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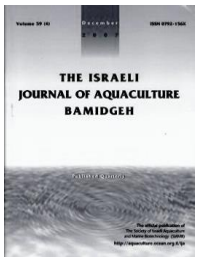

Dietary Soybean Lecithin Enhances Growth Performance, Feed Utilization Efficiency and Body Composition of Early Juvenile Milkfish, *Chanos chanos*

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Keywords: lecithin; phospholipids; soya bean; *Chanos chanos*; juveniles

Abstract

The study was conducted to evaluate the influence of dietary soybean lecithin (SBL) on the growth performance, feed utilization, and survival of early juvenile milkfish. Five experimental diets containing increasing levels of SBL (0 g, 0.75 g, 1.5 g, 3.0 g and 6.0 g/100 g diet) were formulated and fed to triplicate groups of early juvenile milkfish (≈ 9.0 mg) for 50 days. Results showed that dietary lecithin inclusion improved Weight Gain (WG), Specific Growth Rate (SGR), Survival, Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) of milkfish juvenile. Polynomial regression analysis showed that the optimum dietary level of SBL to promote optimum growth in milkfish early juvenile is 3.56g/100g diet. Beyond this inclusion level, growth performance indices tend to decline. Higher carcass total protein and lipid contents were observed in treatment groups fed diets with optimum inclusion levels of SBL compared to the other treatments. Collectively the findings suggest that incorporating SBL at an optimum dose of 3.56g/100g diet enhances survival, overall growth performance, and improves carcass composition of early juvenile milkfish. Dietary SBL inclusion could be a practical approach in improving the efficiency of juvenile milkfish nursery production to meet the requirements of the expanding cage aquaculture production systems.

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Introduction

Industrial milkfish production is currently expanding and gaining dominance in terms of production volume among other aquaculture produce in Southeast Asian countries particularly in Malaysia, Philippines, and Indonesia. Despite the development and expansion of milkfish aquaculture industry in these regions, milkfish production is still hampered by the shortage of fry supply due to low hatchery production, seasonality, and poor spawning performance of broodfish, and inefficient nursery production (FAO, 2014). As pond production intensifies coupled with the expansion of production in cages at municipal waters, demand for juveniles has increased and production of juveniles from the nursery has been identified as a significant constraint to the sustainability and expansion of production (Yap, 2007).

Unlike earthen pond grow-out systems that require direct fry stocking, milkfish sea-cage culture systems requires bigger juveniles (≈30-100g) which are adapted to artificial formulated feeds and can withstand strong water currents. This production system is highly dependent on nursery produced milkfish juveniles reared intensively at high densities in nursery ponds and trained to accept formulated diets. Intensification of nursery production systems has significantly increased juvenile production output as compared to the conventional extensive plankton-based nursery production method. However, efficiency of production in terms of survival and juvenile fish quality is not yet fully optimized and is plagued with low survival (30-40%) and poor performance of juvenile fish in cage grow-out conditions. This inefficient production yield is attributed to the lack of formulated diet adequate for the needs of juveniles since information on the physiology and nutrition of juvenile milkfish is scarce. There have been many earlier studies detailing the nutrient requirements and assimilation of milkfish but most of these were done at the larval stage (Villegas, 1990, Estudillo et al., 1998, Tutas et al., 2013,) and post juvenile (≈50-100g) fish (Benitez, 1989). The importance of the nutrient requirements of early juvenile milkfish (post-metamorphosis, 10mg-10g) for efficient fingerling production in nurseries has just been realized but is yet to be defined.

