PATTERN OF LIPID AND ESSENTIAL FATTY ACID CHANGES IN EARLY DEVELOPMENT OF TIGER GROUPER, *Epinephelus fuscoguttatus* LARVAE

Muhammad Marzuqi, Ketut Suwirya, and Nyoman Adiasmara Giri

ABSTRACT

Development of early stage of larvae depends on body energy content. One of energy source in early larval stage is lipid. Lipid in eggs and early larval development stage of tiger grouper (*Epinephelus fuscoguttatus*) is used for energy and to maintain permeability of membrane cells. Therefore, lipid class for energy decreases and lipid class for maintaining membrane increases. Lipid quality in larvae depends on the content of essential fatty acid. Lipid from larval stage of neurula; 0, 1, 3, 4, 7, 12, 15, 17, and 20 days after hatching were observed on non polar, polar lipid, and essential fatty acid. The result of experiment showed that proportion of non polar lipid from neurula phase to three days larvae (D3) decreased from 81.9% to 36.5% and polar lipid increased from 18.1% to 63.5%. This result showed, that non polar lipid is used for energy and polar lipid for maintaining membrane of cell body. Essential fatty acid, such as EPA and DHA also decreased in non polar and polar lipid. Decreasing essential fatty acids was higher in non polar lipid than these in polar lipid.

KEYWORDS: tiger grouper larvae, lipid, and fatty acid

INTRODUCTION

Development of early stage of eggs and larvae depends on energy content of the body. One of energy source in early life stage of eggs and larvae is lipid. Total length of the larvae increases in line with age. Total length of larvae day-3 longer than that of as day-1 larvae (no fed), so larvae use energy for development and membrane maintenance in the cell body.

Lipid in eggs and in early development larval stage of tiger grouper (*Epinephelus fuscoguttatus*) is used for energy and membrane of cells body maintenance. Therefore, lipid class for energy decreases and lipid class for maintaining membrane increases. Lipid quality in eggs and larvae depends on essential fatty acid content. n-3 highly unsaturated fatty acids (HUFA) are eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are importance in marine fish. Similar fatty acids were reported on striped jack fish (Takeuchi *et al.*, 1992), *Oplegnatus fasciatus* (Kanazawa *et al.*, 1993), red sea bream (Furuita *et al.*, 1996) and humpback grouper *Cromileptes altivelis* (Suwirya *et al.*, 2001). Group of n-3 high unsaturated fatty acids (EPA and DHA) have been reported as important constituents of cell membrane, especially in the brain, and retina (Watanabe *et al.*, 1993) and are needed during early life stages to assure normal visual and neural development (Sargent *et al.*, 1993).

The observation was conducted on early life stage of eggs (neurula phase) and larvae. The purpose of the study was to get biochemical information in utilization for lipid for egg and larval development of tiger grouper (*Epinephelus fuscoguttatus*).

MATERIAL AND METHOD

Samples for analyzing polar, non polar lipid and essential fatty acid were collected from different larval stages: neurula eggs, larvae day-0, day-1, day-3, day-4, day-7, day-12, day-15, day-17, and day-20. These sample used for polar lipid, non polar lipid and fatty acid analysis. The eggs of tiger grouper were incubated in eight fiber tank of 100 liter volume at density of 30 eggs/liter. The eggs were har-

Research Institute for Mariculture Gondol, Bali

vested on neurula phase, day-0, day-1 from two tank, respectively, using plankton net and stored at -20°C before analysis.

The larvae of day-4, day-7, day-12, day-15, day-17, and day-20 were collected from hatchery production tank. Larvae of 7, 12, and 15 day old were transferred to three fiber tanks of 100 liter at density of 15 pcs/liter and no feed for 0, 12, and 24 hour and than harvested.

