AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Optimum dietary levels of Vitamin A (retinyl palmitate) for growth and reduction of incidence of operculum deformity in milkfish (*Chanos chanos*) fry

Leslie B. Tutas, Augusto E. Serrano Jr., Rex F. M. Traifalgar, Valeriano L. Corre

Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines. Corresponding author: A. E. Serrano Jr., serrano.gus@gmail.com

Abstract. A feeding trial was conducted to assess the effects of dietary level of Vitamin A on growth, survival and incidence of deformity in milkfish fry from 21 to 51 days posthatch (dph). Milkfish fry were fed with experimental diets containing increasing levels of Vitamin A (0 IU, 100 IU, 1000 IU, 2000 IU and 4000 IU 100g-1 feed) for 30 days. Results showed that Vitamin A did not affect survival of milkfish fry at all dietary levels but affected specific growth rate (SGR) and the incidence of open operculum deformity. Values for the proportion of the absence of deformity in milkfish fry (minus that for the highest level of 4000 IU 100g-1 diet) fit the quadratic model for the estimate of the optimum dietary Vitamin A level; maximum response (*Imax*) was estimated to be 1,079 IU.
Key Words: Vitamin A, retinyl palmitate, *Chanos chanos*, open opercular deformity.

Introduction. Milkfish is one of the most popularly cultured species in the Philippines, as well as in Indonesia and Taiwan. Currently, the milkfish industry in the Philippines is expanding but problems of inadequate fry supply and high farming cost are considered as the limiting factors (BFAR 2008). Since milkfish production comes almost solely from aquaculture, supplies of fry for grow out plays an important role in the development of the industry. Larval mass mortalities and incidence of opercular and branchiostegal deformities are the major problems limiting its sustainability. Proportions of abnormalities in fry captured from the wild are not similar to those reared in tanks (Hilomen-Garcia 1997; Martinez et al 2006). High mortality and slow growth are associated with the deformities in the branchiostegal membrane and operculum of fry used in grow-out culture.

Enrichment of rotifers with highly unsaturated fatty Acids (HUFAs) and Vitamin C before offering to milkfish fry have been done to eliminate larval deformities but is met with limited success. The mechanism leading to the development of abnormalities in the opercular region of milkfish fry is not yet fully understood (Martinez et al 2006). In vertebrate morphogenesis, Vitamin A takes part in determining the body patterning and growth (Fernández et al 2011). It has been suspected to play an important role in the occurrence of skeletal malformation in hatchery-reared fish larvae (Cahu et al 2003).

Vitamin A, a fat-soluble vitamin, is derived from retinoid and carotenoid. It occurs in the form of an alcohol (retinol), aldehyde (retinal), esters (retinyl actetate/palmitate), acid (retinoic acid) and pro-Vitamin A carotenoids (b-carotene, a-carotene, etc.). Vitamin A is a stimulus for new cell growth, an aid in maintaining resistance to infection and is essential in maintaining epithelial cells (Halver & Hardy 2002). Moreover, excess or deficiency in Vitamin A in fish have been documented to result in poor growth, exophthalmia, abnormal bone formation, hemorrhages of the eye and the base of the fins. In addition, retinoic acid, the active form of Vitamin A, is essential in embryonic

development and formation of body and organ axes in vertebrates (as it affects the expression of *Hox* genes (Hamre et al 2010; Villeneuve et al 2005).

In higher vertebrates, excess intake of vitamin A affects an individual over a short period of time at any stage of its life. In fishes, most studies conducted that involve the effects of Vitamin A focus on fry starting 8 days post-hatching (dph) and are done at an average of 40 day-culture period. In milkfish, the transformation stage is between 21 dph to 51 dph (Kumagai 1990; Martinez et al 2006). Since previous studies start at 8 dph, there is no data available on the effect of deficiency or excess levels of Vitamin A in the critical metamorphosis stages of larval milkfish. This study aims to determine the level of dietary retinyl palmitate that would elicit optimum growth, survival and normal development in 21-day old milkfish fry.

Material and Method

Experimental diets. Five experimental diets were prepared containing various levels of Vitamin A (retinyl palmitate) at 0, 100, 1000, 2000 and 4000 IU 100 g^{-1} (Diets 1-5) of formulated diet in a randomized complete block design (RCBD) with three replicates for each dietary treatment (Table 1).

