

**PHENOTYPE, CAROTENOID LEVEL AND GENE EXPRESSION LINKED TO
SKIN COLORATION IN RED TILAPIA (*Oreochromis sp.*) AS AFFECTED BY
CAROTENOID-RICH DIET**

ERVEE P. LANDINGIN

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological
Sciences, College of Arts and Sciences, Central Luzon State University,
Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree of

**BACHELOR OF SCIENCE IN BIOLOGY
(Major in Biotechnology)**

JUNE 2019

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF APPENDICES	ix
LIST OF APPENDIX TABLES	x
LIST OF APPENDIX FIGURES	xi
ABSTRACT	xii
INTRODUCTION	1
Background of the Study	1
Objective of the Study	4
Significance of the Study	4
Scope and Limitation of the Study	5
Time and Place of the Study	6
REVIEW OF RELATED LITERATURE	7
Tilapia Production	7
Nile tilapia (<i>Oreochromis niloticus</i>)	8
Red Tilapia	9
<i>csflra</i> gene	11
<i>Bcdo2b</i> gene	12
MATERIALS AND METHODS	14
Collection and Preparation of Extracts and Experimental Diets	14
Experimental Fish and Treatment Set-Up	14
Measurement of Carotenoid Level	15
Phenotypic Effect on the Color of the Fish	16
Observation of Coloration on Red Tilapia	16
Gene Expression of <i>Bcdo2b</i> and <i>csflra</i>	16
RNA Extraction	16
Quantitative RT-PCR	17
Data Gathered	18
Statistical Analysis	18

RESULTS AND DISCUSSION	20
Phenotypic Effect on the Color of the Fish	20
Carotenoid Level Measurement	21
Expression of <i>Bcdo2b</i> and <i>csf1ra</i> genes	21
SUMMARY, CONCLUSION AND RECOMMENDATIONS	29
Summary	29
Conclusion	29
Recommendation	30
LITERATURE CITED	31
APPENDICES	38

LIST OF TABLES

TABLE		PAGE
1	Treatment set-up	15
2	Primers for qRT-PCR of the <i>Bcdo2b</i> gene, <i>csflra</i> gene and the internal standard, β -actin	18
3	Mean skin coloration	20
4	Mean absorbance of carotenoid level	22

LIST OF FIGURES

FIGURE		PAGE
1	Color chart (Velasco <i>et al.</i> , 2018)	16
2	Phenotypic effect on the skin coloration of red tilapia as affected by carotenoid extracts (A – carrot peel; B – water spinach leaves; C – horseradish leaves; and D – control).	21
3	Expression of <i>csflra</i> in Red Tilapia skin coloration	22
4	Expression of <i>Bcdo2b</i> in Red Tilapia skin coloration	24

LIST OF APPENDICES

APPENDIX		PAGE
A	Statistical Analysis	39
B	Photo Documentation	41

LIST OF APPENDIX TABLES

APPENDIX TABLE		PAGE
1	Analysis of variance of the phenotypic skin color of red tilapia	39
2	Tukey's HSD test of the phenotypic skin color of red tilapia	39
3	Analysis of variance of carotenoid level	39
4	Tukey test of carotenoid level	39
5	One-Sample statistics of <i>csflra</i> gene	40
6	One-Sample test of <i>csflra</i> gene	40
7	One-Sample statistics of <i>Bcdo2b</i> gene	40
8	One-Sample test of <i>Bcdo2b</i> gene	40

LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	Experimental set-up	41
2	Carotenoid Extraction	42
3	Removal of the skin of red tilapia	43
4	Carotenoid level measurement	44
5	RNA extraction	44

ABSTRACT

LANDINGIN, ERVEE P., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019, PHENOTYPE, CAROTENOID LEVEL AND GENE EXPRESSION LINKED TO SKIN COLORATION IN RED TILAPIA (*Oreochromis* sp.) AS AFFECTED BY CAROTENOID-RICH DIET**

Adviser: **KHRISTINA G. JUDAN-CRUZ**, Ph. D.

Production, marketability and consumer preference of red tilapia often depends upon the intensity of coloration. Hence, color enhancement is now a priority among commercial red tilapia farmers and new approaches to develop coloration are now geared to improve and satisfy market acceptability and sophistication. This study evaluated the effects of carotenoid-rich diets on the skin coloration, carotenoid level and expression of carotenoid-linked genes.

