Journal of Coastal Development ISSN: 1410-5217
Volume 8, Number 3, June 2005 : 187-204 Accredited: 69/Dikti/Kep/2000

Original paper

FATTY ACID PROFILES IN FISH SILAGE MADE FROM VARIOUS MARINE FEEDSTUFFS AS POTENTIAL NUTRITION SOURCES FOR AQUACULTURE FEEDS

Agung Sudaryono *

Aquaculture Study Program, Faculty of Fisheries and Marine Science, Diponegoro University
Jl. Hayam Wuruk 4-A, Semarang, Indonesia.

Received: May, 1, 2005 ; Accepted: May, 15, 2005

ABSTRACT

This study was designed to evaluate fish silage made from various marine feedstuffs (shrimp head, blue crab waste, mud crab waste, squid and tigawaja trash fish) as potential sources of n-3 fatty acids for aquaculture feeds. The marine feedstuffs and the fish silage were analysed for fatty acids contents. Results of fatty acid analysis showed that all the fish silage had higher levels of PUFA (polyunsaturated fatty acid) of linolenic acid (LNA; 18:3n-3) and HUFA (highly unsaturated fatty acid) of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) than the feedstuffs. This study indicates that the bioactive substances contents of n-3 fatty acids (LNA, EPA, DHA) in squid can be significantly improved by manufacturing the fish silage. Fish silage from squid was found to be the most potential source for PUFA (LNA; 5.08 vs 10.72) and HUFA (EPA; 8.07 vs 17.50 and DHA; 7.18 vs 18.08 g/100 g lipid) among the others and suitable for aquaculture feeds.

***Keywords*:** Fish silage; Marine feedstuffs; n-3 fatty acid; PUFA; EPA; DHA

* **Correspondence**: Phone/Fax.: (024) 7450049, E-mail: agungsud@telkom.net

INTRODUCTION

A sustainable aquaculture production can be achieved by one of the ways through improvements in feed formulation, nutrition and technology. The development of cost-effective formulated feed was based on 1) fulfillment of nutritional requirement (energy, protein, lipids, minerals, and vitamins) for targeted aquaculture species and 2) sustainable supply of feedstuffs in high nutritional quality, digestibility and biological values (Latscha, 1991). A nutritionally uncompleted and unbalanced feed can result in adverse effects on fish and shrimp so that it reduces the aquaculture production. Aquaculture feeds with nutritional deficiency can produce a low fish production with a poor health status. Therefore, availability of a nutritionally completed and balanced feed is a critical point and very important requirement for aquaculture industry (Latscha, 1991).

Omega-3 fatty acids are essential nutrients in formulated diets for fish and crustacean aquaculture in order to improve their growth, survival, stress resistance and immune agains disease (Kanazawa *et al,* 1977, 1978, 1979b; Pascual, 1986; Falk-

Petersen *et al*., 1986; Izquierdo, 1996; Mourente and Odriozola, 1990a; Rainuzzo *et al.,* 1991; Nwanna *et al*., 2002). Lipids are required by aquaculture species as a source of energy and nutrients transportation of vitamins A, D, E, and K. Many researchs showed that enrichment of aquaculture feeds with linoleic acid (LOA) and linolenic acid (LNA) have significantly resulted in improvement of growth and survival of fish and shrimp.

Some marine feedstuffs (shrimp head, blue crab waste, mud crab waste, squid, and tigawaja trash fish), good sources of n-3 fatty acids can be improved their nutritional status through the manufacture of fish silage with addition of formic acid. Configuration of unsaturated n-3 fatty acids (eicosapentaenoic acid, EPA; 20:5n-3 and docosahexaenoic acid, DHA; 22:6n-3 in fish silage made from some marine feedstuffs determines the potency of their utilization as a essential source of n-3 fatty acids in aquaculture feeds. This study was designed to explore the contents of n-3 fatty acids extracted from fish silage made from various marine feedstuffs (shrimp head, blue and mud crabs, squid, and trash fish).

MATERIALS AND METHOD

Feedstuffs

Various fresh marine feedstuffs (shrimp head, blue crab waste, mud crab waste, squid, tigawaja trash fish) were obtained from a seafood processing industry in Rembang Regency and a traditional fish market in Semarang, Cental Java Province.

Fish Silage Preparation

The feedstuffs were collected, washed, grounded and mixed with 3% formic acid to manufacture fish silage. The feedstuffs were incubated for 7 days under anaerob and no light conditions and steered 4-5 times a day. Before manufacturing the silage, some samples of the raw feedstuffs were taken for proximate and fatty acid analysis.