Table 1

Diet composition per 100 g

Composition	*Diet 1 (Control)*	*Diet 2 (100 IU)*	*Diet 3 (1000 IU)*	*Diet 4 (2000 IU)*	*Diet 5 (4000 IU)*
Fish meal (g)	29.0	29.0	29.0	29.0	29.0
Squid meal (g)	30.0	30.0	30.0	30.0	30.0
Dextrin (g)	8.0	8.0	8.0	8.0	8.0
Cassava flour (g)	20.0	20.0	20.0	20.0	20.0
Mineral mix (g)	2.0	2.0	2.0	2.0	2.0
Carboxymethyl cellulose (g)	1.7	1.7	1.7	1.7	1.7
Vitamin mix (mg)	0.2	0.2	0.2	0.2	0.2
Fish oil (g)	4.0	4.0	4.0	4.0	4.0
Binder (g)	5.0	5.0	5.0	5.0	5.0
Retinyl palmitate (IU)	0.0	100	1000	2000	4000

All dry ingredients including squid meal and fish meal were defatted and sieved prior to diet preparation while retinol palmitate was serially diluted using fish oil to obtain the desired level. All wet ingredients were weighed and mixed with the dried ingredients. The binder was then dissolved in 80 mL hot water and gradually added to the diet mixture. The diet was then dried in an oven at 80 ^{o}C for 4 h and was passed through a 100 µ sieve. Diets were stored at 4 ^{o}C until use.

Experimental fish and dietary trial conditions. Twenty–one day post-hatched (dph) milkfish fry were obtained from the milkfish hatchery of Southeast Asia Fisheries Development Center Aquaculture Department (SEAFDEC - AQD), Tigbuan, and transported to the hatchery of the University of the Philippines Visayas, Miagao, Iloilo, Philippines. At the start of the experiment, 30 fish were sacrificed to measure the initial weight and upon termination, all fish were batch weighed. Fry were acclimatized to the laboratory conditions before transferring to the experimental containers. Fish were randomly distributed to 15 aquaria (30 L) at a density of 30 fry per aquarium in a static system. Aquaria were provided with continuous mild aeration and about 70 % water replacement with premixed seawater (salinity of 30 ppt) was done every 2 days.

Fry were fed at 20 % body weight (BW), three times daily (800, 1200, 1600), and was decreased to 15 % BW after the 15th day of experiment. Thirty percent of the water was replaced every 48 h. Sampling was done after the 15th day and feeding rate

was adjusted correspondingly. Upon termination of the experiment on the 30th day, fry were batch weighed and sacrificed to check for deformity. Vertebral columns were not checked for malformations since it was already developed during the post-flexion stage (14dph). Also, length was not recorded.

Response parameters. Feed conversion efficiency (FCE) and specific growth ate (SGR) were estimated as follows:

FCE = (Fish weight gain/feed fed) x 100
SGR = (Log_e final weight – Log_e initial weight) / Time (days)

Fish were individually checked for opercular deformity. The fish was held with both hands with the ventral side on top and with the thumb, the head was slightly pulled downwards and checked for opercular malformations such as folding or incomplete formations, open branchiostegal rays or membrane opening.

Estimate of optimum level of dietary Vitamin A. The dietary optimum level of Vitamin A was estimated using the quadratic model (Forster 2000) with the value for the diet containing 4000 IU treated as an outlier so that the data satisfactorily fit the quadratic equation. Had we included the highest dose, the best fit would be a polynomial equation to the third power; this would not have reflected the 'actual' behavior of the curve with a minimum value corresponding to a numerically negative response (thus, was not realistic). A quadratic equation of the form $R = a + bI + cI^2$ was used to fit the response data where R is the measured response (% normal operculum); I is the dietary Vitamin A level; and a, b, and c are constants that are calculated to provide the best fit of the data. The value of I that produces the optimum response I_{max} is calculated as $I_{max} = -0.5\ (b/c)$.

Statistical analysis. Results are given as mean ± standard error of the mean. Data expressed as percentage (SGR, FCE, survival, and incidence of open operculum deformity) were logged- transformed prior to analyses. Means were compared by One-way ANOVA and if differences were significant, Tukey post hoc analysis was performed. In all statistical analyses, the level of significant difference was set at α= 0.05 using the software SPSS version 16.0.

Results and Discussion. Results showed that there were no significant differences among treatments in feed conversion efficiency and survival (Table 2). However, significant differences in SGR and incidence of open operculum deformity were observed. The dose-response curve involving the proportion of milkfish fry that did not exhibit the deformity was fitted to a quadratic equation and the estimated dietary level that elicited the maximum response (i.e. maximum percent normal operculum fry) was determined to be 1,079 IU (Figure 1).