Carotenoids were extracted from dried *Daucus carota* peel, *Ipomoea aquatica* leaves, and *Moringa oleifera* leaves using ethanol. Fish samples in aquaria were fed twice a day for four months using the feeds mixed with carotenoid extracts. The phenotypic effect of the carotenoid extracts was measured in the skin of the red tilapia through a color chart. Carotenoid level was measured through uv-vis spectrophotometer. The expression of *csf1ra* and *Bcdo2* in the skin was done through gene expression analysis using qPCR.

Results on the skin coloration and carotenoid level showed most efficient color enhancement using *Daucus carota* carotenoid extract. *Ipomoea aquatica* leaf carotenoid extract also showed significantly higher values in skin coloration and carotenoid level than commercial feeds alone. Expression of carotenoid- and coloration-linked genes as

affected by the extracts showed upregulation in *csflra* and downregulation in *Bcd02b* which verifies the observed color enhancement and higher carotenoid level in the skin.

This study highlights the potential of the plant extracts for integration and development of feeds for color enhancement in red tilapia. Given that intense redness in red tilapia increases its market value, this can substantially increase market sophistication, thus, enriching the red tilapia industry.

LITERATURE CITED

- Amaya, E., Becquet, P., Carné, S., Peris, S., & Miralles, P. (2014). Carotenoids in Animal Nutrition. Belgium: Fefana Publication.
- Arous, W. H., El-Bermawi, N. M., Shaltout, O. E., & Essa, M. A. E. (2014). Effect of adding different carotenoid sources on growth performance, pigmentation, stress response and quality in red tilapia (*Oreochromis Spp*). *Middle East Journal of Applied Sciences*, 4(4), 988-999.
- Asian Development Bank (ADB). (2005). Impact evaluation study on the development of genetically improved farmed tilapia and their dissemination in selected countries. Impact Evaluation Study, Asian Development Bank: Manila, Philippines.
- Babino, D., Palczewski, G., Widjaja-Adhi, M. A. K., Kiser, P. D., Gloczak, M., & von Lintig, J. (2015). Characterization of the Role of β -Carotene 9,10-Dioxygenase in Macular Pigment Metabolism. *The Journal of Biological Chemistry*, 290(41), 24844-24857.
- Berry, S. D., Davis, S. R., Beattie, E. M., Thomas, N. L., Burrett, A. K., Ward, H. E., Stanfield, A. M., Biswas, M., Ankersmit-Udy, A. E., Oxley, P. E., Barnett, J. L., Pearson, J. F., van der Does, Y., MacGibbon, A. H. K., Spelman, R. J., Lehnert, K., & Snell, R. G. (2009). Mutation in bovine β -carotene oxygenase 2 affects milk color. *Genetics*, 182(3), 923-926.
- Braasch, I., Salzburger, W., & Meyer, A. (2006). Asymmetric evolution in two fish-specifically duplicated receptor tyrosine kinase paralogons involved in teleost coloration. *Molecular Biology and Evolution*, 23(6), 1192-1202.
- Chandra-Hioe, M. V., Rahman, H. H., & Arcot, J. (2017). Lutein and β -Carotene in Selected Asian Leafy Vegetables. *Journal of Food Chemistry and Nanotechnology*, 3(3), 93-97.
- Chintala, S., Li, W., Lamoreuz, M. L., Ito, S., Wakamatsu, K., Svlderskaya, E. V., Bennett, D. C., Park, Y-M., Gahl, W. A., Huizing, M., Spritz, R. A., Ben, S., Novak, E. K., Tan, J., & Swank, R. T. (2005). *Slc7a1* gene controls production of pheomelanin pigment and proliferation of cultured cells. *Proceedings of the National Academy of Sciences of the United States of America*, 102(31), 10964-10969.
- Choi, S., & Koo, S. (2005). Efficient syntheses of the keto-carotenoids canthaxanthin, astaxanthin, and astacene. *Journal of Organic Chemistry*, 70(8), 3328-31.