In larval fish, it is known that lipids, specifically phospholipids (PL), serve as an important source of energy and critically influence larval development, gut maturation, stress resistance, and overall growth performance (Sargent et al., 1999, Cahu et al., 2003, Gisbert et al., 2005). Dietary phospholipids are usually supplied in the form of lecithin in the formulation of fish diets. This nutrient has been reported to elicit lipid emulsification properties that in effect enhance digestion and absorption of feed nutrients including oil soluble vitamins (Kanazawa et al., 1979). Lecithin has also been documented as an antioxidant (King et al., 1992) and a feed-attractant that enhances the acceptability and palatability of formulated diets (Harada, 1987). Significant improvement in growth and survival of several larval fish species including Ayu sweetfish, *Plecoglossus altivelis* (Teshima et al.,1987), rainbow trout, *Salmo gairdneri* (Poston, 1990a), European sea bass and turbot juveniles (Geurden et al., 1997) have been attributed to the inclusion of dietary lecithin in formulated diets fed to these developing fish larvae. Generally, the positive response on growth performance of larval fish to external provision of dietary lecithin is attributable to the limited capacity of the larvae to biosynthesize phospholipids that are essential for enterocyte lipid absorption and a precursor of chylomicrons, functioning as a major lipid transporter molecule of the circulatory system (Fontagne et al. 2000).

Though there have been a number of reports highlighting the quantitative dietary requirement of larval teleost for lecithin (Cahu et al., 2003, Fontagne et al., 2000, Gisbert et al., 2005) information on the requirements of juvenile milkfish for lipids, remains relatively unknown. There is therefore a need to define the quantitative nutrient requirements of juvenile milkfish, and a crucial need to develop formulated diets for intensive nursery culture. The present study aims to evaluate and define the optimum dietary inclusion levels of soya bean lecithin (SBL) on growth, survival, feed utilization efficiency and body composition of early juvenile stage milkfish.

Materials and Methods

Experimental Animal and Design.

Milkfish post-larvae were obtained from the hatchery of the Aquaculture Department, Southeast Asia Fisheries Development Center at Tigbauan, Iloilo, Philippines. Day 22 (post hatch) larvae were selected and acclimatized to laboratory conditions and fed with a commercial milkfish starter diet and pre-metamorphosed rotifers, to the juvenile stage. Following acclimation, 375 early juvenile milkfish (average weight 9.0 mg) were collected and equally distributed into fifteen 30 L aquaria, following a completely randomized design consisting of five experimental treatments run in triplicate. Each experimental aquarium was provided with seawater via a flow-through system with an exchange rate of 5 tank-volumes a day. Salinity was maintained 30-32 ppt, and ample aeration was provided to maintain optimal oxygen concentrations. Removal of organic wastes was done by siphoning and regular monitoring of water quality parameters (salinity, DO, pH, temperature, ammonia and nitrite concentrations) were done twice daily to ensure optimal water quality.

Diets and Feeding.

Prior to the preparation of the test diets, squid meal and fish meal were defatted by ether Soxhlet extraction to remove the phospolipid (PL) contents. Fish oil PL was removed by precipitation with cold acetone as outlined by Liu et al. (2008). Cold acetone was added to the feed oil at a ratio of 5:1 (v:v) for about 20 minutes, PL gradually formed insoluble precipitates that settled. The upper layer of oil was separated from the precipitate and the acetone was recovered from the oil by evaporation. The process was repeated 5 times to ensure complete removal of the PL. The remaining oil was collected and kept in a dry refrigerated place until used.

Experimental diets were sieved through a 100µm mesh and the dry ingredients, including vitamins and mineral mix were thoroughly mixed in a mechanical food mixer. Five experimental diets were formulated at five inclusion levels (0g, 0.75 g, 1.5 g, 3.0 g and 6.0 g/100 g diet) of Soya Bean Lecithin (Sigma-Aldrich, Chemical Co. St. Louis, MO, USA). SBL was weighed and mixed with fish oil, and oil-soluble vitamins were gradually added and mixed with the wet ingredients with water to form a moist dough. The resulting dough was cold-pelleted with a laboratory pelletizer. Pellets were collected, oven-dried at 60^0C, ground and sieved to appropriate size (300 µm) and then stored at 8^0C until use. The composition and major nutrient contents of the basal diet is presented in Table 1.

