4.4	4.9	4.7
ArA/EPA	1.4	1.8	3.2	2.4	1.0	0.9	2.1	1.9

a): Mean value of 4 samples. b): Mean value of 2 pooled samples.

Discussion

Ovary, eggs and newly-hatched larvae from SEAFDEC AQD's broodstock, which were reared with formulated broodstock diets, had high DHA levels especially in polar lipids (14.2%, 29.6% and 24.5%, respectively). However, larval DHA levels decreased drastically from day-0-larvae (24.5% in polar lipids) to day-14-postlarvae (4.7% in polar lipids) and then increased to a constant level in 45-day old fry. Functional and energetic consumption of endogenous DHA in the larvae with no exogenous supply may result in a decrease in DHA level, since *Nannochloropsis* and rotifers fed plankton did not contain substantial amounts of DHA. The starter diets, which had significant amounts of DHA and were fed from day 15, may recover the body DHA level in 45-day old fry. The decrease in DHA level of hatchery-produced fry during the first two weeks after hatching does not seem to be a normal nutritional condition, since the wild-caught fry had 2.7 times (polar lipids) and 5.6 times (neutral lipids) higher DHA levels than the hatchery-produced fry. Fingerling milkfish appear to have the ability to bioconvert EPA to DHA[5,6]. However, it seems that milkfish larvae have the ability to elongate EPA to DPA but can not elongate and/or desaturate DPA to DHA via 24:6n-3 fatty acid, because EPA and DPA levels in the hatchery-produced larvae increased inversely and remarkably during the period from day 0 to day 14, while the DHA level decreased during this period. The decrease in DHA level and the increase in DPA level suggest that milkfish larvae require dietary DHA for growth and survival. Judging from the ample publications proving the essentiality of DHA in larval growth and development of marine fish, the low DHA level observed in the hatchery-produced fry

Table 4. Major fatty acid composition of total lipids of formulated diets and live food

Fatty acids	Formulated diets		Live food	
	Broodstock	Fry	*Nannochloropsis* sp.	Rotifers[a)]
14:0	3.0	4.8	7.1	4.5
16:0	21.1	18.5	35.6	32.0
16:1n-7	4.3	6.2	27.4	16.3
18:0	4.9	4.0	0.9	5.6
18:1n-9	17.4	19.5	3.6	5.2
18:1n-7	3.3	3.6	0.6	4.8
18:2n-6	23.8	11.5	1.6	3.2
18:3n-6	0.2	0.2	0.3	0.5
18:3n-3	2.5	1.5		
20:1	2.2	7.9		1.1
20:2n-6	0.2	0.5		0.1
20:3n-6	0.1			0.7
20:4n-6	0.5	0.3	3.8	5.1
20:5n-3	3.2	2.8	14.0	8.3
22:4n-6	0.1			0.7
22:5n-6	trace			
22:5n-3	0.8	0.6		2.8
22:6n-3	3.1	2.8		
Others	9.3	15.3	5.1	9.1
Σsaturate	30.2	28.6	44.6	44.1
Σmonoene	29.1	38.8	31.8	27.7
Σn-6	24.9	12.8	5.9	10.7
Σn-3	10.6	9.1	14.0	12.2
Σn-3HUFA	7.4	6.7	14.0	11.3
DHA/EPA	1.0	1.0	0	0
DHA/ArA	6.2	10.1	0	0
ArA/EPA	0.2	0.1	0.3	0.6

a): *Nannochloropsis* sp.-fed rotifers.

appears to bring about physiological and physical disorders.

Tamaru et al.[16] investigated the effects of rotifers cultured with various combinations of baker's yeast and *Nannochloropsis oculata* on the growth and survival of milkfish larvae 10 days after posthatching, and they reported that survival was not affected by the combinations and growth was poorest in the larvae fed the rotifers cultured in *N. oculata*. Although the authors discussed the relationship between the performance and DHA levels of the rotifers, they assessed the effects of the live food combinations on survival and growth only in 10-day old larvae, and they did not examine the effect of DHA enrichment on growth and survival.

On the other hand, SEAFDEC AQD has investigated the availability of DHA-enriched live food in milkfish fry production[7,10,11]. Growth at day 40 posthatching and survival at day 26 posthatching in a high salinity stress test were significantly higher in milkfish fed either n-3HUFA-enriched *Chlorella* sp. or n-3HUFA + vitamin C-enriched *Chlorella* sp. than in those fed *Chlorella* sp. without the nutritional treatment[10] (the *Chlorella* sp. was later identified as *Nannochloropsis* sp.). The HUFA + vitamin C treatment alone decreased the incidence of opercular deformities of 40-day old fry[10].