- Clotfelter, E. D., Ardia, D. R., & McGraw, K. J. (2007). Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. *Behavioral Ecology*, 18, 1139-1145.
- Colihueque, N., & Araneda, C. (2014). Appearance traits in fish farming: progress from classical genetics to genomics, providing insight into current and potential genetic improvement. *Frontiers in Genetics*, 5(251), 1-8.
- Das, A. P., & Biswas, S. P. (2016). Carotenoids and pigmentation in ornamental fish. *Journal of Aquaculture & Marine Biology*, 4(4), 1-3.
- Diepeveen, E. T., & Salzburger, W. (2011). Molecular characterization of two endothelin pathways in East African cichlid fishes. *Journal of Molecular Evolution*, 73, 355–368.
- Ekpe, L., Inaku, K., & Ekpe, V. (2018). Antioxidant effects of astaxanthin in various diseases—a review. *Journal of Molecular Pathophysiology*, 7(1), 1-6.
- El-Sayed, A-F. M. (2006). *Tilapia culture*. Cambridge, USA: CABI Publishing.
- Eriksson, J., Larson, G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., Strömstedt, L., Wright, D., Jungerius, A., Vereijken, A., Randi, E., Jensen, P., & Andersson, L. (2008). Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS Genetics*, 4(2), 1-8.
- Fisheries Situationer. (2017). Philippine Statistics Authority. Retrieved from https://psa.gov.ph/sites/default/files/Fisheries%20Situationer%2C%20January%20-%20March%202017_0.pdf
- Fitzsimmons, K. (2000). Future trends of tilapia aquaculture in the Americas. In B.A. Costa-Pierce & J.E. Rakocy (Eds), *Tilapia Aquaculture in the Americas*, Vol. 2 (pp. 252–264). Baton Rouge, Louisiana, United States: The World Aquaculture Society.
- Frohnhofer, H. G., Krauss, J., Maischein, H-M., & Nüsslein-Volhard, C. (2013). Iridophores and their interactions with other chromatophores are required for stripe formation in zebrafish. *Development*, 140, 2997-3007.
- Garcia-Chavarria, M., & Lara-Flores, M. (2013). The use of carotenoid in aquaculture. *Research Journal of Fisheries and Hydrobiology*, 8(2), 38-49.
- Grammer, G. L., Slack, W. T., Peterson, M. S., & Dugo, M. A. (2012). Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) establishment in temperate Mississippi, USA: multi-year survival confirmed by otolith ages. *Aquatic Invasions*, 7(3), 367-376.

- Guerrero, R. D. III. (2013). The production and trade of tilapia in the Philippines. Retrieved from <http://www.infopesca.org/sites/default/files/complemento/actividadesrecientes/adjuntos/1369//4%20Philippines-Production%20and%20Trade%20of%20Tilapia.pdf>
- Gupta, S. K., Jha, A. K., Pal, A. K., & Venkateshwarlu, G. (2007). Use of natural carotenoids for pigmentation in fishes. *Natural Product Radiance*, 6(1), 46-49.
- Hernández-Sánchez, F., & Aguilera-Morales, M. E. (2012). Nutritional Richness and Importance of the Consumption of Tilapia in the Papaloapan Region. *Revista electrónica de Veterinaria*, 13(6), 1-12.
- Hilsdorf, A. W., Penman, D. J., Farias, E. C., & McAndrew B. (2002). Melanophore appearance in wild and red tilapia embryos. *Pigment Cell Research*, 15(1), 57-61.
- Hui, N., Guo-qing, H., Hui, R., Qi-he, C., & Feng, C. (2005). Application of derivative ratio spectrophotometry for determination of β -carotene and astaxanthin from *Phaffia rhodozyma* extract. *Journal of Zhejiang University Science*, 6B(6), 514-522.
- Islam, M. A., Das, D. R., Khalequzzaman, S. M., Kamal, D., & Halim, K. M. A. (2006). Extensive culture of red tilapia with four stocking densities at Beel Kotalia, Bagerhat, Bangladesh. *Pakistan Journal of Biological Sciences*, 9(10), 1965-1969.
- Jaswir, I., Noviendri, D., Hasrini, R. F., & Octavianti, F. (2011). Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. *Journal of Medicinal Plants Research*, 5(33), 7119-7131.
- Jourdan, M., Gagné, S., Dubois-Laurent, C., Maghraoui, M., Huet, S., Suel, A., Hamama, L., Briard, M., Peltier, D., & Geoffriau, E. (2015). Carotenoid Content and Root Color of Cultivated Carrot: A Candidate-Gene Association Study Using an Original Broad Unstructured Population. *PLoS One*, 10(1), 1-19.
- Kiefer, C., Hessel, S., Lampert, J. M., Vogt, K., Lederer, M. O., Breithaupt, D. E., & von Lintig, J. (2001). Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. *Journal of Biological Chemistry*, 276(17), 14110-14116.
- Lin, M. Q., Ushio, H., Ohshima, T., Yamanaka, H., & Koizumi, C. (1998). Skin color control of the Red sea bream (*Pagrus major*). *LWT-Food Science and Technology*, 31(1), 27-32.
- Lobo, G. P., Isken, A., Hoff, S., Babino, D., & von Lintig, J. (2012). BCDO2 acts as a carotenoid scavenger and gatekeeper for the mitochondrial apoptotic pathway. *Development*, 139, 2966-2977.