Table 1. Composition of Experimental Basal Diet

Ingredients	*g/100 g diet*
Fish meal	29
Squid Meal	30
Dextrin	8
Cassava Flour	15
α-cellulose	6
Lecithin	0
[a]Vitamin mix	1
[b]Mineral mix	2
Agar (Binder)	5
Proximate Composition	
Dry Matter	90
Crude Protein	42
Crude Lipid	9.86
Crude Ash	4.02

[a]Vitamin mix; Vitamin A, 1,200,000 IU/kg; Vitamin D3, 200,000 IU/kg; Vitamin E, 20,000 mg/kg; Vitamin B1, 8000 mg/kg; Vitamin B2, 8000 mg/kg; Vitamin B6, 5000 mg/kg; Vitamin B12 1%, 2000 mcg/k; Niacin, 40,000 mg/kg; Calcium Pantothenate, 20,000 mg/kg; Biotin, 40 mg/kg; Folic Acid, 1,800 mg/kg; Ethoxyquin, 500 mg/kg

[b]Mineral Mix: Fe, 40 000 mg/kg; Mn, 10,000 mg/kg; Zn, 40,000 mg/kg; Cu, 4000 mg/kg; I, 1,800 mg/kg; Co, 20 mg/kg; Se, 200 mg/kg

Each tank was allocated to a respective experimental diet fed at 10% of the fish biomass, given three times a day at 08:00, 12:00, and 16:00 hour throughout the 50-day experimental period.

Biochemical Assay.

All analyses were conducted in triplicate. Proximate composition analyses of the diets and carcass were conducted following the established methods of AOAC (2003). Moisture content was quantified by oven drying at 105°C. Experimental diets crude protein was determined by macro-Kjeldahl total protein N analysis and for carcasses Biuret protein quantification was used. Total lipid was determined by the Bligh and Dyer method and total ash by furnace combustion at 55O°C for 12 h.

Growth Performance

Performance was determined as follows:

Specific Growth Rate (SGR) = 100 x (ln [final body weight] – ln [initial body weight])/Culture period (days).

Feed Conversion Ratio (FCR) = Total feed intake (g)/Weight gain (g)

Protein Efficiency Ratio (PER) = Weight gain (g)/Protein intake (g)

% Weight Gain (WG) = (final weight-initial weight (g))/Initial weight (g) x 100

% Survival (S) = (Total number of fish that survived/Total number of fish stocked) x 100

Statistical Analysis.

All data except for weight gain was analyzed using one-way ANOVA using SPSS 16.0. Differences among means were determined by Tukey's test and considered significant at $P<0.05$. Data for weight gain was subjected to a 2^{nd} order polynomial regression analysis to determine the supplementation level that would result to an optimum response.

Results

Following the 50-day feeding trial, the weight gain of juvenile milkfish in response to increasing dietary lecithin inclusion exhibited a second order polynomial curve pattern that increased with the increasing dietary inclusion of lecithin, reaching a maximum peak at 3.0g/100 g, after which, growth tended to decline. The optimum lecithin level needed to elicit a maximum growth response in early juvenile milkfish was estimated at 3.56 g lecithin/100 g diet (Figure 1). Similarly, fish survival was influenced by SBL inclusion levels. Lowest survival occurred in treatments T^0 and $T^{0.75}$. Significantly higher survival was observed in treatments $T^3$and T^6 compared to the control, and treatment with the lowest inclusion levels. Survival trend analysis suggests that milkfish survival peaks at 3.0 g lecithin/100 g diet and tends to decline beyond this inclusion level (Figure 2).

