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QUANTIFYING THE SKIN PIGMENTATIONOF GRAY (*Oreochromis niloticus*) AND RED (*Oreochromis spp.*) TILAPIA FED WITH CAROTENOID RICH PLANTS

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ABSTRACT

The red coloration is more appealing than the paler ones. Sources of coloration may come from feeds, genetics and environment. Carotenoid pigments, obtained by fish from their diets, give most of the bright red, yellow and orange colors and strengthen the immune system and help them grow faster. Natural carotenoids have traditionally been used as dietary coloring agents; however, carotenoids have been found to carry out essential roles in nearly all biological systems.

Supplementing carotenoids from various economical plant sources on aquaculture feed mills promotes growth, high survival rate and darker skin coloration. Thus, making fish farmers and cultivators increased their yield in Nile tilapia as well as increased the market value of Red tilapia by having darker skin coloration.

Keywords: ***Oreochromis spp. Oreochromis niloticus,*** **Skin pigmentation, Carotenoid**

INTRODUCTION

The coloration of skin and flesh of fish is one of the quality attributes of the fish for consumer acceptance or rejection [1]. The red meat of fish is gaining importance in consumer because of their coloration and aesthetic value [2, 3].

High prices of feed-stuffs for the preparation of diets are a great problem for various types of aquaculture, which may represent 40-60% of total operational costs [4, 5, 6].

Pigments are responsible for the wide spectrum of colors in fishes which is an essential prerequisite for the quality as they fetch higher price in the commercial market. As fishes cannot synthesize their own coloring pigments *de novo*, the coloring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in the diet [7]. Varieties of coloring agents are used in aquaculture industry as important criterion for fishes, since their color affect commercial acceptability. Carotenoids have been included in diets of salmonids, crustaceans and other fish, mainly as pigments to provide desirable coloration to these cultured organisms [8]. Carotenoids form one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables and they are of great interest due to their essential biological functions in both plants and animals [9].

The varieties of carotenoids pigment are used in fish diet for color enhancement. The most promising carotenoid that was successfully proven in enhancing color is astaxanthin that shows marked improvement in color in most species brightly coloured ornamental fishes like tetras, cichlids, gouramis, goldfish, koi, danios and many other species. Carotenoids are responsible for pigmentation of muscle in food fish and skin color in ornamental fish [4]. Carotenoid also plays other important functions as pro-vitamin A, antioxidants, immune regulators and they are mobilized from muscle to ovaries which suggest a function in reproduction [10, 11, 12]. It was observed that fishes have a high level of carotenoids in their body so they are more resistant to bacterial and fungal diseases [10].

Red tilapia is a genetic mutant selected from tilapia species in the genus *Oreochromis.* The first reported red tilapia hybrid was produced in Taiwan in the late 1960s and was a cross between a mutant reddish-orange female *O. mossambicus* and a normal male *O. niloticus* and is called the Taiwanese red tilapia [13]. The Philippines red tilapia strain was suggested as a triple hybrid of *Oreochromis niloticus x O. mossambicus x O. hornorum* [14, 15, 16] or of *O. niloticus x O. mossambicus x O. aureus* [13] whereas the *O. mossambicus* red tilapia was considered a hybrid of the white mutant *O. mossambicus x O. niloticus* [17]. *O. niloticus* red tilapia was described as a purebred red mutant of *O. niloticus* originating in Egypt [18].

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

Carrot (*Daucuscarota*) peel, Water spinach (*Ipomoea aquatica*) leaves, Horseradish (*Moringa oleifera*) leaves and were collected from Science City of Muñoz, Nueva Ecija. Matured leaves of water spinach and Horseradish and carrot peels without rots were used in the study.

The leaves were dried in oven with temperature of 40°C for about 1 to 2 hours until the plant samples were completely dried and suitable for grinding. The oven dried leaves were then pulverized and sieved to get fine particles of dried plant samples.

Extraction of Carotenoid Pigments

Acetone was used to extract carotenoid pigments [2]. One hundred fifty grams (150g) of dried plant samples were immersed individually in 750 ml of acetone for 24 hours. The extracts were filtered and air dried and kept refrigerated until use.

Preparation of Experimental diets

About 500 mg and 1000 mg of the collected pigment extract were dissolved in 500 ml of ethyl alcohol separately and mixed in 1 kilogram of commercial feed. The prepared diets were air-dried until the alcohol completely evaporated and were kept in container until use.

Experimental Fish

One-month old Red tilapia (*Oreochromis spp.*) and Nile tilapia (*Oreochromis niloticus* L.) were used in the study. Nile tilapia fingerlings were obtained from the Freshwater Aquaculture Center (FAC), Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines.

