COLOR AND CAROTENOIDS IN TILAPIA FISH FED DIFFERENT CAROTENOIDS

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Abstract – The aim of this study was to evaluate the effects of different carotenoids present in tilapia fish (*Oreochromus niloticus*) feed on the color and the carotenoid content of the fillets. Nine hundred sixty tilapia fish weighing 20 – 40 g were randomly distributed in 24 tanks to receive the experimental diets during 80 days. The treatments consisted of six diets, as follows: one diet without any carotenoid, as the negative control (T1), one diet containing astaxanthin (T2) and four diets containing different concentrations of bacterial *Rubrivivax gelatinosus* biomass (T3, T4, T5 and T6), in which the main carotenoids are spirilloxanthin, spheroidene and hidroxyspheroidene. Color was measured objectively in the CIE*Lab* space and the carotenoids were extracted with solvents and estimated from the absorbance of the final solution. Except the treatment with 175 mg/kg of bacterial biomass (T3), the other treatments provided higher redness to flesh color than negative control. All treatments containing carotenoids provided higher carotenoids contents in the fillets than the negative control group. It was concluded that both the synthetic astaxanthin and *R. gelatinosus* biomass added into tilapia fish diets were able to increase the carotenoids concentration and the redness of fillets.

I. INTRODUCTION

The appearance of a food item plays a role in consumer evaluation and, hence, choice. For food, color is ranked alongside freshness as one of the main criteria governing selection (1). The distinctive red color of some fish makes them an option besides white fish, so adding value to the product and creating a trend in market (2). In farmed fish, the restriction to krill and phytoplankton prevents flesh to reach the red-pink color as seen in fish living in wilderness (3). Since the color of the muscle is such an important quality attribute, carotenoids are used as colorants to provide pigmentation for farmed fish (3, 4). Besides their role as colorants in food, some carotenoids may also have antioxidant properties, preventing the deterioration caused by rancidity in food products (5). Moreover, some authors claim for positive effects on human health due to the ingestion of carotenoids, like the pro vitamin A role (6), the influence in the immune response (1, 7, 8), and the anti-cancer and anti-coronary disease activities (9, 10).

Currently, carotenoids may be obtained from multistep chemical synthesis or from solvent-based extraction procedures from natural sources like plants, yeasts, molds, algae and bacteria (4, 11). Synthetic astaxanthin is widely used in aquaculture since it shows good ability to deposit in the skin, muscles, gonads and eggs of fish (12). Nevertheless, the market for natural colorants has been showing an increasing importance due to the restriction to artificial additives in foods (13). In this scenery, carotenoids produced by *Rubrivivax gelatinosus*, a phototrophic bacterium with the ability to grow in industrial liquid byproducts (14, 15), appear as an alternative for the use of synthetic colorants.

Although color is a feature for salmonids, this study was conducted with tilapia (*Oreochromus niloticus*) due to the ease of farming this kind of fish in Brazil. However, the production of red tilapia flesh might aggregate value to the product and provide another option for consumers, so representing a new trend in market.

The aim of this study was to evaluate the effects of different colorants present in tilapia fish feed on the color and the carotenoid content of fillets.

II. MATERIALS AND METHODS

Experimental design, treatments and fish farming

A completely randomized design with six treatments and four replicates was adopted for the study. Nine hundred sixty tilapia fish (*Oreochromus niloticus*) weighing 20 – 40 g were randomly distributed in 24 tanks to receive the experimental diets. The treatments consisted of six diets, as follows: one diet without any carotenoids as the negative control (T1), one diet containing astaxanthin 10% (Carophyll Pink, T2) and four diets containing *R. gelatinosus* biomass (with spirilloxanthin, spheroidene and hidroxyspheroidene as the main oxycarotenoids) at different concentrations (T3, T4, T5 and T6), according to Table 1. The bacterial biomass contained 57% protein, 11% lipids, 4% minerals and 3 mg oxycarotenoids per gram and was grown in the fish industry effluent (16).

Feeding was performed thrice a day during the trial, which lasted for 80 days. During the experimental period, residues deposited in the bottom of the tanks were removed by siphoning once a week and the water quality parameters (pH, dissolved oxygen, nitrate, ammonia, chlorine) were monitored every two days. Proper aeration was ensured by using an air compressor and temperature was maintained at 28ºC. At the end of the experiment, fishes were stunned in a benzocaine solution, slaughtered by sectioning the gills and cut in fillets for the analyses.

Color assessment

The objective color (*L* - lightness, *a* - redness, *b* - yellowness) was assessed on the central point of the medial side of fresh fillets (Figure 1) with a portable colorimeter MiniScan XE Plus (Hunterlab) previously standardized with black and white tiles, using illuminant D65 and 10º for the observer angle. Three consecutive measurements were performed for obtaining the average of color attributes.