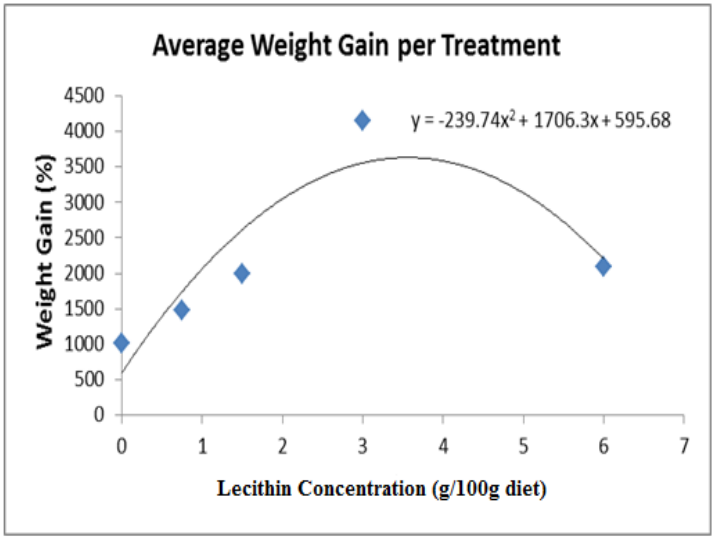

Figure 1. Average weight gain of milkfish larvae fed with different levels of soybean lecithin. Polynomial regression analysis showed that optimum lecithin level is at 3.56%.

In relation to survival, specific growth rate (SGR) followed a similar pattern of response. Treatment group T^3 exhibited the highest SGR (9.12), twice higher than that of the T^0 (5.93) group. The other treatments $T^{0.75}$, $T^{1.5}$, and $T^{6.0}$ exhibited higher SGR values than T^0 but were lower than T^3. The control group T^0, exhibited the lowest SGR rate. Trend analysis suggested that SGR tends to decline beyond the inclusion of 3.0g lecithin/100g diet (Figure 3). FCR responses to dietary SBL inclusion are presented in Figure 4. Dietary treatments without SBL and the low-level dietary inclusion exhibited significantly higher FCR values. Best FCR values occurred in groups $T^{1.5}$, and $T^{3.0}$. Feed conversion ratio significantly increased below and above these inclusion levels.

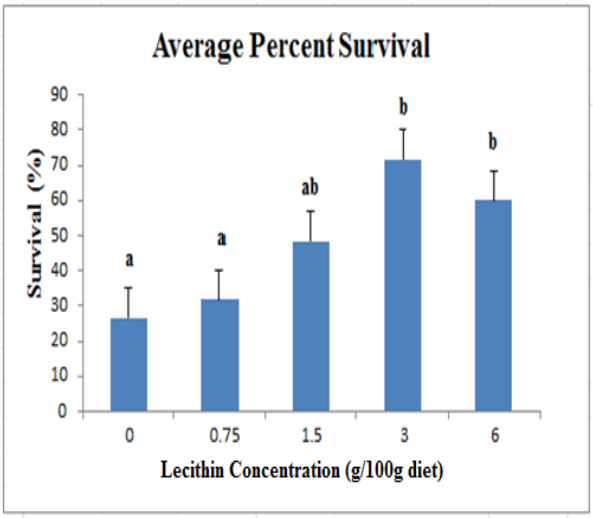

Figure 2. Average Survival (%) of milkfish larvae fed with different levels of soybean lecithin. Bars with similar superscripts are not significantly different.

Similar to other growth-related indices, the highest PER was with $T^{3.0}$, followed by $T^{1.5}$, $T^{0.75}$, and $T^{6.0}$ respectively (Figure 5). No significant differences were found between treatments with $T^{0.75}$, and $T^{6.0}$. Lowest PER was in the T^0 group, indicating that inclusion of soybean lecithin is necessary for better protein utilization for growth.

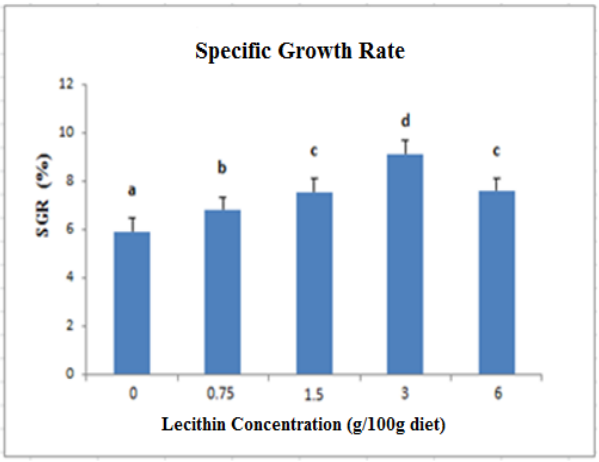

Figure 3. Specific Growth Rate (SGR) of milkfish larvae fed with different levels of soybean lecithin. Bars with similar superscripts are not significantly different.