Table 2

Survival, growth and nutrient utilization of milkfish fry fed the experimental diets

Dietary Vitamin A level	*Survival*	*SGR*	*FCE*	*% Normal operculum (100-IOD*)*
0 IU	60.03 ± 8.8	1.78 ± 0.32^{b}	21.63 ± 4.58^{b}	16.50 ± 8.96^{d}
100 IU	51.10 ± 9.7	1.18 ± 0.26^{c}	24.89 ± 4.15^{ab}	37.03 ± 1.97^{b}
1000 IU	51.13 ± 8.7	2.41 ± 0.13^{a}	35.63 ± 0.21^{a}	64.97 ± 5.61^{a}
2000 IU	60.00 ± 3.3	1.27 ± 0.14^{d}	28.00 ± 1.31^{b}	26.03 ± 5.94^{c}
4000 IU	45.57 ± 4.4	1.88 ± 0.23^{b}	25.88 ± 4.09^{ab}	20.43 ± 10.3^{c}
P	NS	≤0.05	NS	≤0.05

*Incidence of open operculum deformity

Milkfish fry fed with the control diet and with diets containing extreme levels of Vitamin A (i.e. 2000 and 4000 IU) resulted in very low proportion of normal milkfish (i.e. high incidence of open operculum deformity, IOD), while those fed diets containing 1000 IU $100g^{-1}$ diet showed significantly the highest proportion of normal milkfish fry (i.e. lowest IOD) (Table 2). The other forms of opercular deformity such as the folded operculum and incomplete operculum deformities were not observed in all groups in the present study.

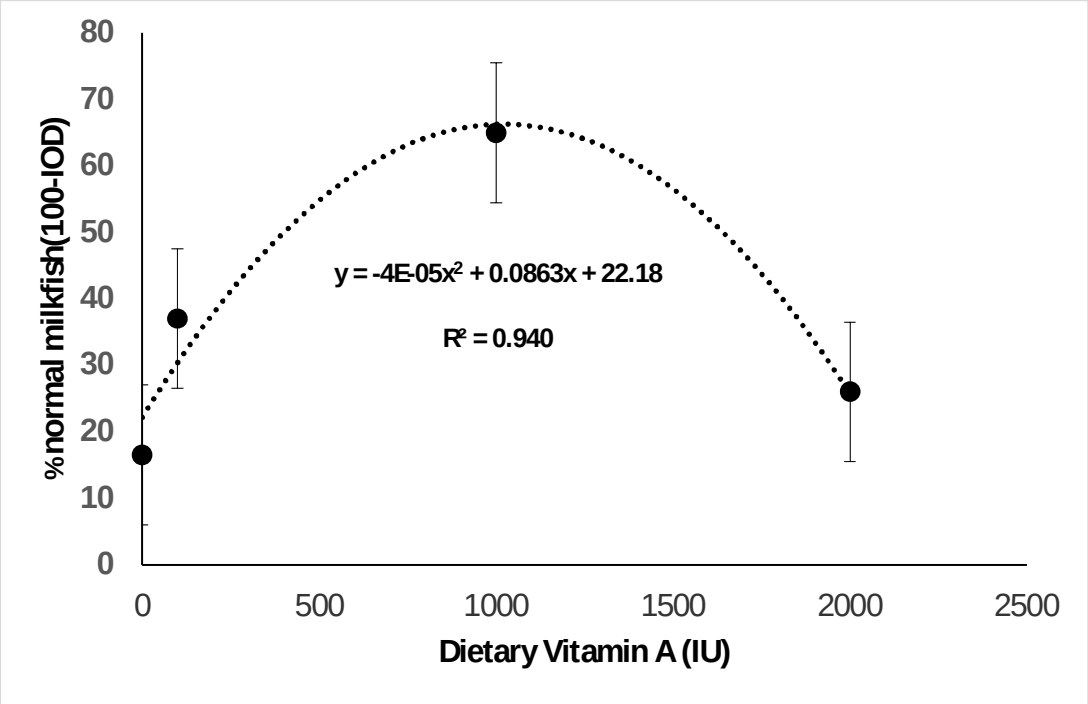

Figure 1. Quadratic fitting of proportion of normal milkfish (i.e. absence of open operculum deformity) to determine the optimum dietary level of Vitamin A for normal operculum.