Experimental design

The study followed a completely randomized design and consisted of three (3) treatments with 3 replications per treatment for each of the plant samples.

Stocking and Sampling

Each aquarium was stocked with 30 fry of Red and Nile tilapia individually per replicates for the 3 plant samples. Initial weights of the fry were determined prior to stocking and sampling was conducted two weeks after stocking to measure weight and survival rate. Final sampling was conducted after 30 days of rearing. Changing of water was done thrice a week to ensure the cleanliness of each aquarium.

Feeding of Fish

The experimental fish were fed four times a day. Feeding rate was 100% of fish body weight for the duration of 30 days.

Data gathered

The data on initial weight, final weight, gain weight, absolute growth,

specific growth rate, and survival rate were determined using the formula;

1. $$\% \text{ survival} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}} \times 100$$

2. $$\text{Specific growth rate (SGR)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Total number of cultured days}} \times 100$$

3. Gain in weight = final weight – initial weight

Observation of Coloration on Fish Fry

The fry was photographed using digital camera. A color chart (Figure 1 and Figure 2) was developed and used in differentiating the degree of skin coloration of Red and Nile Tilapia fry in all treatments were assigned with values (1-10) to quantify the color.

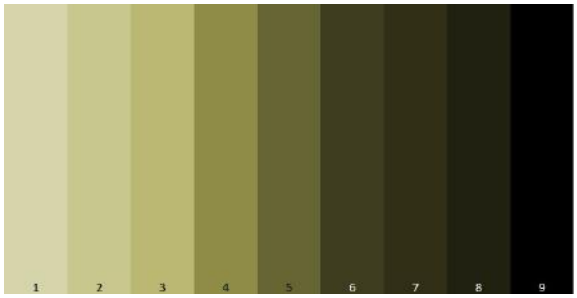

Figure 1: Color chart for Nile Tilapia (*Oreochromis niloticus* L.)(Velasco, R. R., 2018)

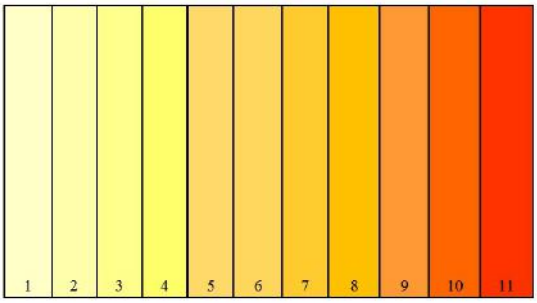

Figure 2: Color chart for the Red Tilapia (*Oreochromis spp.*) (Velasco, R. R., 2018)

Statistical analysis

The data gathered were analyzed using one-way Analysis of Variance (ANOVA) in a completely randomized design (CRD) followed by Duncan's Multiple Range Test (DMRT) for the comparison of means.

RESULTS AND DISCUSSIONS

CARROT (*Daucuscarota*) PEEL

Growth Performance

Fishes generally exhibit what we call indeterminate growth. Most fishes continue to grow throughout their lives. Their growth rates are determined by food supply, habitat, water quality and competition, among other factors. Table 2 shows the growth performance of red tilapia fingerlings in terms of gain in weight, specific growth rate and survival rate after 30 days of culture.

Body Weight Gain

Red tilapia in Treatment III fed with 1000 mg of carotenoid pigments had the highest gain in weight of 1.82 g, followed by those in Treatment II fed with 500 mg of carotenoid pigments with gain in weight of 1.46 g. Red tilapia in Treatment I (commercial feed) obtained lowest gain in weight with 1.27 g.

Specific Growth Rate

Specific growth rate is widely used in articles dealing with growth of aquatic organisms under experimental conditions. It is used to investigate growth and growth patterns correlates in both basic and applied research [22]. Red tilapia in Treatment with 1000 mg of carotenoid pigments had the highest specific growth

rate of 5.20 %, followed by those in Treatment with 500 mg of carotenoid pigments. Red tilapia in Treatment control (commercial feed) had the lowest specific growth rate of 4.89 %.

Survival Rate

The highest survival rate was likewise obtained in Treatment III (1000 mg of carotenoid pigments) with 98.88 %, followed by Treatment II (500 mg of carotenoid pigment) with 96.66%. The lowest survival rate was in Treatment I (commercial feed) with 93.33 %.