Fatty Acid Analysis

The feedstuffs and the fish silage were analysed for the contents of fatty acids following the method of AOAC (1990).

For fatty acid nanlysis, the lipids were first extracted from pooled samples of each marine feedstuffs and fish silage by the method of Bligh and Dyer (1959). Fatty acid methyl ester (FAME) were derivitised by the method of Van Wijngaarden (1967), and separated by capillary gas chromatography (Hitachi 263-50) using split injection on a 30 mm x 0.25 mm i.d. fused silica column coated with 0.25 µm of Durabond-23 (J and W Scientific, Folsom, California). Column temperature was held at 160^{o}C for 10 min and then elevated at 3^{o}C/min to 210^{o}C, where it was held until all FAME of interest had been eluted. FAME were quantified by comparison with the response of an internal standard (heneicosanoic acid methyl ester). FAME were identified by comparing their retention times with those of authentic standards (Sigma Chemical Company, St. Louis, Missouri). Fatty acids contents were calculated by the following equations.

Sample concentration =
total concentration – solvent concentration
Fatty acid (mg/100g) =
$\frac{\text{fatty acid concentration}}{\text{sample concentration}} \times 100$

The data were subjected to a quantitatively descriptive analysis to compare the nutritive contents among fish silage made from various fisheries products.

RESULTS AND DISCUSSION

Results of the fatty acids analysis for the raw feedstuffs and the fish silage are showed in Tables 1 and 2, respectively. Fatty acids analysed are saturated fatty acids of miristic, palmitic, stearic and unsaturated fatty acids of oleic, linoleic, linolenic, arachidonic, eicosapentaenoic (EPA), docosahexaenoic (DHA).

Feedstuff of tigawaja trash fish has the highest level for the miristic fatty acid (6.22 g/100 g), while other feedstuffs (squid, blue crab, shrimp waste) in the form of fish silage tend to have a lower in the meristic (saturated fatty acid, C14:0) than that in the feedstuffs. However, the meristic content of mud crab waste feedstuff increases in the form of the fish silage (6.04 g/100 g) compared to that of the feedstuff (4.55 g/100 g). For another saturated fatty acid, the palmitic (C16:0) decreases in the fish silage except for the mud crab feedstuff (15.76 g/100 g vs. 20.44 g/100 g). The palmitic is available in the highest level in both all feedstuffs and fish silage. Stearic fatty acid contents also sharply decrease in the fish silage except for that in tigawaja fish feedstuff having a similar level to the fish silage (4.55-4.89 g/100 g).

According to the polyunsaturated fatty acid (PUFA) analysis, there is a reduction in the oleic (C18:1n-9) of the feedstuffs after processing to fish silage. The linoleic (C18:2n-6) contents in squid and mud crab silage are higher than those in tigawaja fish, blue crab and shrimp waste. Squid silage was found to have the highest linolenic (C18:3n-3) content in the study. However, squid silage had a lower arachidonic (C20:4n-6) acid level than that in mud crab, blue crab, shrimp waste, and tigawaja trash fish silage. An improvement in PUFA (LNA, C18:3n-3) and HUFA (EPA, C20:5n-3 and DHA, C22:6n-3) levels almost occurs to all the feedstuffs manufactured to be the fish silage (Figures 1, 2 and 3).

Table 1. Fatty acid profiles of various marine feedstuffs

No	Fatty acids	Fatty Acid Contents (g/100 g lipid)				
		Squid	Trash Fish	Mud crab Waste	Blue crab waste	Shrimp Head
1.	Miristic	1.78	6.22	4.55	2.91	2.75
2.	Palmitic	29.76	35.13	31.22	15.76	23.14
3.	Stearic	4.29	4.55	5.89	19.14	7.89
4.	Oleic	31.37	14.83	33.17	44.95	44.45
5.	Linoleic	1.69	5.14	8.31	4.53	4.92
6.	Linolenic	5.08	3.92	0.46	0.42	3.23
7.	Arakidonic	5.25	10.35	2.64	1.91	3.81
8.	EPA	8.07	1.43	1.42	4.25	7.41
9.	DHA	7.18	4.64	4.36	6.27	9.73

