Voyager: Voll. V, Dec. 2014, 26-33: 2014
ISSN :0976-7436 : INDEXED AND ABSTRACTED

EFFECT OF METHOXSALEN ON THE SKIN PIGMENTATION OF BLUE MORPH (*PSEUDOTROPHEUS LOMBARDOI*)

S.Maria Vargies*, T.A. Sethuramalingam**
PG Research Department of Advanced Zoology & Biotechnology
St. Xavier's College, Palayakottai, Tamil Nadu

Abstract

The present study was carried out to evaluate the effect of a photosensitizer Methoxsalen through dietary supplementation on the pigmentation / melanophores change in blue morph (Pseudotropheus lombardoi). Three types of Methoxsalen containing experimental diets (25,100,500ppm) and the feed without methoxsalen was considered as a control diet were fed to four groups of fishes with three fishes in each group forming the triplicates was introduced into the glass aquaria.The experimental duration was run for 60 days. All the fishes were fed with control diets for another 15 days to see the sustainability of the pigmentation due to action of methoxsalen. The fish were fed ad libitum at 11:00 am every day and after the feeding time the fish were exposed the sunlight for 1hour (12:00 to 1:00 pm) to let the photosensitizer work inside body of the fish. The scales were removed from the lateral side of the fishes from the control and experimental diets fed fishes and examined under compound microscope for melanophores and pigmentation. It was found that the melanophores aggregation and the darkening of scales and number of pigment granules were dose- dependent 500 ppm methoxalene contained diet produced a high intensity of mealanophores and pigmentation of skin which has a direct impact on market value of ornamental fishes.

Key words: *Methoxsalen, photosensitizer, blue morph (Pseudotropheus lombardoi), skin colouration.*

Introduction

Ornamental fish culture is one of the most important fields of Aquaculture and now days it has becoming a source of income day by day for the rural people. (Basudev Mandal *et al.*, 2012) Ornamental fish has gained prominence in international trade and plays a significant role in the economy of developing countries like India (Chandy, 2014). There are various factors involved in ornamental fish culture and among these quality and quantity of food, growth and pigmentation for body colouration is the most important feature (James, 1998; Esakiammal *et al.*, 2009). Pigment-based coloration in fish can be expressed permanently flexibly changing gradually in a manner dependent on season, age or mating status or feed depending on an individual's motivational state (Alagappan *et al.*, 2004). Fish are coloured in nature often show faded coloration under intensive relationship between dietary carotenoid and pigmentation exists in them (Halten *et al.*, 1997). The pigments that can be used to provide color fishes these are melanins, carotenoids, pteridines, and purines. Carotenoids, which are lipid soluble, yellow to red colors. In the flesh, the carotenoids are the dominant pigment (Simpson *et al.*, 1981). They are used in aquaculture feeds to provide the color associated with consumer product, such as the bright vibrant colors of ornamental fish (Mukherjee *et al.*, 2010). The body colors of fish are the predominantly dependent on the presence of chromatophores. Pigments also play a role in determining the fish colour. Fish are capable of producing some pigments, but others must be supplied in the diet of most fish, colour enhancing diets may contain additional natural pigments to enhance colours of ornamental fish (Alagappan, 2004; Esakkiammal *et al.*, 2008). People involved in the trade of ornamental fish are constantly exploring methods of enhancing skin colouration. Colour enhancement through the use of carotenoids in feed has been confirmed by a number of authors (Fey and Mayers, 1980; Ako *et al.*, *2000* ; Kiessling *et al.*,2003; Alagappan *et al.*, 2004). (Ako *et al.* 2000) reported intense colouration of freshwater red velvet swordtail (*Xiphorus helleri*), rainbow fish (*Psedomugil furcatus*) and topaz cichlid (*Cichlasoma myrnae*) when fed diet containing carotenoid rich strain of *Spirulina platensis* and *Haematococcus pluvialis*. Sinha and Asimi (2007) reported that the China rose (*Hibiscus rosasinensis*) petal is a potent natural carotenoid source for gold fish (*Carassius auratus*) to enhance its colour. Ezhil *et al.* (2008) reported encouraging results on colouration of red swordtails

(*Xiphophrous helleri*) fed diet containing marigold petal meal. In addition to enhanching colouration of the fish, the different pigments used in the diets are also reported to give better growth (Ezhil *et al.*, 2013) in blue morph. The enhancement of colouration can be done by administering pigment enriched feed. However, detailed studies of colours enhancement in ornamental fish by use of pigment added fish feed is lacking.