- McAndrew, B. J., Roubal, F. R., Roberts, R. J., Bullock, A. M., & McEwen, I. M. (1988). The genetics and history of red, blond and associated color variants in *Oreochromis niloticus*. *Genetica*, 76, 127–137.
- Minchin, J. E. N., & Hughes, S. M. (2008). Sequential actions of Pax3 and Pax7 drive xanthophore development in zebrafish neural crest. *Developmental Biology*, 317, 508–522.
- Moreira, A. A., Marques Moreira, H. L., & Silva Hilsdorf, A. W. (2005). Comparative growth performance of two Nile tilapia (Chitralada and Red-Stirling), their crosses and the Israeli tetra hybrid ND-56. *Aquaculture Research*, 36, 1049–1055.
- Ng, W-K., & Hanim, R. (2007). Performance of genetically improved Nile tilapia compared with red hybrid tilapia fed diets containing two protein levels. *Aquaculture Research*, 38, 965-972.
- O'Quin, C. T., Drilea, A. C., Conte, M. A., & Kocher, T. D. (2013). Mapping of pigmentation QTL on an anchored genome assembly of the cichlid fish, *Metraclima zebra*. *BMC Genomics*, 14(287), 1-8.
- Parichy, D. M., Ransom, D. G., Paw, B., Zon, L. I., & Johnson, S. L. (2000). An orthologue of the kit related gene *fms* is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish, *Danio rerio*. *Development*, 127, 3031–3044.
- Parichy, D. M., & Turner, J. M. (2003). Temporal and cellular requirements for Fms signaling during zebrafish adult pigment pattern development. *Development*, 130, 817-833.
- Pasarin, D., & Rovinaru, C. (2018). Sources of carotenoids and their uses as animal feed additives – a review. *Animal Science*, 61(2), 74-85.
- Patterson, L. B., & Parichy, D. M. (2013). Interactions with iridophores and the tissue environment required for patterning melanophores and xanthophores during zebrafish adult pigment stripe formation. *PLOS Genetics*, 9(5), 1-14.
- Pemsl, D. E., Chen, O. L., Tongruksawatta, S., Garcia, Y., Vera-Cruz, E., Abella, T., & Waibel, H. (2008). In: Proceedings of the 14th biennial conference of the International Institute of Fisheries Economic and Trade(IIFET), Nha Trang, Vietnam, Jul 22-26 2008.
- Plaza, M., Herrero, M., Cifuentes, A. & Ibáñez, E. (2009). Innovative natural functional ingredients from microalgae. *Journal of Agricultural and Food Chemistry*, 5, 7159–7170.

- Pongmaneerat, J. & Watanabe, T. (1991). Nutritive value of protein of feed ingredients for carp *Cyprinus carpio*. *Nippon Suisan Gakkaishi*, 57, 503-510.
- Quigley, I. K., Manuel, J. L., Roberts, R. A., Nuckels, R. J., Herrington, E. R., MacDonald, E. L., & Parichy, D. M. (2005). Evolutionary diversification of pigment pattern in Danio fishes: differential fins dependence and stripe loss in *D. albolineatus*. *Development*, 132, 89-104.
- Rajace, A. H. (2011). Genetic approaches to the analysis of body colouration in Nile tilapia (*Oreochromis niloticus* L.). Published doctorate thesis. Institute of Aquaculture, University of Stirling Stirling, Scotland, UK.
- Ross, L.G. (2000) Environmental physiology and energetics. In M.C.M, Beveridge, & B.J. McAndrew (Eds), *Tilapias: Biology and Exploitation* (pp. 89–128). London: Academic Publishers.
- Rousset, D., Agnes, F., Lachaume, P., Andre, C., & Galibert, F. (1995). Molecular evolution of the genes encoding receptor tyrosine kinase with immunoglobulin-like domains. *Journal of Molecular Evolution*, 41, 421-429.
- Saini, R. K., Shetty, N. P., & Giridhar, P. (2014). Carotenoid content in vegetative and reproductive parts of commercially grown *Moringa oleifera* Lam. Cultivars from India by LC-APCI-MS. *European Food Research and Technology*, 238, 971–978.
- Salzburger, W., Mack, T., Verheyen, E., & Meyer, A. (2005). Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology*, 5(17), 1-15.
- Salzburger, W., Braasch, I., & Meyer, A. (2007). Adaptive sequence evolution in a color gene involved in the formation of the characteristic egg-dummies of male haplochromine cichlid fishes. *BMC Biology*, 5(51), 1-13.
- Schmid, M., Nanda, I., Guttenbach, M., Steinlein, C., Hoehn, M., Scharl, M., Haaf, T., Weigend, S., Fries, R., Buerstedde, J. M., Wimmers, K., Burt, D. W., Smith, J., A'Hara, S., Law, A., Griffin, D. K., Bumstead, N., Kaufman, J., Thomson, P. A., Burke, T., Groenen, M. A., Crooijmans, R. P., Vignal, A., Fillon, V., Morisson, M., Pitel, F., Tixier-Boichard, M., Ladjali-Mohammed, K., Hillel, J., Mäki-Tanila, A., Cheng, H. H., Delany, M. E., Burnside, J., & Mizuno, S. (2000). First report on chicken genes and chromosomes 2000. *Cytogenetics and Cell Genetics*, 90(3-4), 169-218.
- Sefc, K. M., Brown, A. C., & Clotfelter, E. D. (2014). Carotenoid-based coloration in cichlid fishes. *Comparative Biochemistry and Physiology, Part A*, 173, 42-51.