Total lipids of larval samples were extracted with method of Bligh & Dryer (1959). Lipids were separated into neutral lipid and polar lipids in silica cartridges (Sep-pack, Waters S.A. Milford) (Juaneda & Rockquelin, 1985). The fatty acid metylesters were prepared using Boron Trifluoride and Dichloro-methane with method of Metcalfe & Schmitz (1961). Methylester was analyzed using Chromatography of Antex-3,000 with ionization detector and stainless column BFT 10% DEGS (Diethyleneglycolsuccinate) 80/100 1900la-III. The sample was injected at temperature of 200°C. After 1 minute, the temperature increased at 0,8°C/minute to 230°C and kept at this temperature for 1.5 minutes. Both injector and detector were set by temperature controller and maintained at 220°C. Nitrogen (30 mL/minute) was used as gas carrier. Essential fatty acid integration graphic method was compared with standard solution and the data presented into percentage of total essential fatty acid.

Samples were collected from the same old larvae in triplicate. The average proportion of polar and non polar lipid is presented descriptively. Essential fatty acid was conducted with collect polar lipid every replicated of stage, respectively. The same method was conducted in non polar lipid.

RESULT AND DISCUSSION

Proportion of non polar lipid class of eggs (neurula stage) to day-3 larvae decreased from 81.9% to 40.8% and stable on larvae day-4 to day-20 with range of 43.2%—48.9% (Table 1). EPA and DHA of non polar lipid content on eggs (neurula stage) highly related with larvae on day-1 to day-4. Both of essential fatty acids started to increase after day-4, and larvae started to feed (Figure 1). Proportion of DHA in eggs and larvae on day-0 had higher EPA, However on day- 1 larvae to day-3 showed higher EPA to DHA. In this condition cell membrane developed and DHA is for maintenance. Decreasing non polar lipid in unfed larval body indicated that non polar lipid is energy for metabolism.

Proportion of polar lipid class indicated increased from 18.1% to 59,2% in eggs (neurula phase) to larvae day-3 (Table 1). However, EPA and DHA contents in polar lipid decreased in eggs (neurula phase) to larvae day-3, and then increased again from day-4. DHA was lower than EPA in larvae day-0 to day-4. In contrast, on day-4 DHA content was higher than EPA (Figure 2). These trends are correlated with larval development and no supply DHA from outside of larvae day-0 to day-3 because of unfed. In contrast, with after day-4, started to feed. DHA was unstable in unfed larvae compared with EPA.

Proportion EPA and DHA in polar lipid was higher than EPA, and DHA in non polar lipid (Figure 1 and 2). It indicated that both of fatty acids contained higher in membrane than used as energy. EPA and DHA in polar lipid were more permanent to maintain permeability of cell body.

Larval samples collected from larvae day-7, day-12, and day-15 fasted for 12 to 24 hours indicated that proportion of polar lipid increased and non polar lipid decreased. Both EPA and DHA in polar and in non polar lipid decreased (Table 2). It showed the same trend with larvae on day-0 to day-3. Increasing polar lipid and decreasing non polar lipid of larvae

Table 1. Proportion of polar and non polar lipid of larval body of tiger grouper in early life stage (%)

Lipid class	Stage									
	Eggs	DO	D1	D3	D4	D7	D12	D15	D17	D20
Non polar (%)	81.9	71.1	60.2	40.8	48.9	39.9	36.5	38.7	40.3	43.2
Polar (%)	18.1	28.9	39.8	59.2	51.1	60.1	63.5	61.3	59.7	56.8
Total (%)			100		100	100	100	100	100	100