Gapasin & Duray[11] proved by using a more potent DHA supplement that milkfish, which were first reared with DHA-enriched rotifers and *Artemia* during the first 25 days after hatching and subsequently in nursery ponds for another 60 days, showed improved survival in 25-day old fry, no difference in growth for the first 25 days but higher growth for the latter 60 days and a low incidence of opercular deformities in 80-day old juveniles compared to the fish reared with the live food without DHA

Table 5. The time course change of major fatty acids (mean% ± S.E.) in polar lipids after hatching

Fatty acids	Day posthatching						Body weight of fingerlings	
	D 0	D 7	D 10	D 14	D 21	D 45	13.1 g	22.8 g
14:0	0.21 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
16:0	25.1 ± 1.6	28.8 ± 1.9	25.1 ± 0.0	25.5 ± 1.3	26.1 ± 1.2	24.4 ± 0.6	25.0 ± 1.4	25.6 ± 1.9
16:1n-7	0.6 ± 0.1 a	3.1 ± 0.1 cd	4.2 ± 0.0 e	5.2 ± 0.3 f	3.7 ± 0.3 de	2.8 ± 0.2 c	1.7 ± 0.4 b	1.6 ± 0.1 b
18:0	10.1 ± 0.1	13.8 ± 1.5	11.2 ± 0.0	11.1 ± 0.6	11.4 ± 0.4	11.8 ± 0.4	9.6 ± 0.3	9.8 ± 0.3
18:1n-9	14.1 ± 1.5 b	10.1 ± 0.1 a	8.8 ± 0.1 a	7.8 ± 0.2 a	9.0 ± 0.7 a	13.7 ± 2.0 b	15.5 ± 0.1 b	15.6 ± 1.0 b
18:1n-7	2.5 ± 0.0 a	3.1 ± 0.1 b	3.3 ± 0.1 b	4.1 ± 0.1 c	4.1 ± 0.3 c	4.3 ± 0.2 c	3.8 ± 0.1 bc	3.7 ± 0.0 bc
18:2n-6	4.6 ± 1.1 b	2.3 ± 0.4 a	3.6 ± 0.0 ab	2.2 ± 0.3 a	2.2 ± 0.2 a	5.1 ± 1.5 b	9.0 ± 0.3 c	9.5 ± 0.1 c
18:3n-6	0.3 ± 0.1	0.4 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:3n-3	0.2 ± 0.0	trace	trace	0.6 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20:1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	0.8 ± 0.2	0.5 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
20:2n-6	0.7 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	trace	0.2 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
20:3n-6	2.9 ± 0.6 b	1.5 ± 0.2 a	1.6 ± 0.0 a	1.3 ± 0.1 a	1.3 ± 0.3 a	0.9 ± 0.2 a	1.4 ± 0.1 a	1.4 ± 0.1 a
20:4n-6	6.2 ± 1.0 b	6.3 ± 0.6 b	7.5 ± 0.0 b	7.9 ± 0.1 b	7.6 ± 1.1 b	5.0 ± 2.0 ab	2.9 ± 0.1 a	2.8 ± 0.2 a
20:5n-3	2.1 ± 0.2 a	6.0 ± 0.6 bc	9.6 ± 0.1 de	10.8 ± 0.9 e	6.9 ± 1.0 cd	3.9 ± 0.8 ab	2.9 ± 0.1 a	3.1 ± 0.2 ab
22:4n-6	0.4 ± 0.1	0.9 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.2 ± 0.2	0.7 ± 0.4	0.3 ± 0.0	trace
22:5n-6	0.6 ± 0.2	0.6 ± 0.0	trace	0.5 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.0
22:5n-3	0.9 ± 0.2 a	4.5 ± 0.4 b	8.2 ± 0.0 c	8.9 ± 0.5 c	7.7 ± 1.0 c	2.8 ± 1.4 ab	1.4 ± 0.0 a	1.5 ± 0.1 a
22:6n-3	24.5 ± 2.9 d	10.7 ± 0.9 b	5.1 ± 0.0 a	4.7 ± 1.1 a	6.8 ± 0.5 a	13.2 ± 0.4 bc	15.1 ± 0.9 c	15.9 ± 0.5 c
Others	3.0 ± 0.5	7.0 ± 0.4	8.8 ± 0.1	7.8 ± 1.5	10.1 ± 2.5	9.0 ± 1.3	8.6 ± 0.2	6.5 ± 1.5
Σsaturate	36.1 ± 1.4	43.8 ± 3.4	37.3 ± 0.0	37.4 ± 0.9	38.7 ± 1.5	37.4 ± 0.6	35.6 ± 1.2	36.6 ± 1.5
Σmonoene	18.1 ± 1.8	17.1 ± 0.1	17.0 ± 0.1	17.3 ± 0.2	17.6 ± 0.4	21.1 ± 1.7	21.7 ± 0.5	21.7 ± 1.0
Σn-6	15.7 ± 3.1	11.8 ± 1.3	14.9 ± 0.0	13.4 ± 0.2	13.0 ± 1.5	12.8 ± 0.9	14.9 ± 0.8	14.9 ± 0.5
Σn-3	27.6 ± 2.9	21.4 ± 2.4	22.9 ± 0.1	24.8 ± 0.9	21.8 ± 2.1	20.3 ± 2.3	19.8 ± 1.2	20.7 ± 0.9
Σn-3HUFA	27.5 ± 3.0	21.4 ± 2.4	22.9 ± 0.1	24.6 ± 1.0	21.7 ± 2.2	20.0 ± 2.4	19.5 ± 1.2	20.5 ± 0.7
DHA/EPA	11.6 ± 0.1 e	1.9 ± 0.2 b	0.5 ± 0.0 a	0.5 ± 0.1 a	1.0 ± 0.1 a	3.6 ± 0.5 c	5.3 ± 0.1 d	5.1 ± 0.2 d
DHA/ArA	4.1 ± 1.2 bc	1.7 ± 0.0 a	0.7 ± 0.0 a	0.6 ± 0.1 a	1.0 ± 0.1 a	3.4 ± 0.9 b	5.2 ± 0.1 cd	5.8 ± 0.1 d
ArA/EPA	3.1 ± 0.8 b	1.1 ± 0.1 a	0.8 ± 0.0 a	0.7 ± 0.1 a	1.1 ± 0.2 a	1.2 ± 0.2 a	1.0 ± 0.0 a	0.9 ± 0.0 a