It was clear in the present study that specific growth rate was affected by the level of Vitamin A incorporated in the diet. It was significantly the highest at 1000 IU level and corresponded to the estimated optimum level of 1,079 mg kg^{-1} diet (i.e. IU) diet using the data on the incidence of open opercular deformity. As far as we know, there is still no documented mechanism on how Vitamin A affects the growth of fish. It was highly probable that poor vision and reduced thyroid hormones could have affected the growth of milkfish in the present study since milkfish fry are highly dependent on vision for feeding. Deficiency or excess of Vitamin A cause degeneration of the retina, exophthalmia and reduced growth in salmonids, *Cyprinus carpio, Lepomis panctatus* and *Poecilla reticulata* (Tacon 1992). In higher vertebrates, Vitamin A (either in palmitate or retinoic acid form) is shown to affect thyroid hormone synthesis (Braathen et al 2004; Garcin et al 1984; Taruoura et al 1991). Thyroid hormones affect the metabolic rate, protein synthesis and cell differentiation in fish (Halver & Hardy 2002). Thus, thyroid hormones could have partially affected the growth of milkfish in the present study.

Complete absence of Vitamin A could be lethal to fish (Villeneuve et al 2005) but survival in the present study were not significantly different in fish fed diets containing all levels of the vitamin including those fed the control diet which supposedly did not contain the vitamin. Fish oil presumably contained a very small amount of Vitamin A enough for the survival of the fry within the 30-day feeding trial. High levels of Vitamin A could be toxic to fish and could also affect the spleen and digestive (Halver & Hardy 2002) and this could be the explanation why the performance of milkfish fry at extreme levels of 2000 and 4000 IU were very poor.

Growth and survival data obtained from the present study were comparable to those obtained in the European sea bass (Mazuraisa et al 2009; Villeneuve et al 2005) and in gilthead sea bream (Fernández et al 2008, 2009) where fish fed diets containing extreme dosages of Vitamin A exhibit lower growth rates. In the European sea bass, the highest growth rate is exhibited by the fish fed the diets with 0.5 mg 100 g^{-1} diet (i.e. 1000 IU 100g^{-1}) which was similar to the result of the present study.

Results of the present study indicated that Vitamin A affected the development of milkfish operculum in fry stages. Fish opercula are primarily consisted of protein, collagen, and calcium (Davis & Lusk 2012). Collagen is the underlying matrix of the bone which provides its shape and matrix (Ott et al 2003). In higher vertebrates, collagen is decreased by retinol in a dose-dependent manner, but the studies conducted are *in vitro* and the degree of effects of Vitamin A is also dependent on the incubation period (Dickson & Walls 1985). Excessive levels of Vitamin A overstimulates cells called osteoclasts, which break down bone, and may interfere with the bone supporting activities of Vitamin D such as calcium absorption (Andrews 2011). The open operculum deformity observed in the present study was in conformity to the observations in the European sea bass (Mazuraisa et al 2009; Villeneuve et al 2005), gilthead sea bream (Fernández et al 2008) and Senegalese sole (Fernández et al 2008, 2009) where higher levels of Vitamin A resulted in higher malformation in the cranial, more particularly in the jaw and opercular regions. In sea bass, high dietary Vitamin A levels strongly affected early development (specifically before 20 dph) and the effects decrease in intensity after this stage (Cahu et al 2003). This was not observed in the present study probably because the opercula of the fish were already developed prior to the feeding experiment.

Conclusions. Vitamin A did not significantly affect survival and food conversion efficiency but significantly affected the specific growth rate and incidence of open opercular deformity when the supplemented diets were fed from 21 to 51 dph. The estimated optimum dosage of Vitamin A that resulted in the lowest incidence of open operculum deformity in milkfish fry was 1,079 IU 100g^{-1} diet.

References

Andrews T., 2011 Excess vitamin A and bone fractures. http://www.livestrong.com/article/418460-excess-vitamin-a-and-bone-fractures

BFAR. 2008 Fisheries Commodity Road Map: Milkfish. Quezon City, Philippines.

Braathen M., Derocher M., Wiig Ø., Sørmo E., Lie E., Skaare J., Jenssen B. M., 2004 Relationships between PCBs and thyroid hormones and retinol in female and male polar bears. Environ Health Perspect 112(8):826-833.

Cahu C., Zambonino J.-L., Takeuchi T., 2003 Nutritional components affecting skeletal development in fish larvae. Aquaculture 227:245-258.