The current work was undertaken to study the effect of a photosensitizer, Methoxsalen on the body pigmentation of the blue morph (*Pseudotropheus lombardoi*) In general, the photosensitizer (Methoxsalen) is used for various pigmentation disorders in human beings like psoriasis, vitiligo, leukoderma etc. Methoxsalen belongs to furocoumarin group of organic chemical compounds which absorb UV light and act as photosensitizer based on the interaction of light and a photoactive drug, to bring about beneficial results in a biological system, such as the skin. Chromatophores are pigment containing cells located beneath the scales and result in change in the appearance of fishes in the most common types of chromatophores are melanophores. These contain black or brown coloured melanin crystals and intensity of these cells depends on the amount of dispersion of the melanin pigment within the cell. The aggregation and dispersion phenomenon of chromatophores has direct impact relation with the market of ornamental fish (Asimi,2009). The present finding will be very useful to understand the changes in the concentration of melanophores after feeding with methoxsalen through dietary supplementation. . To enhance the body pigmentation of Blue morph, the present study was designed to feed the fishes with varied concentration of methoxsalen to improve the pigmentation in the scales.

Materials and Methods

Three types of experimental feed was prepared by using fishmeal, rice bran, soybean meal, groundnut oil cake, tapioca flow, vitamin and mineral mix and cod liver oil. (Table-1) To each feed methoxsalen in different concentration ie., 25ppm, 100ppm and 500ppm was added to the basal diets. The diet without methoxsalen is considered as the control diet. All the three types of feed were added with *Spirulina maxima* (10%) as carotenoid source. 12 glass aquaria of 40 liter capacity were taken and divided in four groups forming the triplicates. The experimental fish of blue morph (*Pseudotropheus lombardoi*) was collected from local aqua form and stocked for 10 days acclimatization. The experiment was conducted for 60 days duration and in which for

initial 45 days the experimental and control feed were fed to the fishes after which the scale samples were collected from the latral side of the fishes. All the fishes were fed with control diets for another 15 days to see the sustainability of the pigmentation due to action of methoxsalen.

The fish were fed *ad libitum* at 11:00 am everyday and after 1 hour after feeding, they were exposed to sunlight for 1hour (12:00 to 1:00 pm). Exposure to sunlight was to let the photosensitizer work inside the body of the fish. The above procedure was continued for 15 days after which the scales were taken from latral side of the fish and preserved in 6% formalin. The scales were viewed under compound microscope for chromatophores counts. The water quality parameters were analysed using the standard methods (APHA, 1998). The water temperature ranged between 21to 23^0 C. The pH was between 7.1 to 7.3, dissolved oxygen between 6 to 8 ppm and alkalinity was well below 160 ppm.

Results

The scale of the fish fed with control diet showed no pigmentation. The pigments were not present either in the center or on the ridges (plate-1) where as the fish fed with experimental diet 2 (methoxsalen 25ppm) showed a slight darkening of the scale (plate- 2). An increase in the pigmentation number or pigment granules as well as darkening of the scale could be seen the fishes fed with diet-3 (plate- 3). A high number of melanophores could be clearly visualized in the scale of fishes fed with diet-4 (plate-4-51). The development of higher pigmentation of chromatophores and darkening of the scales were highly significant in scales of fish fed with high concentration of methoxsalen (500 ppm).

Discussion

The present study, demonstrated that the feeding diet supplement with the higher dose of photosensitizer methoxsalen (500 ppm) has provided a dispersing action of melanin pigment in the chromotophores in the scales of blue morph. Similar results were observed in fishes fed with natural or artificial diet containing substances in order to enhance colouration. Storebakken *et al.* (1987); Buttle *et al.* (2001) and Kiessling *et al.* (2003) reported an increase in pigment concentration of Atlantic salmon, *Salmo salar.* Hama and Haseawa (1967) while studying the chromatophores of *Oryzias latipes* (medaka) reported an enhanced pigmentation. Ako *et al.* (2000) reported intensification of colour in the freshwater red velvet swordtails (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topqz cichlids (*Cichlasoma myrnae*) when

fed a diet containing 1.5 to 2% of carotenoid rich strain of *Spirulina platensis* and 1% of *Haematococcus pluvialis*. Wallat *et al*. (2005) used six different popular commercial diets to study their effect on skin colouration, survival and growth of red oranda goldfish (*Carassius auratus*) and reported a vibrant colouration in the fish. Fujita and Fujii (1997) using mammalian endothelins in a dose dependent manner to effectively disperse the light scattered organelles in leucophores in medaka, have concurrence with the present study Ezhil and Narayanan (2013) found the enhancement of blue morph by feeding spirulina, mangopeal, curry leaf and beat root and produce a significance effect on the colouration fed with10% of spirulina diets. The mechanism of action of drug methoxsalen, used in the present study, exhibited colour aggregation and dispersion in blue morph (*Pseudotropheus lombardoi*), which is seen in the scales. The present study also conform the result of Asimi (2009) who studied the effect of the mathoxsalen on the melanophore response of blue gourami (*Trichogaster trichopterus*) and found out that the aggregation and dispersion of the melanosphore of scales is greatly influence by supplementation of 500 ppm methoxsalen in the feed.