Values in the same row with a different letter are significantly different ($P < 0.05$).

treatment. These results indicate that the nutritional condition (DHA) in earlier stage (day 0 to day 14) can affect later performance (growth, deformity, etc.) in post-fry stage, even after shifting feeds from cultured live food to natural food in nursery ponds or artificial feeds. In other words, the improvement of DHA status during the hatchery stage will lead to increased growth, quality and survival of fry and consequently the production of milkfish.

SEAFDEC AQD has developed formulated larval diets for milkfish, which contain 2.8% DHA (Table 4)[7]. The performance of larvae fed combinations of the formulated diets and live food was comparable to that of larvae fed live food alone. This result indicates that the formulated diets could reduce the dependence of milkfish larvae on live food. However, the total replacement of live food by the formulated diets is not yet possible in the larval stage, especially between day 2 and day 15. In other words, DHA, possibly with vitamin C, -enriched live food is required for the normal development of milkfish larvae.

Thus, although SEAFDEC AQD has possessed knowledge on the importance of DHA in milkfish fry production[7,10,11], the knowledge has not been applied to the fry production. When the importance of DHA in the improvement of growth, survival and deformity is considered, it is obvious that hatcheries should produce milkfish fry with DHA-enriched live food. There might have been some obstacles or environments that prevented DHA enrichment of milkfish in domestic hatcheries. At least, DHA enrichment does not seem to be a cost-cutting technology. It is interesting to estimate the cost of DHA enrichment (Table 7). The stocking density of hatched larvae, the density of rotifers in tank, the duration of rotifer-feeding and the average survival to harvest at day 21 were according to hatchery operations developed by SEAFDEC AQD. Given 1,000 to 1,500 rotifers/mL of a

Table 6. The time course change of major fatty acids (mean% ± S.E.) in neutral lipids after hatching

