# GROWTH AND COLOUR PIGMENTATION OF *CLARIAS GARIEPINUS* FED WITH FEED FORMULATED WITH CARROT (*DAUCUS CAROTA*) AND TURMERIC (*CURCUMA LONGA*)

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## ABSTRACT
*The experiment assessed the growth and pigmentation of Clarias gariepinus feed with two carotenoid-containing feed ingredients; carrot and turmeric. The work was carried out in a completely randomized design with three treatments which were in triplicates. Treatment 1, 2, and 3 were feed formulated with 10% turmeric inclusion, 10% carrot inclusion, and the control with no carrot or turmeric included, respectively. Fish feed wereformulated, and the fish were fed 5% of their body weight, twice daily. Sampling was done biweekly and feed weights were adjusted accordingly. Water quality parameters were monitored and recorded, growth parameterswere calculated from the fish weight. The initial and final carotenoid content of the fish were measured as well as organoleptic test. The result of the physicochemical parameters showed that there were no significant differences ($p>0.05$) amongst the treatments. The growth result also showed no significant differences ($p>0.05$) amongst the treatments. The carotenoid content of the fish showed significant differences ($p<0.05$) amongst the treatments for the final carotenoid content and the change in the level of carotenoid content. Treatment 2 provedto have higher carotenoid content than the others. Feed with carrot inclusion did better amongst the other treatments and show greater pigmentation.*

**Keywords**: colouration, carotenoid, catfish, fish feed, vitamin A.

## INTRODUCTION

The catfish industry is developing rapidly in many countries all around the world with a significant result in an expansion of product availability and the quality and quantity of species cultured. In the United States, for example, the catfish industry is a mature industry with a long history of success (Hanson and Sites, 2011). Nevertheless, the industry faces a lot of challenges as it evolves and adapts to shifting markets, some of them include: disease and survival issues, increasing feed costs, and quality concerns (Adedeji and Okocha, 2011) Efforts are currently underway to help improve the catfish industry using a wide range of technologies, such as new hybrid species application, feed formulation modifications and improved quality control of catfish fillets among others.

Maintaining the natural skin colour or pigmentation of cultured fish is of immense importance in aquaculture especially for commercial purposes, because it has a direct impact on consumer's product acceptance or rejection as well as the market price of the product. A variety of natural and synthetic carotenoids are available to enhance colouration in the flesh of catfish (Yanar *et al.*, 2007). Colour, which is an important test in food quality control or analysis, is an important parameter of food quality in many food industries. Colour is a determinant factor to consumer's acceptability and price of food. It is considered as one of the most important sensory attributes of any food because is a precursor to consumer judgment of other sensory and non-sensory characteristics (Clydesdale, 1991). In the seafood industry, the skin and fillet colour of fish together with texture, flavour, and odour are used as sensory attributes dictating value in the market (Shpigel *et al.*, 2006).

Both astaxanthin and canthaxanthin, which are synthetic carotenoids have been used efficiently either alone or in combination as dietary additives for muscle pigmentation in salmonids (Bjerkeng, 2000). Synthetic carotenoid pigments are commercially available as feed additives, however, they are expensive and uptake levels are poor, estimated between 5% and 10% (Gatlin *et al.*, 2007). Furthermore, there is increasing consumer awareness about the safety of these synthetic feed additives. As a result, there is an increase in interest in the use of natural carotenoids for some fish and shrimp species of economic concern. Carotenoids are a group of over 600 natural lipid-soluble pigments that are primarily produced in bacteria, algae, fungi, and plants. Carotenoids are the main pigments of many aquatic animals as well as ornamental tropical fishes (Meyers, 1994), responsible for various colours like yellow, red, and other related colours. Fishes like every other animal, do not synthesize carotenoids (Fujii, 2000), they have to obtain carotenoids from their feed intake. Fishes can modify alimentary carotenoids and store them in the integument as well as other tissues and organs such as flesh, gonads, kidney, liver, intestines, and only in very small amounts in the brain. Wild carnivorous fishes obtain most of their carotenoid requirements by feeding on small crustaceans and other vertebrates previously fed on algae (Tlusty and Hyland, 2005). However, when fish are removed from the wild, from their natural sources of food, and brought under rearing

conditions, fish depend entirely on added dietary carotenoid intake to achieve their natural colouration, hence, a direct relationship between dietary carotenoids and pigmentation exists in them (Halten *et aI.*, 1997).