Table 1. Treatments of the experiment

Treatment	Diets
T1	Basal diet
T2	Basal diet + Carophyll Pink (350 mg/kg)
T3	Basal diet + *R. gelatinosus* biomass (175 mg/kg)
T4	Basal diet + *R. gelatinosus* biomass (350 mg/kg)
T5	Basal diet + *R. gelatinosus* biomass (700 mg/kg)
T6	Basal diet + *R. gelatinosus* biomass (1400 mg/kg)

Fig. 1. Central point of the medial side of fresh fillets

Carotenoid concentration

Fresh fillets were frozen at - 30 °C and then lyophilized in a vacuum chamber during 48 h. The powdered flesh used to asses on carotenoid concentration was obtained by grinding the lyophilized samples with a mortar and a pestle. For the carotenoids extraction, 1 mL DMSO was added to 0.1 g of the powdered flesh in Eppendorf tubes, followed by vortexing for 10 s and sonication in a 40 °C water bath for 15 min. Next, 500 µL acetone was added and the contents were again vortexed for 10 s and centrifuged at 400 x g for 4 min. For the phase separation, the extract was transferred to another tube and added of 500 µL diethyl ether and 250 µL deionized water. The upper phase was transferred to another tube and dried under N_2 stream. Saponification was performed overnight with 200 µL 6% KOH:ethanol (1:9), followed by another partition with diethyl ether and deionized water and final drying with N_2. For the carotenoid quantification, the dried extract was diluted in 2 mL ethanol and the absorbance was read in spectrophotometer at 475 nm. Extinction coefficient 2500 was used for the calculations and the carotenoids in powdered flesh of tilapia fish were expressed as total carotenoids, in mg/kg.

Statistical analyses

Data on color and carotenoids concentrations were analyzed by ANOVA and Tukey´s test to check on differences between means. Data were analyzed by the Action Supplement procedures of Excel. All statements of significance were based on $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 2 shows the results for the objective color of tilapia fillets. Lightness and yellowness were not significantly influenced by treatments (P = 0.3072 and P = 0.4480, respectively) whist the other treatments with carotenoids (except T3), provided higher redness to flesh color than negative control.

According to Diler et al. (17), diets for farmed fish must be supplemented with either natural or synthetic pigments not to be rejected by consumers due to the pale and greyish colors of their flesh. Results found in this study certify the ability of astaxanthin and *R. gelatinosus* biomass to provide redness improvement in farmed fish.

Other authors had already found an increase in trouts pigmentation due to the use of synthetic astaxanthin in the diets (18, 19). Nevertheless, although *R. gelatinosus* biomass had already been tested for improving broilers meat color (20), this was the first time it was used as a colorant for fish feeding, what renders the results presented herein very relevant. So, it was shown that dosages around 370 mg biomass/kg feed and above are necessary to increase redness in tilapia muscles, providing *a* values similar to those provided by the synthetic pigment.

Table 3 shows the concentration of carotenoids in the fillets. From that it can be seen that in all the treatments with colorants, the carotenoid contents were higher than for the negative control group (P<0.05). Some other authors also found the deposition of carotenoids from diets in fish fillets (18, 19, 21, 22). However, in this study, there seemed to be a saturation effect for the deposition of these substances in muscles since, regardless of the type and the quantity administered, the amounts of carotenoids found in the flesh were the same. That means that the carotenoids in *R. gelatinosus* biomass have the same ability as astaxanthin to deposit in muscles. These are very promising results since the frequent consumption of fish fed carotenoids might work as a carotenoid supplementation and so bring beneficial effects to human health.

The results found in this study show that *R. gelatinosus* biomass may find a role as a pigmenting additive for tilapia fish and so be considered as an option besides the synthetic additive studied.

Table 2. Color attributes for tilapia fish fillets

	Color attribute*		
Treatment	***L***	***A***	***B***
T1	52.25 ± 0.40^a	1.21 ± 0.26^b	10.95 ± 0.77^a
T2	51.84 ± 2.34^a	2.48 ± 0.26^a	11.37 ± 0.41^a
T3	50.68 ± 1.46^a	1.37 ± 0.17^b	10.87 ± 0.19^a
T4	51.85 ± 0.53^a	2.08 ± 0.31^a	10.52 ± 1.41^a
T5	50.89 ± 0.98^a	2.66 ± 0.31^a	12.01 ± 2.21^a
T6	50.18 ± 1.80^a	2.49 ± 0.30^a	11.88 ± 0.85^a
CV	1.58	30.25	5.25
P	0.3072	>0.0001	0.4480

* Means followed by different letters in the column are significantly different (P < 0.05)

Table 3. Carotenoid concentration (mg/kg) in tilapia fish fillets

Treatment	**Carotenoid concentration (mg/kg)***
T1	3.3 ± 0.41^b
T2	5.7 ± 0.16^a
T3	5.3 ± 0.21^a
T4	5.3 ± 0.22^a
T5	5.7 ± 0.90^a
T6	5.8 ± 0.54^a
CV	18.58
P	>0.0001

* Means followed by different letters are significantly different (P < 0.05)

IV. CONCLUSION

Both the synthetic astaxanthin and the *R. gelatinosus* biomass added into tilapia fish diets increased the carotenoids concentration and the redness of fillets.