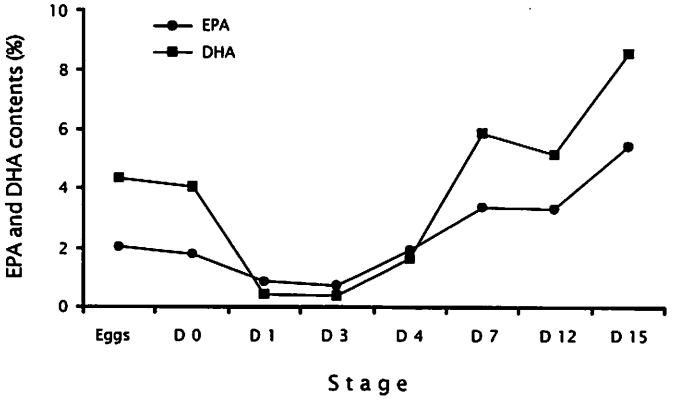

Figure 1. EPA and DHA contents in nonpolar lipid of early larvae stage

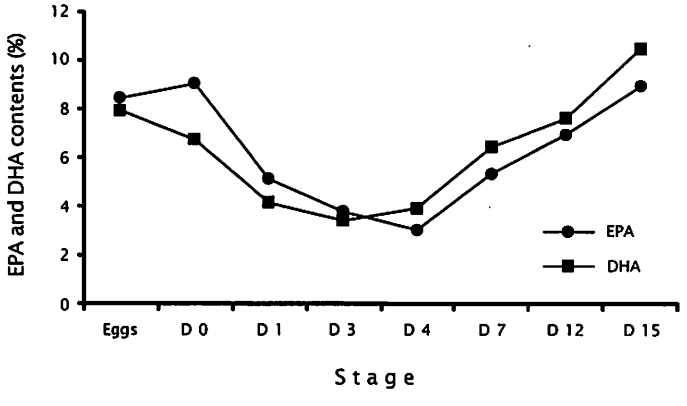

Figure 2. EPA and DHA contents in polar lipid of early larval stage

day-0 to day-3 were faster on day-7, day-12, and day-15, showing that polar lipid used for maintaining membrane cells and non polar lipid used as energy for their growth and development.

These result similar to Watanabe (1988) that lipid was in addition to energy sources, it also important in maintaining structure of cell, body and flexibility integrity of bio membrane.

Larvae of day-7, day-12, and day-15 were fasted for 12 to 24 hours, the ratio of DHA: EPA indicated decreased in polar and non polar lipid, respectively. In metabolism, DHA used more than EPA or EPA can be made from essential fatty acid of lower chain n-3.

EPA and DHA content in polar and non polar lipid of eggs (neurula phase) were higher than those in the larvae. Nutritional values were used by larvae for larval survival and normal development before exogenous feeding.

EPA and DHA concentration of polar and non polar lipid in larvae of day-12 and day-15 fasted for 12 hour increased. These indicated that other fatty acids were used as energy for maintaining of metabolism process. Tandler *et al.* (1995) reported that increasing n-3 fatty acid related to unsupplemented DHA or other HUFAs were used metabolism process.

EPA and DHA concentration of polar and non polar lipid in larvae day-12 and day-15 fasted

Table 2. Non polar and polar lipid proportion and EPA and DHA contents in each lipid class in larvae fasted for 0, 12, and 24 hours

Lipid class/ EPA and DHA	Larval stage and fasting period							
	D 7		D 12			D 15		
	0	12	0	12	24	0	12	24
Nonpolar	39.96	27.73	36.55	35.29	27.44	43.21	41.47	35.48
EPA	3.37	7.29	3.1	4.28	1.7	5.48	5.68	5.07
DHA	5.89	2.87	5.15	5.11	2.04	8.58	9.97	6.78
Other fatty acids	30.70	17.57	28.3	25.9	23.7	29.15	25.82	23.63
Ratio DHA/EPA	(1.75)	(0.39)	(1.66)	(1.12)	(1.2)	(1.57)	(1.75)	(1.34)
Polar	60.04	72.67	63.45	64.71	72.56	56.79	58.53	64.52
EPA	5.30	4.84	6.93	9.35	7.87	8.99	9.38	7.74
DHA	6.46	3.57	7.62	7.86	6.44	10.54	8.57	5.95
Other fatty acids	48.28	64.26	48.90	47.5	58.25	37.26	40.58	50.83
Ratio DHA/EPA	(1.22)	(0.74)	(1,10)	(0.84)	(1.1)	(1.17)	(0.91)	(0.77)
Total (NL+PL)	100	100	100	100	100	100	100	100

for 12 hour decreased. These indicated that there is degradation of fatty acid in body of larvae. Larvae used energy for survive in unfed condition. Similar to Chang & Idler (1960), that in fish lipid is importance as energy during the unfed period.