Table 2. Fatty acid profiles of fish silage made from various marine feedstuffs

No	Fatty Acids	Fatty Acid Contents (g/100 g lipid)				
		Squid	Trash Fish	Mud crab Waste	Blue crab waste	Shrimp Head
1.	Miristic	1.35	5.02	6.04	1.48	1.84
2.	Palmitic	28.24	30.31	20.99	20.44	20.44
3.	Stearic	1.37	4.89	0.84	3.21	1.38
4.	Oleic	10.77	16.94	21.18	20.30	20.30
5.	Linoleic	7.13	4.58	7.50	3.44	3.21
6.	Linolenic	10.72	5.09	0.75	1.38	3.44
7.	Arakidonic	1.99	6.00	6.75	4.35	4.35
8.	EPA	17.50	2.45	3.84	7.50	7.50
9.	DHA	18.08	5.61	3.89	8.04	8.04

This improvement of LNA and HUFA in fish silage compared to those of the origin feedstuffs might have been attributed to enzymatic process (fermentation) resulted in a change of the substances (Buckle *et al*., 1978). However, mostly some fatty acid contents of miristic (C14:0), palmitic (C16:0), stearic (C18:0), and oleic (C18:1n-9) became lower in fish silage. The decreased change of those fatty acid contents might have resulted in higher contents of LNA and HUFA in fish silage. This is in agreement with Guillou *et al.* (1995) that there was a higher HUFA content in shrimp waste silage of *Pandalus borealis* compared to the origin feedstuff (EPA 43.9% vs 24.7% and DHA 45.5% vs 20.3%). This was particularly evident that improvement in HUFA contents of the fish silage compared to those of the feedstuffs is very required and closely relevant to the potential use of the fish silage as n-3 fatty acid sources in feed formulation for aquaculture. Nutritive value of arctic char (*Salvelinus alpinus*) improved when fed with the diets containing high HUFA (EPA and DHA) (Pigott and Tucker, 1987).

It was reported by Kanazawa and Teshima (1977) and Kanazawa *et al.* (1979a) in *Liptopenaeus japonicus*, Kanazawa *et al*. (1979b) in *Penaeus monodon* and *Penaeus mergueinsis* that these penaeids have shown the absence of *de novo* synthesis of linoleic (C18:2n-6), linolenic (C18:3n-3), EPA (C20:5n-3) and DHA (C22:6n-3) acids from saturated fatty acids (acetate or palmitic acid). Some of these fatty acids may therefore be essential nutrients for penaeids. Furthermore, Kanazawa *et al.* (1977, 1978, 1979b) showed that juveniles of *L. japonicus* had a higher weight gain with diets containing linoleic acid, linolenic acid, EPA and DHA than fed with diets containing oleic acid (C18:1n-9). A similar performance has also reported by Pascual (1986) for *P. monodon*.

Dietary supplements of n-3 PUFAs have been shown to improve reproductive performance in shrimp (Millamena, 1989; Middleditch *et al*., 1979, 1980). The successful addition of the polychaete Glycera dibranchiate to penaeid maturation diets is thought to be due, in part, to its high n-3:n-6 ratio of 9.83 (Lytle *et al*., 1990).

It was confirmed by (Falk-Petersen *et al*., 1986; Castell, 1999) that EPA and DHA are two n-3 fatty acids (HUFA) which are essential for development of embryo and larva of some marine finfish; halibut, red sea bream (Izquierdo, 1996), gilthead sea bream (Mourente and Odriozola, 1990a), and cod (Rainuzzo *et al.,* 1991). It was concluded that growth, survival and activity of marine animal aquaculture species (mainly for mariculture larval species) are significantly influenced by enrichment of EPA and DHA in diets.