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REFERENCES

1. Baker, R. & Günther, C. (2004). The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. Trends in Food Science and Technology 15: 484–488.
2. Takahashi, N. S., Tsukamoto, R. Y., Tabata, Y. A. & Rigolino, M. G. (2008). Truta salmonada. Panorama da Aquicultura 18: 28-33.
3. Breithaupt, D. E. (2007). Modern application of xanthophylls in animal feeding e a review, 2007. Trends in Food Science & Technology 18: 501-506.
4. Bhosale, P. & Bernstein, P. S. (2005). Microbial xanthophylls. Applied Microbiology and Biotechnology 68: 445–455.
5. Stahl, W. & Sies, H. (2003). Antioxidant activity of carotenoids. Molecular Aspect of Medicine, 24: 345-351.
6. Yeum, K.-J., Aldini, G., Russell, R. M. & Krinsky, N. I. (2009). Antioxidant/ pro- oxidant actions of carotenoids. In G. Britton et al., Carotenoids, vol. 5: Nutrition and health (pp. 235-268). Basel, Boston, Berlin: Birkhäuser verlag.
7. Chew, B P. & Park, J. S. (2009). The immune system. In G. Britton et al., Carotenoids, vol. 5: Nutrition and health (pp. 363-382). Basel, Boston, Berlin: Birkhäuser verlag.
8. Park, K., Gross, M., Lee, D. H., Holvoet, P., Himes, J. H., Shikany, J. M. & Jacobs Jr., D. R. (2009). Oxidative stress and insulin resistance. Diabetes Care 32: 1302:307.
9. Johnson, E. J. & Krinsky N. I. (2009). Carotenoids and coronary heart disease. In G. Britton et al., Carotenoids, vol. 5: Nutrition and health (pp. 287-300). Basel, Boston, Berlin: Birkhäuser verlag.
10. Rock, C. L. (2009). Carotenoids and cancer. In G. Britton et al., Carotenoids, vol. 5: Nutrition and health (pp. 269-286). Basel, Boston, Berlin: Birkhäuser verlag.
11. Chociai, M. B., Machado, I. M. P., Fontana, J. D., Chociai, J. G., Busato, S. B. & Bonfim, T. M. B. (2002). Cultivo da levedura *Phaffia rhodozyma (Xanthophyllomyces dendrorhous)* em processo descontínuo alimentado para produção de astaxantina. Brazilian Journal of Pharmaceutical Sciences. 38: 457-462.
12. Meyers, S. P. & Chen, H.M. (1982). Astaxanthin and its role in fish culture. In Proceeding of the warmwater fish culture (pp. 153–165), Louisiana State University, United States.
13. Squina, F. M. & Mercadante, A. Z. (2003). Análise, por CLAE, de carotenoides de cinco linhagens de *Rhodotorula*. Brazilian Journal of Pharmaceutical Sciences 39: 309-318.
14. Ponsano, E. H. G., Lacava, P. M. & Pinto, M. F. (2003). Chemical composition of *Rhodocyclus gelatinosus* biomass produced in poultry slaughterhouse wastewater. Braz. Arch. Biol. Technol. 46:143–147.
15. Lima, L. K. F., Ponsano, E. H. G. & Pinto, M. F. (2011). Cultivation of *Rubrivivax gelatinosus* in fish industry effluent for depollution and biomass production. World Journal of Microbiology and Biotechnology 27: 2553 – 2558.
16. Ponsano, E. H. G., Lima, L. K. F. & Torres, A. P. C. (2011). From a pollutant byproduct to a feed ingredient. In D. Matovic, Biomass – detection, production and usage (pp. 461-472). Rijeka: Intech.
17. Diler, I. & Dilek, K. (2002). Significance of pigmentation and use in aquaculture. Turk. J. Fish. Aquat. Sci. 2: 97-99.
18. De La Mora, G. I., Arredondo-Figueroa, J. L., Ponce-Palafox, J. T., Barriga-Soca, I. & Vernon-Carter, J. E. (2006). Comparison of red chilli (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhyncus mykiss*) fillet pigmentation. Aquaculture 258: 487–495.
19. Yesilayer, N. & Erdem, M. (2011). Effects of oleoresin paprika (*capsicum annum*) and synthetic carotenoids (canthaxantin and astaxanthin) on pigmnetation levels and growth in Rainbow trout *Oncorhynchus mykiss* W. J. Anim. Vet. Adv. 10: 1875-1882.
20. Ponsano, E. H. G., Avanço, S. V., Grassi, T. L. M., Minello, M. C. S., Santo, E. F. E., Pinto, M. F. & Garcia Neto, M. (2012). Microbial oxycarotenoids in broilers chicken rearing. In Proceedings 58th International Congress of Meat Science and Technology (DIETQUALP-105), 12-17 August 2012, Montreal, Canada.
21. Katsuyama, M., Komori, T. & Matsuno, T. (1987). Metabolism of three steroisomers of astaxanthin in fish, rainbow trout and tilapia. Comp. Biochem. Physiol. 86: 1-5.
22. Czeczuga, B., Semeniuk, J., Czeczuga-Semeniuk, E., Semeniuk, A. & Klyszejko, B. (2013). Amount and qualities of carotenoids in fillets of fish species fed natural feed in some fisheries of West African Coast. Afr. J. of Biotechnol 12: 1443-14.