On larvae of day-4, day-7, day-12, and day-15, DHA in both polar and non polar lipid increased and higher than EPA with ratio 1.10—1.75. High DHA content in fed larvae indicated that DHA was more superior than EPA for growth and survival. The same trend occurred in larvae of Knife Jaw (*Oplegnatus fasciatus*) and Striped Jack (*Caranx delicatissimus*), as reported by Kanazawa (1993) that EPA and DHA are important constituents of cell membrane. However, some study indicated that DHA was more superior than EPA as essential fatty acid (Watanabe, 1983).

Essential fatty acid was higher and stable in larvae day-15 compared to in larvae day-7 and day-12. Larvae of day-15 used energy from essential fatty acid lower for metabolism during unfed. These showed that larvae of day-15 had better vitality than that of larvae day-7 and day-12.

Essential fatty acid in total lipid of early stages of tiger grouper larvae presented in Table 3. It showed that decreasing of EPA slower than DHA in larvae of day-0 to day-3. These showed EPA and DHA ratio of 0.85 in day-0 and 1.11 in day-3. However, fed larvae showed DHA and EPA content increased and showed EPA and DHA ratio between 0.90 and 0.77 in larvae day-4 to day-15, respectively.

Before the larvae were fed larvae develop to utilize essential fatty acid of palmitat (16:0) compared to n-3 HUFA. These indicated essential fatty acid of palmitat (16:0) ratio with n-3 (Table 3) of 3.91 in eggs (neurula phase) and 2.99 in day-3. Larvae started to feed on day-4. Table 3 showed that palmitat concentration in day-4 highly increased, while n-3 HUFA concentration was not different from larvae day-3. Essential fatty acid of palmitat (16:0) ratio to n-3 HUFA in larvae day-4—15 (Table 3) decreased from 6.95 to 1.75. These were caused by low level of n-3 HUFA in diet and increasing level in the body was late.

CONCLUSION

Non polar lipid class was used as energy and polar lipid was used for maintaining membrane cells in early larval stages of tiger grouper (*E. fuscoguttatus*). *E. fuscoguttatus* eggs contained higher DHA than that of EPA, showed that DHA was unstable and important for growth and maintaining vitality of tiger grouper larvae.

Table 3. Fatty acid composition of lipid of tiger grouper larvae in neurula stage; D-0, D-1, D-3, D-4, D-7, D-12, and D-15 (% area)

Fatty acid	Stage							
	Eggs	DO	D1	D3	D4	D7	D12	D15
Myristic acid (14:0)	5.54	4.05	3.71	2.09	4.61	2.74	2.83	2.83
Palmitat (16:0)	29.96	34.68	26.87	20.16	36.74	33.52	32.90	30.11
Palmitoleic acid (16:1n-9)	18.78	17.54	12.42	11.17	15.63	18.02	12.29	18.41
Oleic acid (18:1n-9)	15.82	13.54	9.51	8.17	10.57	8.99	8.59	8.75
Linolenic acid (18:3n-3)	20.60	20.15	32.05	40.17	22.54	20.04	16.86	16.52
Arachidonate acid (20:4n-6)	1.24	0.28	2.02	3.79	0.73	1.04	0.86	0.96
EPA (20:5n-3)	**2.88**	**4.09**	**3.56**	**3.55**	**2.50**	**4.53**	**5.61**	**7.48**
DHA (22:6n-3)	**4.78**	**4.81**	**3.86**	**3.20**	**2.79**	**6.23**	**6.34**	**9.70**
NN*	0.40	0.86	0.00	7.70	3.88	4.90	4.35	5.20
n-3 HUFA	7.66	8.90	6.22	6.75	5.29	10.76	12.33	17.18
Ratio EPA:DHA	0.60	0.85	1.33	1.11	0.90	0.73	0.83	0.77
Palmitat : n-3 HUFA	**3.91**	**3.89**	**3.62**	**2.99**	**6.95**	**3.11**	**2.66**	**1.75**

* non-identified fatty acid

REFERENCES

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physol.*, 37: 911—917.