Fatty acids	Day posthatching						Body weight of fingerlings	
	D 0	D 7	D 10	D 14	D 21	D 45	13.1 g	22.8 g
14:0	0.4 ± 0.1 a	1.7 ± 0.4 ab	2.0 ± 0.0 ab	2.3 ± 0.2 bc	2.9 ± 0.7 bc	3.6 ± 0.4 c	2.1 ± 0.1 bc	2.2 ± 0.1 bc
16:0	30.8 ± 0.2	20.4 ± 0.6	23.9 ± 0.0	25.0 ± 2.2	26.3 ± 2.2	25.1 ± 2.4	23.0 ± 0.4	24.8 ± 0.7
16:1n-7	2.3 ± 0.6 a	8.9 ± 0.7 b	10.8 ± 0.1 bc	13.8 ± 1.9 c	10.3 ± 1.9 bc	9.9 ± 1.5 bc	5.8 ± 0.8 ab	6.1 ± 0.2 ab
18:0	8.8 ± 2.1	8.4 ± 0.6	10.0 ± 0.0	11.1 ± 1.3	9.8 ± 0.9	7.8 ± 1.9	5.0 ± 0.6	5.2 ± 0.1
18:1n-9	29.5 ± 0.5 c	8.0 ± 0.3 a	6.6 ± 0.1 a	6.3 ± 1.1 a	10.1 ± 2.1 a	19.9 ± 5.2 b	24.9 ± 0.9 bc	26.1 ± 0.1 bc
18:1n-7	4.8 ± 0.3	3.6 ± 0.1	4.0 ± 0.1	5.4 ± 0.2	5.6 ± 0.9	6.3 ± 1.3	4.3 ± 0.0	4.4 ± 0.2
18:2n-6	4.8 ± 0.9 ab	3.1 ± 0.2 a	4.9 ± 0.2 ab	2.5 ± 0.2 a	3.5 ± 1.0 a	11.0 ± 4.8 b	18.3 ± 0.4 c	17.2 ± 0.2 bc
18:3n-6	0.3 ± 0.1	0.7 ± 0.1	0.9 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	1.8 ± 1.1	0.2 ± 0.0	0.2 ± 0.0
18:3n-3	0.2 ± 0.1	0.4 ± 0.0	trace	0.4 ± 0.2	0.4 ± 0.2	0.8 ± 0.2	1.2 ± 0.0	1.1 ± 0.0
20:1	2.4 ± 0.2	1.0 ± 0.1	1.1 ± 0.0	1.2 ± 0.2	2.3 ± 0.6	1.7 ± 0.3	2.0 ± 0.0	2.1 ± 0.0
20:2n-6	0.6 ± 0.1	0.2 ± 0.0	trace	trace	0.2 ± 0.0	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
20:3n-6	1.5 ± 0.3	1.6 ± 0.2	1.2 ± 0.0	1.0 ± 0.1	0.9 ± 0.3	0.5 ± 0.0	1.0 ± 0.0	0.8 ± 0.1
20:4n-6	1.8 ± 0.4 ab	7.2 ± 1.0 d	5.0 ± 0.2 c	3.1 ± 0.6 bc	3.8 ± 0.6 c	1.3 ± 0.4 ab	1.0 ± 0.1 ab	0.6 ± 0.0 a
20:5n-3	0.7 ± 0.0 a	11.1 ± 0.5 d	8.5 ± 0.3 cd	6.3 ± 2.0 bc	5.1 ± 0.6 b	1.6 ± 0.2 a	1.5 ± 0.0 a	1.1 ± 0.1 a
22:4n-6	0.2 ± 0.0	1.1 ± 0.1	0.9 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	trace	0.2 ± 0.0	trace
22:5n-6	0.2 ± 0.1	0.3 ± 0.0	trace	trace	0.2 ± 0.0	trace	trace	trace
22:5n-3	0.3 ± 0.0 a	5.3 ± 0.6 c	3.9 ± 0.1 c	2.9 ± 0.8 bc	3.0 ± 0.6 c	1.1 ± 0.3 ab	1.1 ± 0.0 ab	0.9 ± 0.0 a
22:6n-3	6.5 ± 0.5 bc	7.1 ± 1.5 c	1.1 ± 0.0 a	0.6 ± 0.3 a	1.7 ± 0.6 a	1.9 ± 0.4 a	3.9 ± 0.1 ab	2.7 ± 0.1 a
Others	4.2 ± 0.1	11.4 ± 1.3	16.3 ± 1.1	18.1 ± 2.3	14.2 ± 1.6	7.4 ± 3.2	3.9 ± 0.7	3.7 ± 0.7
Σsaturate	40.2 ± 1.8	30.5 ± 1.5	36.1 ± 0.2	38.5 ± 3.1	39.4 ± 3.2	36.6 ± 4.0	30.2 ± 0.0	32.5 ± 0.6
Σmonoene	39.0 ± 0.7 d	21.5 ± 0.8 a	22.3 ± 0.3 ab	26.8 ± 2.1 bc	28.3 ± 1.7 c	37.8 ± 2.6 d	36.9 ± 0.1 d	38.7 ± 0.2 d
Σn-6	9.4 ± 1.6	13.9 ± 1.2	12.8 ± 0.4	7.2 ± 1.2	8.8 ± 1.8	14.4 ± 3.6	21.6 ± 0.5	19.6 ± 0.4
Σn-3	7.8 ± 0.5	23.8 ± 2.4	13.6 ± 0.4	10.3 ± 2.9	10.3 ± 1.8	5.1 ± 0.5	8.3 ± 0.0	6.2 ± 0.1
Σn-3HUFA	7.6 ± 0.5	23.8 ± 2.3	13.6 ± 0.4	10.1 ± 3.1	10.0 ± 1.7	4.4 ± 0.5	7.0 ± 0.0	5.1 ± 0.1
DHA/EPA	8.9 ± 0.2 d	0.6 ± 0.1 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.3 ± 0.1 a	1.2 ± 0.3 b	2.6 ± 0.1 c	2.4 ± 0.1 c
DHA/ArA	3.8 ± 1.1 c	1.0 ± 0.1 ab	0.23 ± 0.0 a	0.3 ± 0.0 a	0.5 ± 0.1 a	1.8 ± 0.6 b	3.8 ± 0.1 c	4.2 ± 0.1 c
ArA/EPA	2.6 ± 0.7 b	0.7 ± 0.1 a	0.6 ± 0.0 a	0.5 ± 0.1 a	0.8 ± 0.1 a	0.8 ± 0.2 a	0.7 ± 0.0 a	0.6 ± 0.0 a