Conclusion

The present study is basic step to find out the behavior of melanosphores for the aggregation in the scale of fishes feeding with photosensitizer methoxsalen through dietary supplementation. This report is useful to the feed manufacturers to use methoxsalen to improve the colouration fish. However, more studies have to be done related to the physiological and biochemical parameters of the fish species, before using this phtosensitizer as dietary ingredient.

Table 1: Percentage composition of ingredient used in formulated diets.

Ingredients	%
Fish meal	38
Groundnut Oil cake	22
Soybean meal	12
Rice bran	10
Tapioca Flour	4
Cord lever oil	2
Vitamin mineral mix*	2
Spirulina maxima	10

"Supradyn tablet"-Nicholas-pharma, Pethampur, M.P, India.

Plate1: Scale of blue morph (*Psedotropheus lombardoi*) fed with control diet(250x):

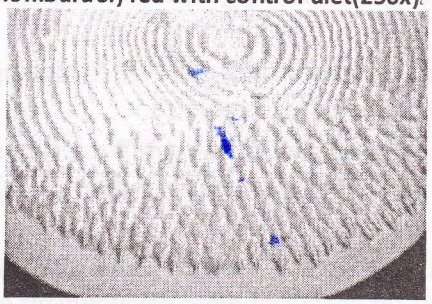


Plate2: Scale of blue morph (*Psedotropheus lombardoi*) fed with *Spirulia maxima* diets containing (25ppm) methoxsalen (100x):

Plate3: Scale of blue morph (*Psedotropheus lombardoi*) fed with *Spirulia maxima* diets containing (100ppm) methoxsalen (100x):

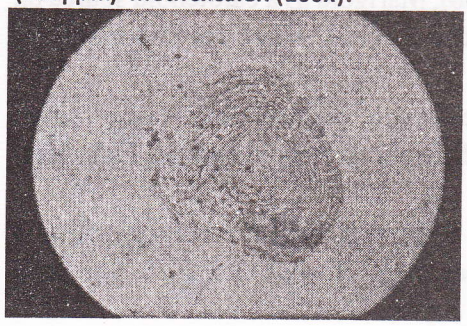


Plate4: Scale of blue morph (*Psedotropheus lombardoi*) fed with *Spirulia maxima* diets containing (500ppm) methoxsalen (100x):

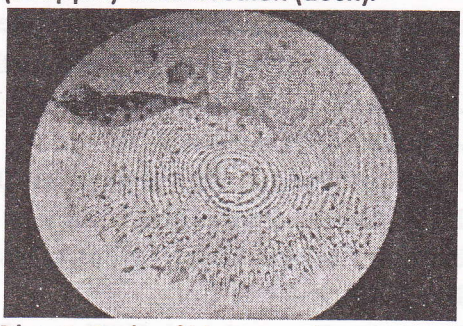
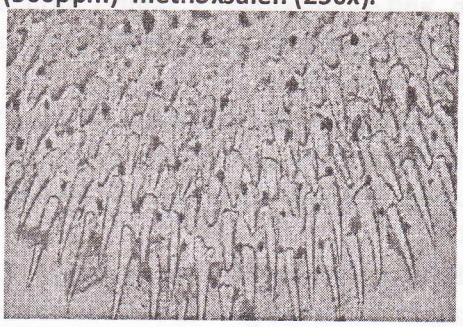


Plate5: Scale of blue morph (*Psedotropheus lombardoi*) fed with *Spirulia maxima* diets containing (500ppm) methoxsalen (250x):



References

Alagappan M., Vijila K. and Archana S. (2004). Utilization of Spirulina algae as a source of carotenoid pigment for blue gouramis (*Trichogasster trichopterus* Pallas). **Aquaricult. Aquatic Sci., 10 (1)**: 1-11.

Ako H., Tamaru C.S., Asano L., Yuen B. and Yamamoto M. (2000). Achieving natural colouration in fish under culture. UJNR Technical Report 28.