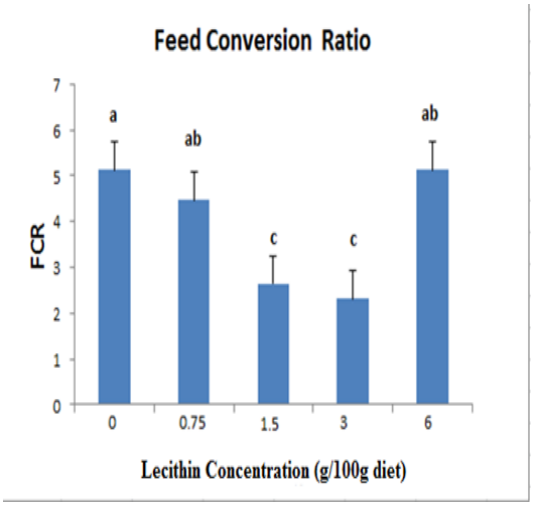

Figure 4. Feed Conversion Ratio (FCR) of milkfish larvae fed with different levels of soybean lecithin. Bars with similar superscripts are not significantly different.

The influence of dietary SBL affects not only growth responses but also affects tissue nutrient composition. Results showed that all experimental groups supplemented with SBL had higher tissue protein and lipid contents than the control treatments (Table 2). Highest tissue protein and lipid contents were observed in treatment $T^{3.0}$, beyond this treatment, decline in tissue protein and lipid contents were observed.

Table 2. Carcass composition (% dry weight basis) of milkfish *Chanos chanos* fed with varying levels of lecithin.

Lecithin Concentration (g/100 g diet)	*Protein*	*Lipid*
0	21.84 ± 0.69[a]	8.03 ± 0.75[ab]
0.75	34.47 ± 0.80[b]	8.39 ± 0.59[ab]
1.5	34.98 ± 0.45[b]	8.64 ± 0.34[ab]
3	41.62 ± 0.86[d]	10.26 ± 0.16[b]
6	31.84 ± 0.52[c]	7.34 ± 0.12[a]

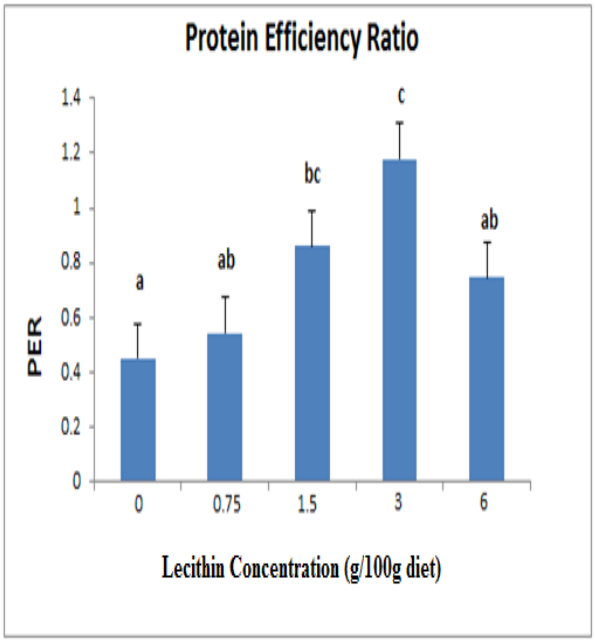

Figure 5. Protein Efficiency Ratio (PER) of milkfish larvae fed with different levels of soybean lecithin. Bars with similar superscripts are not significantly different.