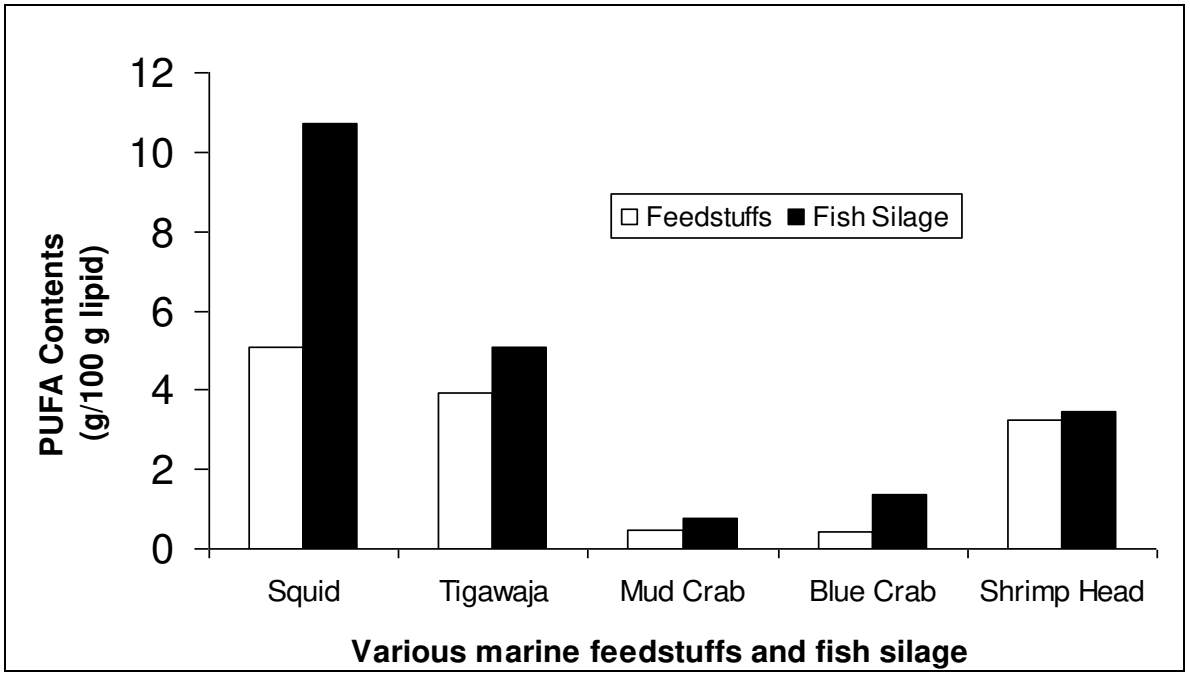


Fig. 1. PUFA (C18:3n-3) Content of various marine feedstuffs and fish silage

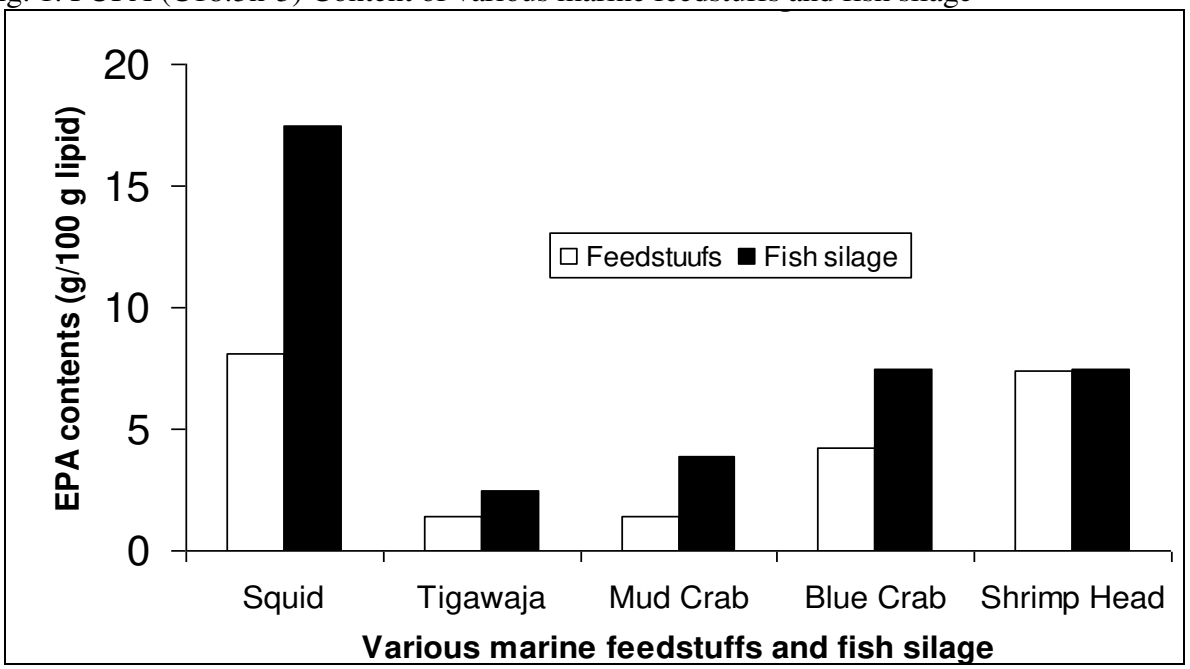
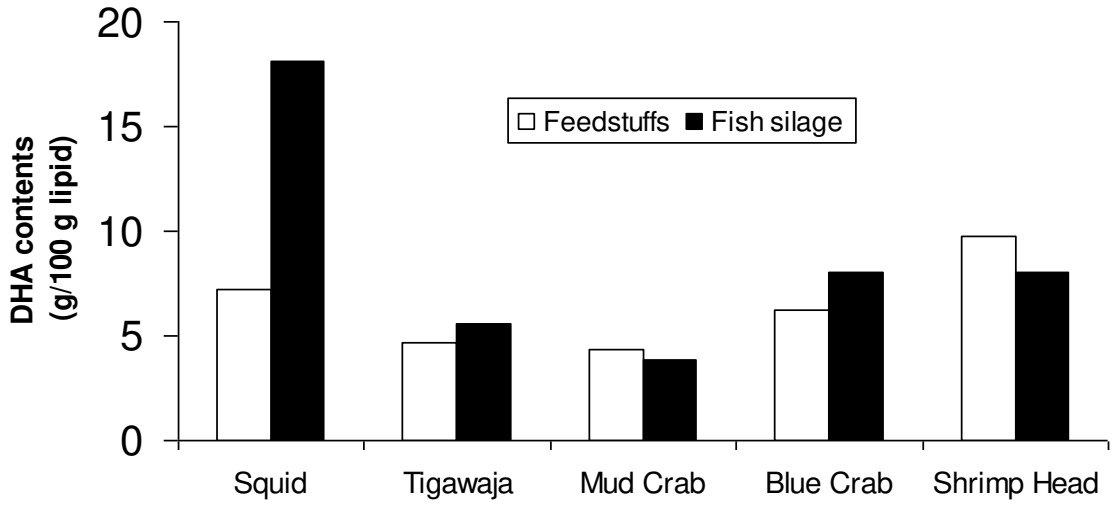