- Smith, I. R., & Pullin, R. S. V. (1984). Tilapia Production Booms in the Philippines. ICLARM Newsletter.
- Solanki, J., Parmar, H., Parmar, A., Parmar, E., & Masani, M. (2016). Freshness evaluation of fish by quality index method (QIM) and instrumental method at Veraval Fish Landing Centre. *International Journal of Processing and Post Harvest Technology*, 7(1), 42-46.
- Southeast Asian Fisheries Development Center, Aquaculture Department (1993). Farming tilapia in the Philippines. *Aqua Farm News*, 11(3), 4.
- Tan, R. L., Garcia, Y. T., D., M-A. L., Tan, I. M. A., & Pemsil, D. E. (2011). Technical efficiency of genetically improved farmed tilapia (GIFT) cage culture operations in the lakes of Laguna and Batangas, Philippines. *ISSAAS Journal*, 17(1), 194-207.
- Tian, R., Pitchford, W. S., Morris, C. A., Cullen, N. G. & Bottema, C. D. (2010). Genetic variation in the beta, beta-carotene-9_, 10_-dioxygenase gene and association with fat colour in bovine adipose tissue and milk. *Animal Genetics*, 41, 253-259.
- Towers, L. (2005). *Farming tilapia: life history and biology*. Retrieved May 26, 2018, from The Fish Site: <https://thefishsite.com/articles/tilapia-life-history-and-biology>.
- Velasco, R. R., Dandan, R.R., Tomas, P. A. G., Torres, J. C., Galvan, J. A., Fernando, S. I. D., De Guia, A. C. M., & Bartolome, R. M. (2018). Quantifying the skin pigmentation of gray (*Oreochromis niloticus*) and red (*Oreochromis spp.*) tilapia fed with carotenoid rich plants. *International Journal of Biology, Pharmacy and Allied Sciences*, 7(8), 1601-1615.
- Wang, L., Zhu, W., Yang, J., Miao, L., Dong, J., Song, F., & Dong, Z. (2018). Effects of dietary cystine and tyrosine on melanogenesis pathways involved in skin color differentiation of Malaysian red tilapia. *Aquaculture*, 490, 149-155.
- Watanabe, W. O., Losordo, T. M., Fitzsimmons, K., & Hanley, F. (2002). Tilapia production systems in the Americas: technological advances, trends, and challenges. *Reviews in Fisheries Science*, 10(3-4), 465-498.
- Wiesmann, C., Muller, Y. A., & de Vos, A. M. (2000) Ligand-binding sites in Iglike domains of receptor tyrosine kinases. *Journal of Molecular Medicine*, 78(5), 247-260.
- Yang, X., Li, J., & Deng, X. (2012). Polymorphism of BCDO2 gene for the yellow shank color in chicken. *China Poultry*, 8(1), 5.

Zhu, W., Wang, L., Dong, Z., Chen, X., Song, F., Liu, N., Yang, H., & Fu, J. (2016). Comparative transcriptome analysis identifies candidate genes related to skin color differentiation in red tilapia. *Scientific Reports*, 6, 1-11.