Values in the same row with a different letter are significantly different ($P < 0.05$).

Table 7. Estimated cost of DHA-enriched milkfish fry

Density of larvae: **30 larvae/L**
Density of rotifers: **15 rotifers/mL/day**
Number of rotifers/larvae/day: $15 \times 10^3/30 =$ **5×10^2 rotifers/larvae/day**
Survival at 21-day old fry: **30%**
Number of rotifers/day for 10^6 fry production: $5 \times 10^2 \times 10^6/0.3 =$ **1.67×10^9**

Rotifer density in culture tank/mL: **1,000 to 1,500/mL**
DHA supplement/10^6 rotifers at the rotifer density: **0.20 g**
DHA supplement/day for 10^6 fry production: $0.20 \times 1.67 \times 10^9/10^6 =$ **3.34×10^2 g**
Duration of feeding of rotifers: **14 days**
DHA supplement for the 14 days for 10^6 fry production: $3.34 \times 10^2 \times 14 =$ **4.7 kg**

Price of the DHA supplement: **US$100/kg = P5,500/kg**
Cost of the DHA supplement for 10^6 fry production: $4.7 \times 5{,}500 =$ **P25,850**
Cost of the DHA supplement for 1 fry production: $57{,}750/10^6 =$ **2.6 centavos**

rotifer-culture density in tank, feeding rate of DHA supplement was 0.20 g/10^6 rotifers/day[14]. Thus, the cost of DHA enrichment for one milkfish fry was estimated to be 2.6 Philippines centavos. The regular price of milkfish fry is 30–35 centavos/fry in Visaya/Mindanao and 20–23 centavos in Luzon (Chavez, personal communication). The estimated cost is equivalent to about 10% of the market price of milkfish fry. Although milkfish fry producers in Visaya and Mindanao have tried to expand their market to Luzon where imported milkfish fry from Indonesia are oversupplied at a lower price, the increase in production cost of 2.6 centavos could be not accepted by milkfish fry producers and milkfish farmers.

Conclusions

Although DHA enrichment may increase fry production costs, the enrichment would eventually increase economic returns through the high growth, high survival rates and low deformity even after being transferred to culture ponds. It is necessary to conduct institutional campaigns to inform the fry producers and milkfish farmers of the importance of DHA enrichment. Financial and marketing support by the government will also be one of the measures to encourage the stable production of domestic milkfish fry with high quality in the Philippines.

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