APHA. (1998). Standard Methods for the Examination of Water and Wastewater, 20th edn.ed. by Clesceri LS, Greenberg AE, Eaton AD, American Public Health Association, DC USA p,1060.

Basudev Mandal, Chandrasekhar Dutta and Uttam Kumar Laha. (2012). Freshwater ornamental fish farming as a tool for socioeconomic development of backward woman selfhelf grops to restore live hood security. **Advanced Biol. Res., 2(1)**:95-98

Buttle L., Cramption V., Williams., P. (2001). The effect of feed pigment type of flesh pigment deposition. **Aqua. Nutr., 32** : 103 -111.

Esakkiammal B., SethuramalingamT.A. , Pathimuthu Joharl A., Rajakumari K. and Anandkumar Y. (2009). Influence of Dietary Carotenoid supplementation on growth and changes in the Development of skin colouration on *Poecilia reticulata.* **Adv. Zoo., 30(1)**: 27 -33.

Esakkiammal B., Sethuramalingam T.A., Rajakumari K. and Anand kumar Y. (2008). Influence of Dietary Catortenoid Concentrations on the growth and skin colour development in coloured mollies *Poecilia sphenops*. **Aqua cult., 9(2):** 177-184.

Ezhil J., Jeyanthi C., Narayanan, N. (2008), Marigold as a carotenoid source on pigmentation and growth of red swordtaii, *Xiphophorus helleri*. **Turkish Fisheries Aq. Sci., 8**: 99-10.

Ezhil J., and Narayan M. (2013), Enhancement of pigmentation in blue Morph *Pseudotrophus lombardoi* through feeding different carotenoid sources, **Fish. Mar. Sci., 5(6):**655-659.

Fujita T. and Fijii R. (1997). Endotheins disperse light-scattering organelles in leucophores of the medata, *Oryzias latipes. Dep. Biomol. Sci. Fac. Sci. Toho Univ, Myzonna, Funabashi, Chiba 274, Japan.*

Halten B., Arnesan A.M., Jobling M., Siikavuopio and Bjerkeng B.(1997). Carotenoid pigmentation in relation to feed intake, growth and social integration in

Arctic char, *Salvelinus alpines* (L.), from two anadromous strains. **Aquacult. Nutr., 3**: 189-199.

Hama T. and Hasegawal H. (1967). Studies on the chromatophores of *Oryzias latipes* (Teleostean fish): Behavior of the pteridine, fat and carotenoid during xanthophore differentiation in the color varieties, **Proc. Jap. Acad., 43**: 901 -906.

James R. (1998). Studies on growth and reproduction in chosen fishes with particular references to nutrition and water quality. **Ph.D. Thesis** submitted to Manonmaniam Sundaranar University, Tirunelveli, TN, India.

Kiessing A., Oisen E., Buttle L. (2003), Given the same dietary carotenoid inclusion, Atlantic salmon, *Salmo salar* (L) display higher blood levels of canthaxanthin than astaxanthin . **Aqua. Nutri., 9**: 253-261.

Mukherjee, A. K., Kalita, P., Unni, B. G., Wann, S. B., Saikia, D., Mukhopadhyay, P. K. (2010) Fatty acid composition of four potential aquatic weeds and their possible use as fish-feed neutraceuticals. **Food Chemistry., 123:** 1252-1254.

Ommen Chandy, (2014). 7th India Inter National Aquashow, Organised by state Fisheries Resource Manageement Society (FIRMA),24-28 January-2014 Department of fisheries, Kerala

Oyas Ahmed Asimi. (2009). To study the effect of photosensitizer on melanophore responses of blue gourami (*Trichogaster trichopterus*), **Cell Ani. Biol., 3(5):83-87**

Simpson, B.K. and Haard, N.F. (1981). The use of proteolyses enzymes to extract carotenoproteins from shrimp processing wastes. **Applied Biochem., 7**: 212-222.

Sinha A. and Oyas Amed Asimi (2007). China rose (*Hibiscus rosasinensis*) petals: a potent natural carotenoid source for goldfish (*Carassius auratus* L), **Aquacult. Res., 38:1123-1128.**

Storebakken T., Foss P., Schiedt K., Austreng E ., Jensen S.L. and Manz U. (1987). Carotenoids in the diets for salmonids IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. **Aquaculture, 65**: 279-292.

Wallat GK., Lazur AM., Chapman F.A. (2005). Carotenoids of different types and concentrations in commercial formulated fish diets affect color and its development in the skin of the Red Oranda variety of Goldfish. **North Am. J. Aquacult. 67**: 42 -51.