Ornamental swordtail *Xipho phorushelleri*, fry fed with natural sources of carotenoid pigments from *Rosa rubiginosa* enhanced the growth performance and survival of fish [5]. Results of this study, likewise revealed that red tilapia fed with diets containing carotenoid pigments from *D. carota* peel had better growth performance and survival rate than those fed without carotenoid pigments in the diet. Carotenes are known to have a positive role in intermediate metabolism of fish [20]. It helps to enhance the nutrient utilization, which may result in growth improvement of fish [21].

Skin Coloration

Table 3 presents the data on skin coloration of red tilapia fingerlings fed with commercial feeds with different concentrations of carotenoid pigments from *Daucus carota* peel after 30 days of culture period.

The skin coloration of Red tilapia was compared using a color chart (Figure 1). Results showed that Red tilapia in Treatment III (1000 mg of carotenoid pigments) had more intense skin coloration with a value of 9.82 ± 0.005, followed by those in Treatment II (500 mg of carotenoid pigments from *D. carota* peel) with a value of 8.82 ± 0.000. Red tilapia in Treatment I (commercial feeds) obtained the lowest skin coloration with a value 6.69 ± 0.205.

Red tilapia fed with feeds supplemented with 1000 mg of carotenoid pigments expressively enhanced skin coloration. Higher concentration of carotenoid pigments from *D. carota* peel on the diet of Red tilapia gave the best result on the skin coloration of Red tilapia fingerlings thus, making its value higher in the market. β-carotene as the principal pigment in carrot gives a red orange appearance [22]. When the fish ingest the β-carotene, these are slowly transformed to the astaxanthin in the gut [23]. When the fish get sufficient amount of β-carotene, excess will be deposited as astaxanthin to give the bright pink color to the flesh and the skin [5]. Environmental factors such as temperature, pH and dissolved oxygen will also determine the skin pigmentation while handling stress and method of sacrifice

also have been known to provide negative influence on coloration of fish skin [24].

Carotenoids are responsible for pigmentation of muscle in food fish, and skin color in ornamental fish. As fish is not capable of synthesizing carotenoids *de novo* there is a need to incorporate carotenoids in the diet of cultured species. Since synthetic carotenoids are known to have deteriorating effects on the environment, there is a great demand for inclusion of natural carotenoids in aqua feed to achieve bright coloration in fish [25].

Water Spinach (*Ipomoea aquatica*)

Growth Performance in Nile Tilapia

Table 4 shows the growth performance of Nile tilapia in terms of gain in weight, specific growth rate and survival rate after 30 days of culture.

Body Weight Gain

Highest body weight gain was obtained in Treatment III, 3.65 g, followed by Treatment I (2.47 g). Lowest body weight gain was in Treatment II (2.26 g).

Specific Growth Rate

Nile tilapia in Treatment III had the highest specific growth rate of 4.81 g, Administration of carotenoid-rich diet also improved growth rates and enhanced reproductive performance of [29]. Several herbal components as flowers, leaves, seeds and roots from different plant species have been shown to enhance growth, non-specific immune response as well as survival rates of such cultivated species like African catfish, *Clarias gariepinus* [27,28], tilapia *Oreochromis mossambicus* [29] and common carp, *Cyprinus carpio* [30,31,32]. Carotenoid from water spinach leaves with the concentration of 1000 mg in the diet gave the best result on the growth performance of Nile tilapia fingerlings thus, giving fish farmers higher yield and income.

Survival Rate

Various sources of dietary carotenoids have positive effect on growth and survival of [33], immune function and disease resistance in higher animals and human [34, 35, 36]. Physiological stress and physical injury are the primary contributing factors of fish disease and mortality in aquaculture [37]. Fish mortality in the study can be probably attributed to the small size of the fish. Being small, they are easily stressed during sampling, cleaning and changing of water in the aquaria thus survival rates obtained in this study were relatively lower.

Skin Coloration

Skin coloration was compared using a color chart (Fig. 2). Results showed that fish fed with commercial feeds with 1000 mg pigment extract had more intense skin coloration compared to other treatments. Higher concentration of

carotenoid pigments from water spinach leaves on the diet gave the best result in terms of the coloration of Nile tilapia fry. In ornamental swordtail *Xiphophorushelleri* fry fed with higher concentration of natural sources of carotenoid enhanced the skin coloration of fish [5]. The color of gourami was enhanced when fed with carotenoid pigment source [38].

Horseradish (Moringaoleifera)

Growth Performance

Table 6 shows the summary data on the growth performance of Nile tilapia fry in terms of gain in weight, specific growth rate and survival rate after 30 days of culture.

Weight gain

Highest weight gain obtained by Nile tilapia was in Treatment III having a mean of 2.095 g, followed by those in Treatment II with 2.085 g and Treatment I (control) obtained the lowest gain in weight of 2.013 g.

