

**EFFECT OF CAROTENOID PIGMENTS FROM WATER SPINACH
(*Ipomoea aquatica*) LEAVES ON THE GROWTH, SURVIVAL AND
SKIN COLORATION OF NILE TILAPIA
(*Oreochromis niloticus*) FRY**

by

PATRICIA ANNE GRAGASIN TOMAS

An Undergraduate Thesis presented to the Faculty of the College of Fisheries in partial
fulfilment of the requirements for the degree of

BACHELOR OF SCIENCE IN FISHERIES

**COLLEGE OF FISHERIES
CENTRAL LUZON STATE UNIVERSITY
Science City of Muñoz, Nueva Ecija
Philippines**

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ABSTRACT

The 30-day-feeding experiment was conducted to evaluate the effect of carotenoid pigments from water spinach (*Ipomoea aquatica*) leaves on the growth, survival and skin coloration of Nile tilapia fry cultured in aquaria. The study had three treatments with three replicates namely: Treatment I – control commercial feeds, Treatment II – commercial feeds with 500mg of carotenoid pigments from water spinach leaves, Treatment III – commercial feeds with 1000mg from carotenoid pigments of water spinach leaves.

Results of the study showed that Nile tilapia in Treatment III had the highest final weight, gain in weight and specific growth rate. Analysis of variance revealed that final weight and gain in weight of Nile tilapia in Treatment III were significantly higher than those in Treatments I and II. Nile tilapia in Treatments II and III had significantly darker skin coloration than those in Treatment I.

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LITERATURE CITED

- Agarwal, S.S. and V.K. Singh. 1999. Immunomodulators studies in Indian medicinal plants and synthetic peptides. Part I: medicinal plants. *Proc. Indian Natl. Sci. Acad.*, 65: 179-204.
- Alishahi, M., M.M. Ranjbar, M. Ghorbanpour, R. Peyghan, M. Mesbah and M. Razi Jalali. 2010. Effects of dietary *Aloe vera* on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). *International Journal of Veterinary Research*, 3: 189-195.
- Anderson, J.S., S.P. Lall, D.M. Anderson and M.A. McNiven. 1993. Evaluation of protein quality in fish meal by chemical and biological assays. *Aquaculture*, 115: 305-325.
- Arulvasu, C., S. Ramya Meena, D. Chandhirasekar and S. Sivaganam. 2013. Evaluation of natural sources of carotenoids pigments from *Rosa rubiginosa* on growth, survival and coloration of *Xiphophorus helleri* fish fry. *European Journal of Biological Science*, 5(2): 44-49.
- Aspengren, S., D. Hedberg, H.N. Sköld and M. Wallin. 2009. New insights into melanosome transport in vertebrate pigment cells. *Int. Rev. Cell Mol. Biol.*, 272: 245-302.
- Austin, D.F. 1980. Convolvulaceae. p. 288-363. *In*: M.D. Dassanayake & F.R. Fosberg (eds.). *A Revised Handbook of the Flora of Ceylon*. Volume 1. Amerind Publishing Co. New Delhi, India. 508 p.
- Bagnara, J.T. and M.E. Hadley. 1973. *Chromatophores and Color Change: The comparative physiology of animal pigmentation*. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, USA. 191 p.
- Bagnara, J.T., J.D. Taylor and M.E. Hadley. 1968. The Dermal Chromatophores unit. *J. Cell Biol.*, 38: 67-79.
- Bardach, J.E., J.H. Ryther and W.D. McLarney. 1972. *Aquaculture: The farming and husbandry of freshwater and marine organisms*. Wiley-Interscience. New York, USA. 868 p.
- Bell, J.G., J. McEvoy, D.R. Tocher and J.R. Sargent. 2000. Depletion of α -tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *American Society for Nutritional Sciences*, 130(7): 1800-1808.