Fig. 2 EPA (C20:5n-3) content of various marine feedstuffs and fish silage

Figure 3. DHA (C22:6n-3) content of various marine feedstuffs and fish silage



As has been proven above that the availability of bioactive contents of n-3 PUFA (LNA) and HUFAs (EPA and DHA) in the diets is essentially required by fish and shrimp for growth and survival especially for the larval stage. It was relevant to this matter, therefore results of this study have shown that the production process of fish silage has effectively provided evidence to improve the LNA, EPA and DHA contents of various marine feedstuffs. As shown in Tables 1 and 2, for example: increased LNA and HUFAs levels of squid feedstuff were found after processing to fish silage (LNA; 5,08 vs 10,72), (EPA; 8,07 vs 17,50) and (DHA; 7,18 vs 18,08), almost all marine feedstuffs also showed a similar performance (Figures 1, 2 and 3).

It was expected that results of the study could become more intense to utilize various marine feedstuffs to be processed into fish silage as potential and essential bioactive nutrition sources of n-3 LNA and HUFA in formulated diets for aquaculture.

All marine feedstuffs used in the study (shrimp head, blue crab waste, mud crab waste, squid, tigawaja fish) has a high potency as sources of n-3 fatty acids (LNA and HUFA) after manufacturing into fish silage products. It was shown that in terms of n-3 fatty acid profile (LNA and HUFA), fish silage of squid showed the best results followed by fish silage of shrimp head and blue crab, respectively. These fish silage were better than those of tigawaja fish and mud crab. The marine feedstuffs (squid, shrimp head, blue crab waste) is available in huge quantity enough especially obtained from seafoods processing plants.

CONCLUSION

Some conclusion addressed from this study are as follows:

1. Palmitic acid is saturated fatty acid available in high levels in both marine feedstuffs and fish silage.

2. A higher content of EPA and DHA were found in fish silage than those in marine feedstuffs.
3. Fish silage of squid is the most potensial as a source of n-3 fatty acids (LNA, EPA and DHA).

ACKNOWLEDGEMENT

We would like to thank Directorate General for Higher Education, Department of National Education that had funded this study through Study and Research Project for Science and Technology with a Letter of Agreement for Implementation of the Basic Research, Ref. No. 68/P2IPT/ DPPM/III/2004 dated the 1st of March, 2004. Special thanks to Dr. Endang Kusdiyantini for her kind support and cooperation in this study. Many thanks to Hartati and Yonedi for their kind assistance in the study.

REFERENCES

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Washington, D.C., 1094 pp.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of lipid extraction and purification. *Can. Biochem. Physiol.*, 35: 911-917.