Specific Growth Rate

The highest specific growth rate was obtained by Nile tilapia in Treatment II with 3.727 %. The observed reduced feed intake was perhaps one of the most important factors responsible for growth of fish. Fish fed with diet supplemented with *Moringa*leaves had lower growth performance and poorer diet utilization because of the methionine deficiency [39].

Survival Rate

Nile tilapia in Treatment III obtained the highest survival rate of 86.250 %. It was observed that fishes with high level of carotenoids in their body were more resistant to bacterial and fungal diseases [10]. *M. oleifera*is a potential plant that could be used to enhance immune responses [40]. *Moringa* leaves are rich in carotenoids, tocopherols and vitamin C and have health-promoting potential in maintaining a balanced diet and preventing free-radical damage that can initiate many illnesses [41]. Carotenoids enhanced growth of Atlantic salmon fry (*Salmo salar*), and improved survival rate in kuruma prawn (*P. japonicus*) [42].

Skin Coloration

Nile tilapia in Treatment III had darker skin coloration with the mean of 7.21. Absorption and deposition of carotenoids are affected by several factors including their kinds, dietary concentration and feeding period [43]. Varieties of carotenoids pigments are used in fish diet for color enhancement [4]. Pigmentation in the skin is responsible for coloration in the fish and carotenoids are the primary source of the pigmentation of the skin in fishes [5]. Dietary carotenoids play an important role in the regulation of skin and muscle color in fish [42].

Concentration of carotenoid in muscle of Atlantic salmon is significantly affected by its source and dietary concentration [43]. Skin pigmentation in tiger barb (*Barbus tetrazona),* has been reported to increase, when fed with diet containing carotenoids from shrimp meal, marigold petals and annatto extract [4].

The study showed that fry fed with 1000 mg of carotenoid pigments from *M. oleifera* leaves obtained darker skin coloration. Ornamental swordtail *Xiphophorushelleri* fry fed with feeds with 2000 mg carotenoid pigments from *Rosa rubiginosa*had enhanced skin coloration compared to those fed with feeds containing lower concentration [5].

Water Spinach (*Ipomoea aquatica*)

Specific Growth Rate for Red Tilapia

Red tilapia in Treatment II (500 mg carotenoid pigments) and Treatment III (1000 mg carotenoid pigments) had the same specific growth rate of 4.51%. Treatment I (no carotenoid pigments) obtained the lowest specific growth rate of 4.45%.

Water spinach has high potential to convert efficiently the nitrogen in bio-digester effluent into edible biomass with high protein content [44].Water spinach is a potential source of feed protein concentrate; the edible portion contain up to 29% crude protein on a dry matter basis and a number of nutrients and minerals [45]. *Ipomoea aquatic* contains very low amount of anti-nutritive factors such as trypsin inhibitor, calcium oxalate, tannin and phytate [46].

Survival Rate

Highest fish survival rate was in Treatment II (500 mg carotenoid pigments) with 100%, followed by fish survival rate in Treatment I, 98.33%. Lowest survival rate was in Treatment III, 95.83%. Carotenoids, particularly those that are vitamin A precursors have received increasing attention in recent years due to their reported health benefits. Already, the effects of carotenoids on aquatic animals are multi-faceted: they enhance larval growth and survival, improve the performance of broodstock and nauplii quality, as well as increase resistance to diseases [47].

Skin Coloration

Fish skin coloration was compared using color chart in Figure 2. The results showed that fish fed with 500 mg carotenoid pigments (TII) had the highest mean skin coloration of 8.81 followed by the fed with 1000 mg carotenoid pigments (T III) with mean skin coloration of 8.60. Lowest value of skin coloration was obtained on fish fed with no carotenoid pigments (TI).

Major carotenoids in *Ipomoea aquatic* were characterized by open-column, thin layer and higher performance

liquid chromatography. Comparing the absorption spectra and retention time with reference standards the compounds were identified as β-carotene, cryptoxanthin, lutein epoxide, violoxanthin and neoxanthin[48]. Carotene composition and contents of *I. aquatica*were analyzed by AOAC and HPLC methods. The carotenoids identified were lutein and β-carotene and were compared to that of reference standards [49]. Provitamin A and carotenoids from *I. aquatic* of different maturity and origin were investigated and revealed that the carotene content of mature plant was high compared to that of young plants [50].