Discussion

Recently, the rising demand for milkfish juveniles for cage grow-out have led to the development of intensive nursery production systems in the aquaculture industry. However, the economic viability of intensive milkfish production is hampered by the lack of developed formulated diets due to the limited information on the nutritional requirements of milkfish juveniles. Phospholipids are known to be an essential nutrient for the development of fish larvae. They play a vital role in larval development, specifically in gut maturation, digestion, tissue growth resistance, and overall growth performance (Sargent et al., 1999, Cahu et al., 2003. The present study investigated the optimum dietary levels of lecithin for milkfish early juvenile. Results suggested that dietary lecithin significantly improves overall biological performances in milkfish in terms of growth, efficiency of nutrient utilization, and survival. The optimum dietary inclusion level of SBL was 3.56 g/100 g diet, comparable to values obtained in the early feeding of rainbow trout *Oncorhynchus mykiss* (Poston, 1990a), rock bream *Oplegnathus fasciatus*, (Kanazawa, 1993), goldfish *Carassius auratus* diet, (Lochmann and Brown 1997), juvenile sturgeon *Huso huso* (Ebrahimnezhadarati et al., 2011), that were all optimal with the inclusion of 4.0 g/100 g diet.

These earlier reports also indicate the significant improvement of growth performance associated with the dietary provision of SBL. Similarly, in the present study, the optimal dose of SBL promoted a five-fold increase in milkfish growth and sixty percent improvement in FCR. The growth enhancement observed with SBL supplementation is attributed to the limited ability of the larvae to bio-synthesize PL to meet the requirement for rapid cellular and tissue growth (Kanazawa et al., 1985). Dietary PL is also essential in the formation of chylomicrons and lipoproteins involved in efficient gut absorption and transport of dietary lipids (Hadas et al., 2003). Additionally, the enhancement of feed palatability (Harada, 1987), reduction of feed-nutrient leaching (Castell et al., 1991) and improvement of digestion (Tocher et al., 2008) are properties of PL associated with enhancement of larval fish performance.

The enhanced growth performance of milkfish juveniles in response to SBL supplementation in the present study is associated with a significant improvement of feed protein utilization (measured as PER) and the significant increase in body protein

and lipid contents. Similar results were also observed in juvenile tilapia *Oreochromis niloticus* which exhibited increased PER and body protein content as a result of being fed diets containing SBL (Ata et al., 2009). Similar to the present findings, significant increase in tissue lipid contents were also reported in other fish species including Atlantic salmon, *Salmon salar* (Poston, 1990b), amberjack, *Seriola dumerili* (Uyan et al., 2009), Dojo loach *Misgurnus anguillicaudatus*, (Gao et al., 2014), juvenile red drum (Craig and Gatlin, 1997) and rainbow trout (Poston, 1990a). The emulsifying property of SBL enhances lipid absorption. Transport and tissue utilization was suggested as the major factor involved in the efficient tissue deposition of lipid and protein in fish larvae (Olsen et al., 1991, Koven et al., 1993). The high protein content of milkfish receiving SBL supplementation could also be attributed to the protein sparing effect of dietary lipids. The efficient utilization of lipids as energy source due to lecithin supplementation may spare protein catabolism for energy production, resulting in increased tissue protein accretion (Catacutan and Coloso, 1995) similar to that observed in the present study.

Though lecithin supplementation at an optimum dose can improve larval performance, excessive dosage inhibits physiological processes associated with growth. In the present study, milkfish juveniles receiving a dose of SBL higher than the optimum resulted in decreased tissue protein and lipid content. These findings are in agreement with those reported in most fish larvae, indicating the detrimental effects of excessive levels of lecithin in the diet (Coutteau, et al., 1997). As a negatively charged molecule, excessive lecithin apparently binds and coats feed particles limiting the enzymatic digestion and absorption of nutrients (Chen and Jenn, 1991).

In conclusion, the optimum dietary dose of 3.56 g lecithin/100 g diet was identified for milkfish early juveniles and the dietary supplementation of SBL is recommended to improve the biological performance in terms of growth, survival, feed utilization efficiency, and body composition of milkfish juveniles.