Castell, J.D. 1999. Review of the lipid requirement of finfish. *In*: K. Tiews and J.E. Havier (eds), Finfish Nutrition and Fish Technology, Vol. 1. Heenemann, Berlin, 59-84.

Falk–Petersen, S., I-B. Falk–Petersen, J.R. Sargent and T. Haug. 1986. Lipid class and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 52, 207–211.

Izquierdo, M.S. 1996. Review article essential fatty acid requirements of cultured marine fish larvae, *Aquaculture Nutrition*. Dpto. Biologia, Univ. de La Palmas de Gran Canaria, Spain, pp. 183–189.

Kanazawa A. 1991. Recent advances in penaeid nutrition in Japan. In: G.L. Allan and W. Dall (eds), Proc. Aquaculture Nutrition Workshop, Salamar Bay, 15-17 April 1991. NSW Fisheries, Barackish Water Fish Culture Research Station, Salamander Bay, Australia: 64-71.

Kanazawa, A., S. Teshima and S. Tokiwa. 1979a. Biosynthesis of fatty acids from palmitic acid in the prawn, *Penaeus japonicus*. *Mem. Fac. Fish*., 28: 17–20

Kanazawa, A., S. Teshima, M. Endo and M. Kayama. 1978. Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus*. *Mem. Fac. Fish*., 27: 35–40

Kanazawa, A., S. Tokiwa, M. Kayama and M. Hirata. 1977. Essential fatty acids in the diet of prawn–I. Effects of linoleic and linolenic acids on the growth. *Nippon Suisan Gakkaishi,* 43: 1111–1114

Kanazawa, A., S. Teshima, S. Tokiwa, M. Kayama. and M. Hirata. 1979b. Essential fatty acids in the diet of prawn–II. Effect of doc-

osahexaenoic acid on growth. *Nippon Suisan Gakkaishi*, 45: 1141–1153.

Latscha, T. 1991. Carotenoids in aquatic animal nutrition. In: D.M. Akiyama and R.K.H. Tan (eds), Proceedings of the Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia, September 19-25, 1991. American Soybean Association, Singapore: 68-79.

Lytle, J.S., Lytle, T.F. and Ogle, J.T. 1990. Polyunsaturated fatty acid profiles as a comparative tool in assessing maturation diets of Penaeus vannameio. *Aqua-culture*, 89: 287-299.

Middleditch, B.S., Missier, S.R., Ward, D.G., McVey, J.B., Brown, A. and Lawrence, A.L. 1979. Maturation of penaeid shrimp: dietary fatty acids. *Proc. World Maricult. Soc.,* 10: 472-476.

Middleditch, B.S., Missier, S.R., Hines, H.B., McVey, J.B., Brown, A., Ward, D.G. and Lawrence, A.L. 1980. Metabolic profiles of penaeid shrimp: dietary lipids and ovarian maturation. *J. Chromatography*, 195: 359-368.

Millamena, O.M. 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilization and hatching in pond reraed *Penaeus monodon. Asian Fish. Sci.*, 2: 127-134.

Mourente, G. and J.M. Odriozola. 1990. Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish Physiol. Biochem.,* 8, 93–101.

Nwanna, L.C., A.M. Balogun and Y.F. Ajenifuja. 2002. Effect of organic-acid fermented shrimp head silage meal on the production of African catfish *Clarias gariepinus*. In: Paper Abstracs of the World Aquaculture Society Conference, Beijing, China 2003. 553 pp.

Pascual, F.P. 1986. Effect of supplemental lecithin and lipid sources on the growth and survival of *Penaeus monodon*, juveniles *in*: J.L. Maclean, L. B. Dizon and L. Y. Hosillos (Editors), First Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines, pp. 615–618.

Pigott, G.M and B.W. Tucker. 1987. Science opens new horizons for marine lipids in human nutrition. *Food Rev. Intl.*, 3: 105-138.

Rainuzzo, J.R., K.I. Reitan and L. Jorgensen. 1991. Fatty acid and lipid utilization in the yolk–sac stage of marine fish larvae. In: Larvi '*91–Fish & Crustacean Larviculture Symposium* (Lavens, P., Sorgeloos, P. P., Jaspers, E. &Ollevier, F. eds), pp. 26–29. Special Publication No. 15, European Aquaculture Society, Gent, Belgium.

Van Wijngaarden, D. 1967. Modified rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem*., 39 (7): 848-849.