CONCLUSION

High priced feed-stuffs for various types of aquaculture diets brought the urge for culturists to find ways on improving the growth, survival and skin coloration of their fish. Supplementing carotenoids from various economical plant sources on aquaculture feed mills promotes growth, high survival rate and darker skin coloration. Thus, making fish farmers and cultivators increased their yield in Nile tilapia as well as Increased the market value of Red Tilapia by having darker skin coloration

Table 1: Treatments used in the study

Treatments	Descriptions
I (control)	commercial feeds
II	commercial feeds with 500 mg of carotenoid pigments from carrot peel extract
III	commercial feeds with 1000 mg of carotenoid pigments from carrot peel extract

Table 2: Mean growth performance (± standard deviation) of Red tilapia (*Oreochromis spp.*) fingerlings fed with different concentration of carotenoid pigment from *Daucuscarota*peel after 30 days of culture.

Parameters	Treatment I	Treatment II	Treatment III
Gain in weight (g)	1.27±0.124a	1.46±0.303a	1.82±0.238a
Specific growth rate (%)	4.98±0.053a	5.00±0.119a	5.20±0.175a
Survival rate (%)	93.33±3.333a	96.66±0.000a	98.88±1.924a

Means in rows with similar letter superscript are not significantly different at 5% level of significance.

Table 3: Mean skin coloration (± standard deviation) of red tilapia fingerlings after 30 days of culture.

	Treatment I	Treatment II	Treatment III
Skin coloration	6.69 ±0.205c	8.82 ± 0.000b	9.82 ± 0.005a

Means in rows with the different letter superscripts were significantly different at 5% level of significance.

Table 4: Means with standard deviation of growth performance of Nile tilapia fed with different concentrations of carotenoid pigments from water spinach leaves after 30 days.

Growth Parameters	Treatment I	Treatment II	Treatment III
Gain in Weight (g)	2.47^{b} (±0.41)	2.26^{b} (±0.32)	3.65^{a} (±0.70)
Specific Growth Rate (g)	4.54^{a} (±0.04)	4.36^{a} (±0.11)	4.81^{a} (±0.30)
Survival (%)	77.77^{a}(±15.03)	73.33^{a} (±15.27)	52.22^{a}(±21.43)

Mean values in rows with the same superscript letters are not significantly different (P<0.05)

Table 5: Summary data on skin coloration of Nile tilapia (*Oreochromis niloticus*) fed with varying levels of carotenoid pigments from water spinach leaves for 30 days.

	Treatment I	Treatment II	Treatment III
Skin Coloration	4.72^{c} (±0.20)	5.35^{b} (±0.21)	5.85^{a} (±0.12)

Mean values in rows with the different superscript letters were significantly different (p<0.05).

Table 6: Mean growth performance (± standard deviation) of Nile tilapia (*O. niloticus*) fry fed with different concentrations of carotenoid pigments from horseradish *(M. oleifera)* leavesafter 30 days of rearing.

Growth Parameters	Treatment I	Treatment II	Treatment III
Weight gain (g)	2.013±0.081[a]	2.085±0.179[a]	2.095±0.310[a]
Specific growth rate (%)	3.665±0.103[a]	3.727±0.200[a]	3.622±0.129[a]
Survival rate (%)	65.833±1.443[a]	72.500±0.000[a]	86.250±12.332[a]

Mean values in rows with the same letters superscript are not significantly different (P<0.05).

Table 7: Summary data on mean skin coloration (± standard deviation) of Nile tilapia (*O. niloticus* L.) fed with commercial feeds containing varying levels of carotenoid pigments from *M. oleifera* leaves after 30 days.

	Treatments		
	Treatment I	Treatment III	Treatment III
Skin Coloration	5.240±0.517[a]	5.990±0.824[a]	7.217±0.478[b]

Mean values in rows with the same letter superscript are not significantly different (P<0.05).

Table 8: Mean (±SD) growth performance of Red tilapia feed with *Ipomeaaquatica* after 30 days of culture Means in rows with similar letter superscript are not significantly different at 5% level of significance

Growth Parameters	Treatments		
	Treatment I	Treatment III	Treatment III
Gain in Weight (g)	0.71[a] (±0.13)	0.80[a] (±0.17)	0.86[a] (±0.15)
Specific Growth Rate (%)	4.45[a] (±0.25)	4.51[a] (±0.23)	4.51[a] (±0.12)
Survival rate (%)	98.33[a] (±1.44)	100[a] (±0.00)	95.83[b] (±1.44)

Table 9. Summary data on skin coloration of Red tilapia (*Oreochromis sp.)* fed with varying levels of caroteniod pigments from water spinach leaves for 30 days.

	Treatment I	Treatment II	Treatment III
Skin Coloration	8.54[a]±0.33	8.81[a]±0.38	8.60[a]±0.80

Mean values in rows with the different letter superscripts are not significantly different

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