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References

AOAC., 2003. Official Methods of Analysis. Vol.I.17th ed. Association of Analytical Chemists Washington, DC, USA.

Ata H., Beckan S., and Olmez, M., 2009. The effects of dietary soybean lecithin in the growth performance, feed conversion and body composition of tilapia (*Oreochromis niloticus*) fry. *J Anim Vet Advances*, 8:1678-1684.

Benitez, L. V., 1989. Milkfish nutrition: a review. In R. D. Fortes, L. C. Darvin, & D. L. de Guzman (Eds.), Fish and crustacean feeds and nutrition: Proceedings of the seminar-workshop on fish and crustacean feeds and nutrition held on 25-26 February 1985 at UPV, Iloilo City (pp. 31-34). Laguna, Philippines: Philippine Council for Aquatic and Marine Research and Development.

Cahu, C., Zambonino Infante, J.L., and Barbosa V., 2003. Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of sea bass (*Dicentrarchus labrax)* larvae fed a compound diet. *British J Nutr.*, 90: 21-28.

Castell, J., Boston, L.D., Conklin, D.E., and Baum, N.A., 1991. Nutritionally induced molt death syndrome in aquatic crustaceans II: The effect of B vitamin and manganese deficiencies in lobster (*Homarus americanus*). *Crustacean Nutr News*, 7:108-114.

Catacutan, M., and Coloso, R., 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 131:125-133.

Chen, H.Y., and Jenn, J.S., 1991. Combined effects of dietary phosphatidylcholine and cholesterol on the growth, survival and body lipid composition of marine shrimp, *Penaeus penicillatus*. *Aquaculture*, 96:167–178.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., and Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. *Aquaculture,* 155:149–164.

Craig S.R., and Gatlin, D., 1997. Growth and body composition of juvenile red drum (*Scinenops ocellatus*) fed diets containing lecithin and supplemental choline. *Aquaculture*, 151:259-267.

Ebrahimnezhadarati M., Saad M., Harmin S.A., Satar M.K. and Kenavi, **A.,** 2011. Effects of phospholipids in diet on growth of sturgeon fish juveniles. *J Fish Aquat Sci.*, 6:247-255.

Estudillo, C. B., Duray, M. N., & Marasigan, E. T., 1998. Growth and survival of milkfish (*Chanos chanos*), seabass (*Lates calcarifer*) and rabbitfish (*Siganus guttatus*) larvae reared at the same density in different sized tanks. *The Israeli Journal of Aquaculture-Bamidgeh*, *50*: 20-24.

FAO, 2014. Cultured Aquatic Species Information Programme *Chanos chanos* (Forsskal, 1775). http://www.fao.org/fishery/culturedspecies/Chanos_chanos/en

Fontagne, S., Burtaire, L., Corraze, G., and Bergot, P., 2000. Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth, and lipid metabolism in common carp (*Cyprinus carpio*) larvae. *Aquaculture*, 190:289–303.

Gao, J., Koshio, S., Wang, W., Li, Y., Huang, S., and Cao, X., 2014. Effects of dietary phospholipid levels on growth performance, fatty acid composition and antioxidant responses of Dojo loach, *Misgurnus anguillicaudatus* larvae. *Aquaculture*, 2: 426–427.

Geurden, I., Coutteau, P., and Sorgeloos, P., 1997. Effect of dietary phospholipid supplementation on growth and fatty acid composition of European seabass *Dicentrarchus labrax L.* and turbot *Scophthalmus maximus* juveniles from weaning onwards. *Fish Physiol Biochem., 16,259–272*.

Gisbert E., Villeneuve L., Zambonino-Infante J.L., Quazugel P. and Cahu, C.L., 2005. Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. *British J Nutr.*, 40:609-618.

Hadas, E., Koven, W., Sklan, D., and Tandler, A., 2003. The effect of dietary phosphatidylcholine on the assimilation and distribution of ingested free oleic acid (18:1n-9) in gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture,* 217:577–588.