Chang, V.M. and D.R. Idler. 1960. Biochemical studies on sockeye salmon during spawning migration. XII. Liver Glycogen. *Can. J. Biochem. Physol.*, 38: 553—558.

Furuita, H., T. Takeuchi, M. Toyota, and T. Watanabe. 1996. EPA and DHA requirements in early juvenile red sea bream using HUFA enriched *Artemia* nauplii. *Fish. Sci.*. 62(2): 246—251.

Juaneda, P. and G. Rocquelin. 1985. Rapid and convenient separation of phospholipids and nonphosphorus lipids from rat using silica cartridges. *Lipids*, 20: 40—41.

Kanazawa, A. 1993. Importance of dietary docosahexaenoic acid on growth and survival of fish larvae. *In* C.S. Lee, M.S. Su and I.C. Liao (Eds.) *Finfish Hatchery in Asia: Proceedings of Finfish Hatchery in Asia '91*. TML Conference Proceeding, 3: 87—95.

Metcalfe, L.D. and A.A. Schmitz. 1961. The rapid preparation of fatty acid ester for gas chromatographic analysis. *Anal. Chem*, 33: 363—364.

Nurdjana, M.L. 1986. *Pengaruh Ablasi Mata Unilateral terhadap Perkembangan Telur dan Embrio serta Kualitas Larva Udang Windu (**Penaeus monodon** Fabr.)*. Disertasi Doktor Ilmu Biologi. Universitas Gadjah Mada, Yogyakarta.

Sargent, J.R., J.G. Bell, M.V. Bell, R.J. Henderson, and D.J. Tocher. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. *In*: B. Lahlou and P. Vitiello (Eds.), *Aquaculture: Fundamental and Applied Research*. Coastal and Estuarine Studies, American Geophysical Union, Washington, 43: 103—124.

Suwirya, K., N.A. Giri, dan M. Marzuqi. 2001. Pengaruh n-3 HUFA terhadap pertumbuhan dan efisiensi pakan yuwana ikan kerapu bebek, *Cromileptes altivelis*. *In* A. Sudradjat *et al.* (Eds.) *Teknologi Budi Daya Laut dan Pengembangan **Sea Farming** di Indonesia*. Departemen. Kelautan dan Perikanan, Jakarta, p. 201—206.

Takauchi, T., T. Arakawa, S. Satoh, and T. Watanabe. 1992. Supplemental effect of phospholipid and requirements of eicosapentaenoic acid and docosahexaenoic acid of juvenile striped jack. *Nippon Suisan Gakkaishi*, 58(4): 707—713.

Tandler, A., M. Harel, W.M. Koven, and S. Kolkovski. 1995. Broodstock and Larvae nutrition in gilthead sea bream, *Sparus aurata*. New findings on its mode of involment in improving growth, survival, and swimb inflation. *The Israeli J. Aquaculture-Bamidgeh*, 47: 95—111.

Watanabe, T. 1983. Importance of docosahexaenoic acid in marine larval fish. *J. World Aquaculture Soc.*, 24: 152—161.

Watanabe, T. 1988. *Fish Nutrition and Mariculture.* JICA textbook, The general aquaculture course, 233 pp.

Watanabe, T., C. Kitajima, and S. Fujita. 1993. Nutritional value of live food organism used in Japan for mass production of fish: a review. *Aquaculture*, 155: 103—115.