- Bendich, A. and S.S. Shapiro. 1986. Effect of β -carotene and canthaxanthin on the immune responses of the rat. *Journal of Nutrition*, 116(11): 2254-2262.
- Bentsen, H.B., A.E. Eknath, M.S.P. DeVera, J.C. Danting, H. Bolivar, R.A. Reyes, E.E. Dionisio, F.M. Longalong, A.V. Circa, M.M. Tayamen, and B. Gjerde. 1998. Genetic improvement of farmed tilapias: growth performance in a complete dialled cross experiment with eight strains of *Oreochromis niloticus*. *Aquaculture*, 160(1-2): 145-173.
- Braasch, I., M. Scharl, J.-N. Volff. 2007. Evolution of pigment synthesis pathways by gene and genome duplication in fish. *BMC Evol. Biol.* 7: 74 p.
- Brown, A.C. 2014. Honesty and carotenoids in a pigmented female fish. Ph.D. dissertation. University of Massachusetts Amherst. Massachusetts, United States. 109 p.
- Bwanika, G., D. Murie and L. Chapman. 2007. Comparative age and growth of Nile tilapia (*Oreochromis niloticus* L.) in Lakes Nabugabo and Wamala, Uganda. *Hydrobiologia*, 589: 287-301.
- Charo-Karisa, H., H. Komen, M. Rezk, R.W. Ponzoni, J.A.M. Van Arendonk and H. Bovenhuis. 2006. Heritability estimates and response to selection for growth of Nile tilapia (*Oreochromis niloticus*) in low-input earthen ponds. *Aquaculture*, 261(2): 479-486.
- Chew, B.P. 1992. Role of carotenoids in the immune response. *J. Dairy Sci.*, 76: 2804–2811.
- Chew, B.P. and J.S. Park. 2004. Functions and actions of retinoids and carotenoids: building on the vision of James Allen Olson. *Journal of Nutrition*, 134: 257S-261S.
- Chitsa, H., T. Mtaita, and J. Tabarira. 2014. Nutrient content of water spinach (*Ipomoea aquatica*) under different harvesting stages and preservation methods in Zimbabwe. *Intl. J. Biol. Chem. Sci.*, 8(3): 854-861.
- Dada, A.A. and M. Ikuerowo. 2009. Effects of ethanolic extracts of *Garcinia kola* seeds on growth and haematology of catfish (*Clarias gariepinus*) brood stock. *African Journal Agriculture Research*, 4: 344-347.
- Dijkstra, P.D., R. Hekman, R.W. Schulz and T.G.G. Groothuis. 2007. Social stimulation, nuptial coloration, androgens and immunocompetence in a sexual dimorphic cichlid fish. *Behav. Ecol. Sociobiol.*, 61: 599–609.

- Diler, I. and K. Dilek. 2002. Significance of pigmentation and use in aquaculture. *Turkish Journal of Fisheries and Aquatic Science*, 2: 97-99.
- Edie, E.E. and B.W.C. Ho. 1969. *Ipomoea aquatica* as a vegetable crop in Hong Kong. *Economic Botany*, 23: 32-36.
- Fallahpour, F., M. Banaee and N. Javadzade. 2014. Effects of dietary marshmallow (*Althaea officinalis* L.) extract on growth performance and body composition of common carp (*Cyprinus carpio*). *International Journal of Advance Biological and Biomedical Research*, 2 (8): 2453-2460.
- Fay, M.F., M.D. Lledo, M.M. Kornblum and M.B. Crespo. 1999. From the waters of Babylon? *Populus euphratica* in Spain is clonal and probably introduced. *Biodiversity and Conservation*, 8: 769-778.
- Fryer, G and T.D. Iles. 1972. *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. Oliver and Boyd. Edinburgh. 641 p.
- Garcia-Chavaria, M. and M. Lara-Flores. 2013. The use of carotenoids in aquaculture. *Research Journal of Fishery and Hydrobiology*, 8(2): 38-49.
- Gothberg, A., M. Greger and B. Bengtsson. 2002. Accumulation of heavy metals in water spinach (*Ipomoea aquatica*) cultivated in the Bangkok region, Thailand. *Environ. Toxicol. Chem.*, 21: 1934-1939.
- Grether, G.F., G.R. Kolluru and K. Nersissian. 2004. Individual colour patches as multicomponent signals. *Biol. Rev.*, 79: 583-610.
- Güroy B., I. Sahin, S. Mantoglu and S. Kayali. 2012. Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. *Aquacult. Int.*, 20: 869-878.
- Hill, G.E. and J.D. Johnson. 2012. The vitamin A-redox hypothesis: a biochemical basis for honest signalling via carotenoid pigmentation. *Am. Nat.* 180, E127-E150.
- Hoening, J.M. and T. Gedamke. 2007. A simple method for estimating survival rate from catch rates from multiple years. *Transactions of the American Fisheries Society*, 136: 1245-1251.
- Immanuel, G., P.R. Uma, P. Iyapparaj, T. Citarasu, S.M. Punitha Peter, M. Michael Babu and A. Palavesam. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia (*Oreochromis mossambicus*). *Journal of Fish Biology*, 74: 1462-1475.