Harada K., 1987. Relationships between structure and feeding attraction activity of certain L-amino acids and lecithin in aquatic animals. *Nippon Suisan Gakkaishi*, 53:2243-2247.

Kanazawa A., 1993. Essential phospholipids of fish and crustaceans. *Fish Nutrition in Practice IV*. *International Symposium on Fish Nutrition and Feeding*, National Institute for Agricultural Research, France. pp. 519-530.

Kanazawa A., Teshima S. and Sakamoto, **M.,** 1985. Effects of dietary lipids, fatty acids and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. *Aquaculture,* 50:39-49.

Kanazawa A., Teshima S., Tokiwa S., Endo M. and Abdel Razek, **F.A.,** 1979. Effects of short –necked calm phospholipids on the growth of prawn. *Bull Japanese Soc Fisheries Sci.*, 45:961-965.

King M.F., Boyd L.C. and Sheldon, **B.W.,** 1992. Effects of phospholipids on lipid oxidation of a salmon oil model system. *J American Oil Chemists Soc*, 69:237-242.

Koven W.M., Kolkovski S., Tandler A., Kissil G.W. and Sklan, D.,1993. The effect of dietary lecithin and lipase as a function of age on n-9 fatty acid incorporation in the tissue lipids of *Sparus aurata* larvae. *Fish Physiol Biochem.*, 10:357-364.

Liu, D.C., Tao, Y.H., Sun, F.Z., and Li, Y.L., 2008. Extraction and Purification of Phosphatidic Acid of C18 Fatty Acids from Powdered Soybean Phospholipids. *Asian J Chem.*, 20: 5595-5600.

Lochmann, R., and Brown, R. J., 1997. Soybean lecithin supplementation of practical diets for juvenile goldfish *(Carassius auratus). J American Oil Chemists' Soc.*, 74: 149.

Olsen, R., Henderson, R.J., and Pedersen, T., 1991. The influence of dietary lipid classes on the fatty acid composition of small cod (*Gadus morhua* L.) juveniles reared in an enclosure in northern Norway. *J Experim Mar Biol Ecol.*, 148:59-76.

Poston, H. A., 1990a. Performance of rainbow trout fry fed supplemental soy lecithin and choline. *Progressive Fish Culturist*, 52:218–225.

Poston, H. A., 1990b. Effect of body size on growth, survival, and chemical composition of Atlantic salmon fed soy lecithin and choline. *Progressive Fish Culturist*, 52:226–230.

Sargent, J., Bell, G., Mc Evoy, L., Tocher, D., and Estevez, A., 1999. Recent developments in the essential fatty acid nutrition in fish. *Aquaculture*, 177:191-1999.

Teshima S., Kanazawa A., Horinouichi K., Yamasali S. and Hirata, **H.,** 1987. Phospholipids of rotifer, prawn and larval fish. *Nippon Suisan Gakkaishi*, 53:609-615.

Tocher D., Bendiksen E., Campbell P., and Gordon Bell, **J.,** 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, 280:21-34.

Tutas L. B., Serrano Jr. A. E., Traifalgar R. F. M., and Corre V. L., 2013 Optimum dietary levels of Vitamin A (retinyl palmitate) for growth and reduction of incidence of operculum deformity in milkfish (*Chanos chanos*) fry. *AACL Bioflux*, 6:464-469.

Uyan, O., Koshio, S., Ishikawa, M., Yokoyama, S., Uyan, S., Ren, T. and Hernandez, L.H.H., 2009. The influence of dietary phospholipid level on the performances of juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. *Aquacult Nutr.*, 15: 550–557.

Villegas, C. T., 1990. The effects on growth and survival of feeding water fleas (*Moina macrocopa Straus*) and rotifers (*Brachionus plicatilis*) to milkfish (*Chanos chanos Forsskal*) fry. *Israeli Journal of Aquaculture-Bamidgeh*, *42*:10-17.

Yap W., 2007. Milkfish production and processing technologies in Philippines. *Milkfish project publication series No. 2*, 96pp.