Davis A. P., Lusk J. D., 2012 Chapter 2. Rio Grande Silvery Minnow Health Assessment. Albuquerque, NM: U.S. Fish and Wildlife Service New Mexico Ecological Services Field Office.

Dickson I., Walls J., 1985 Vitamin A and bone formation. Effect of an excess of retinol on bone collagen synthesis *in vitro*. Biochem J 226(3):789-795.

Fernández I., Darias M., Andree K. B., Mazurais D., Zambonino-Infante J. L., Gisbert E., 2011 Coordinated gene expression during gilthead sea bream skeletogenesis and its disruption by nutritional hypervitaminosis A. BMC Dev Biol 11:7. doi:10.1186/1471-213X-11-7.

Fernández I., Pimentel M. S., Ortiz-Delgado J. B., Hontoria F., Saracquete C., Estévez A., Zambonino J.-L., Gisbert E., 2009 Effect of dietary vitamin A on Senegalese sole (*Solea senegalensis)* skeletogenesis and larval quality. Aquaculture 295:250-265.

Fernández I., Hontoria F., Ortiz-Delgado J., Yannis K. Y., Estévez A., Zambonino-Infante J. L., Gisbert E., 2008 Larval performance and skeletal deformities in farmed gilthead seabream (*Sparus aurata*) fed with graded levels of Vitamin A enriched rotifers (*Brachionus plicatilis*). Aquaculture 283:102-115.

Forster I., 2000 Nutrient requirements. In: Encyclopedia of Aquaculture. Stickney R. R. (ed), pp. 592-600, John Wiley & Sons Inc., Canada.
Garcin H., Higuere P., Amoikon K., 1984 Effects of a large dose of retinol or retinoic acid on the thyroid hormones in the rat. Ann Nutr Metab 28(2):92-100.
Halver J., Hardy R. W., 2002 Fish nutrition. Academic press, London.
Hamre K., Krossøy C., Lock E. J., Moren M., 2010 Roles of lipid-soluble vitamins in the ontogeny of marine fish larvae. Aquac Res 41:745-750.
Hilomen-Garcia G. V., 1997 Morphological abnormalities in hatchery-bred milkfish (*Chanos chanos* Forsskal) fry and juveniles. Aquaculture 152:155-166.
Kumagai S., 1990 Reproduction and early life history of milkfish *Chanos chanos* in the waters around Panay Island, Philippines. PhD Dissertation, Kyushu University, Fukuoka, Japan.
Martinez F., Tseng M. C., Yeh S. P., 2006 Milkfish (*Chanos chanos*) culture: situations and trends. J Fish Soc Taiwan 33(3):229-244.
Mazuraisa D., Glynatsi N., Dariasa M., Christodoulopoulou S., Cahua C., Zambonino-Infante J. L., Koumoundouros G., 2009 Optimal levels of dietary Vitamin A for reduced deformity incidence during development of European sea bass larvae (*Dicentrarchus labrax*) depend on malformation type. Aquaculture 294:262-270.
Ott C., Iwanciw D., Graness A., Giehl K., Goppelt-Struebe M., 2003 Modulation of the expression of connective tissue growth factor by alterations of the cytoskeleton. J Biol Chem 278:44305-44311.
Tacon A. G. J., 1992 Nutritional fish pathology: Morphological signs of nutrient deficiency and toxicity in farmed fish. FAO, Rome, Italy, 75 p.
Taruoura D., Higueret P., Garcin H. D. H., 1991 Effects of a synthetic retinoid, etretinate (RO 10-9359), on the peripheral metabolism of thyroid hormones in rats. National Center for Biotechnology Information 29:595-599.
Villeneuve L., Gisbert E., Delliou H., Chantal L., Cahu C. L., Zambonino-Infante J. L., 2005 Dietary levels of all-trans retinol affect retinoid nuclear receptor expression and skeletal development in European sea bass larvae. Br J Nutr 93:791-801.

Received: 10 July 2013. Accepted: 03 August. Published online: 12 August 2013.
Authors:
Leslie Batingal Tutas, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao 5023 Iloilo, email: lbtutas@gmail.com
Augusto Erum Serrano Jr., University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao 5023 Iloilo, email: serrano.gus@gmail.com
Rex Ferdinand Mallare Traifalgar, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao 5023 Iloilo, email: skerferd@yahoo.com
Valeriano Lopez Corre, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao 5023 Iloilo, email: profcorre@yahoo.com

How to cite this article:
Tutas L. B., Serrano Jr. A. E., Traifalgar R. F. M., 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(5):464-469.