- Ji, S.C., G.S. Jeong, G.S. Im, S.W. Lee, J.H. Yoo and K. Takii. 2007. Dietary medicinal herbs improve growth and some nonspecific immunity of red sea bream, *Pagrus major*. *Fisheries Sciences*, 73: 70-76.
- Johnson, E.A. and G.H. An. 1991. Astaxanthin from microbial sources. *Critical Reviews of Biotechnology*, 11: 297-326.
- Kala, A. and J. Prakash. 2004. Nutrient composition and sensory profile of differently cooked green leafy vegetables. *International Journal of Food Properties*, 7: 659-669.
- Khan, V. 2011. *Oreochromis niloticus* (Nile tilapia). Retrieved from www.sta.uwi.edu on March 11, 2016.
- Kodric-Brown, A. 1998. Sexual dichromatism and temporary color changes in the reproduction of fishes. *Am. Zool.*, 38: 70-81.
- Ligon, R.A and K.L. McCartney. 2016. Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. *Current Zoology*, 62: 1-29.
- Logan, D.W, S.F. Burn and I.J. Jackson. 2006. Regulation of pigmentation in zebra fish melanophores. *Pigment Cell Res.*, 19: 206-213.
- Lovshin, L.L. 1997. *Criteria for selecting Nile tilapia and red tilapia for culture*. Retrieved from www.pdacrsp.oregonstate.edu on March 11, 2016.
- Ly, J., P. Samkol, C. Ty and T.R. Preston. 2002. Nutritional evaluation of aquatic plants for pigs; pepsin/pancreatin digestibility of seven plant species. Retrieved from <http://www.cipav.org.co/lrrd/lrrd14/1/ly141b.htm> on March 11, 2016.
- Maan, M.E., M.P.Q.J. van der Spoel, J.J.M. van Alphen and O. Seehausen. 2006. Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behav. Ecol.*, 17: 691-699.
- McGraw, K.J. and D.R. Ardia. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am. Nat.*, 162: 704-712.
- Moav, R., G.W. Wohlfarth, G.L. Schroeder, G. Hulata and H. Barash. 1977. Intensive polyculture of fish in freshwater ponds. I. Substitution of expensive feeds by liquid cow manure. *Aquaculture*, 10 (1): 25-43.

- Nagy, E.B., A.H. Walaa, S.E. Omayma and E.A.E. Mohamed. 2015. Effect of adding different carotenoid sources on growth performance, pigmentation, stress response and quality in red tilapia *Oreochromis* spp. Retrieved from <https://www.was.org> on July 20, 2016.
- Nakano, T., T. Kanmuri, M. Sato and M. Takeuchi. 1999. Effect of astaxanthin rich red yeast (*Phaffia rhodozyma*) on oxidative stress in rainbow trout. *Biochimica et Biophysica Acta*, 1426: 119-125.
- Nya, E.J. and B. Austin. 2009. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 32: 963-970.
- Olvera-Novoa, M.A., S.G. Campos, M.G. Sabido and C.S. Martinez Palacios. 1990. The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). *Aquaculture*, 90: 291-302.
- Pakravan, S., A. Hajimoradloo and R. Ghorbani. 2011. Effect of dietary willow herb *Epilobium hirsutum* extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*. *Aquaculture Research*, 43: 861- 869.
- Peterson, M.S, W.T. Slack and C.M. Woodley. 2005. The occurrence of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus) in coastal Mississippi, USA: ties to aquaculture and thermal effluent. *Wetlands*, 25: 112-121.
- Pongmaneerat, J. and T. Watanabe. 1991. Nutritive value of protein of feed ingredients for carp *Cyprinus carpio*. *Nippon Suisan Gakkaishi*, 57: 503-510.
- Popma, T. and M. Masser. 1999. Tilapia life history and biology. Southern Regional Aquaculture Center Publication No. 283. Stoneville, Mississippi, USA. 4 p.
- Rao, Y.V., B.K. Das, P. Jyotirmayee and R. Chakrabarti. 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, 20: 263 – 273.
- Rottmann R.W., R. Francis-Floyd, and R. Durborow. 1992. The Role of Stress in Fish Disease. Retrieved from <https://wwwwww.darc.cms.udel.edu/AquaPrimer/stress474fs.pdf> on May 26, 2017.
- Sahu, S., B.K. Das, B.K. Mishra, J. Pradhan and N. Sarangi. 2007. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology*, 23: 80-86.

- Shahidi, F., A. Metusalach and J.A. Brown. 1998. Carotenoid pigments in seafood and aquaculture. *Critical Reviews of Food Science and Nutrition*, 38: 1-67.
- Sivaram, V., M.M. Babu, T. Citarasu, G. Immanuel, S. Murugadass and M.P. Marian. 2004. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemental diets gains *Vibrio harveyi* infections. *Aquaculture*, 237: 9-20.
- Stafford, E.A. and A.G.J. Tacon. 1985. The nutritional evaluation of dried earthworm meal (*Eisenia foetida*, Savigny, 1826) included at low levels in production diets for rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture Management*, 16: 213-222.
- Soosean, C., K. Marimuthu, S. Sudhakaran and R. Xavier. 2010. Effects of mangosteen (*Garcinia mangostana* L.) extracts as a feed additive on growth and haematological parameters of African catfish (*Clarias gariepinus*) fingerlings. *European Review of Medicine Pharmacy Sciences*, 14: 605-611.
- Tacon, A.G.J., E.A. Stafford and C.A. Edwards. 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. *Aquaculture*, 35: 187-199.
- Trewavas, E. 1983. Tilapiine Fishes of the Genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum (Natural History). London, UK. 583 p.
- Umar, K.J., L.G. Hassan, S.M. Dangoggo and M.J. Ladan. 2007. Nutritional composition of water spinach (*Ipomoea aquatica* Forsk.) leaves. *Journal of Applied Sciences*, 7(6): 803-809.
- Van Heel, W.A. 1971. Notes on some more tropical labyrinth seeds. *Blumea*, 19: 109-111.
- Von Lintig, J. 2010. Colors with functions: elucidating the biochemical and molecular basis of carotenoids metabolism. *Annu. Rev. Nutr.*, 30: 35-56.
- Von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist and H. Wittzell. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B*, 266: 1-12.
- Yu, M.C., Z.J. Li, H.Z. Lin, G.L. Wen and S. Ma. 2008. Effects of dietary bacillus and medicinal herbs on the growth, digestive enzyme activity, and serum biochemical parameters of the shrimp *Litopenaeus vannamei*. *Aquaculture International*, 16: 471-480.