Reports have shown that feed rich in carotenoids content led to growth enhancement in rainbow trout, *Oncorhynchus mykiss* (De la Mora *et al*., 2006), Atlantic salmon fry, *Salmo salar* (Kiessing*et al*., 2003), and goldfish, *Carassius auratus* (Sinha and Asimi, 2007). Minerva and Maurilio (2013) stated that Carotenoids are responsible for muscle pigmentation in food fish and skin colour in ornamental fish. Various artificial colouring agents are used in the aqua industry to impart colour to the muscles and skin of fishes as their skin colour is highly dependent on the carotenoids present in the diet. A detailed study on colour enrichment in fish is still lacking. Different natural resources such as the inclusion of crustacean and crustacean processing wastes, chestnut flowers, marigold petal meal, capsicum, etc. are employed to alleviate this problem, but none has performed so effectively (Matsuno, 2001).

Turmeric belongs to the genus; *Curcuma*. Scientific name: *Curcuma longa***,** which is ginger or *Zingiberaceae* family of root herbs, of Curcumin, a polyphenolic compound in the root. It is the primary pigment that is responsible for the deep orange colour of turmeric. Many studies have suggested that curcumin may have anti-tumor, antioxidant, anti-arthritic, anti-amyloid, anti-ischemic, and anti-inflammatory properties (USDA, 2018). Carrots on the other hand are enriched with phytochemicals of high importance, such as carotenoids and phenolic compounds (Stahl and Sies, 2005) that could be extracted and utilised as natural additives (e.g. colourants). The storage root of many carrot cultivars exhibits a characteristic colour which may be due to the accumulation of high levels of carotenoids, this is different from most plants (Manuel and Claudia, 2013).

Carotenoids in addition to their role of pigmentation have various biological effects in fish such as supplying pro-vitamin A, anti-oxidation (involving lipid peroxidation), and immune enhancement (Stahl and Sies, 2005). A lack of nutrients and feed low in carotenoid content can result in retarded growth, faded colouration, and a degraded nutrient profile of the fish. Carotenoids also have a positive effect on the nutrition of larvae and the survival of young fry (Arulvasu *et al*., 2013). Colour maturation, growth, enhanced image, and quality of hybrid catfish are important quality determinants for market value criteria (Chow *et al*., 2016), hence the use of carotenoid in catfish diet.

One of the greatest challenges in the fish industry is to replicate the accurate natural colour of the fish in their captive environment. This study is aimed to achieve that by comparing and checking the effectiveness of feed formulated with dried carrot and turmeric for enhancement of colouration in fish, its growth rate, as well as consumer's preference of the colouration of the catfish fillet.

## MATERIALS AND METHODS

The experiment was carried out in the Department of Fisheries and Aquatic Resources Management laboratory, Michael Okpara University of Agriculture Umudike, in the south-eastern zone of Nigeria. It lies between latitude $5^0$ 26´N and longitude $7^0$ 33´E and with a minimum temperature of $22^0$C and altitude which falls within the range of (122m) 40ft above sea level.

The experiment was carried out in a completely randomized design with three treatments and three replicates each. A total of 90 individual fish were used for the experiment with a mean weight of 3.99g ± 0.03. Treatments were assigned as follows: Treatment 1 was formulated with 10% turmeric inclusion in the feed; treatment 2 was formulated with 10% carrot inclusion in the feed, while treatment 3 was formulated without any inclusion of either carrot or turmeric in the diet and served as the control. The fish were randomly distributed into the 9 experimental units which were 70 litre plastic bowls at a stocking density of 10 fish per bowl. *Clarias gariepinus* fingerlings were used for the experiment. They were purchased from the fish farm and brought to the laboratory. The fish were acclimated for one week before the commencement of the experiment.

The feed ingredients used for feed formulation were as follows; fishmeal, soya bean meal, maize, oil, salt, vitamin premix, carrot, and turmeric. Carrot and turmeric were sundried and were milled for easy inclusion with other feed ingredients. Ten (10%) percent of both turmeric and carrot, respectively were used and added to the experimental diets. Fishmeal, vitamin premix, and soya bean were purchased from a reputable source. Maize was bought from the market and milled. Cassava starch was used as a binder. The feed ingredients were ground, mixed thoroughly, and pelleted using a hand pelleting machine. The pelleted feed was dried in an oven and stored at room temperature.

The fish were fed twice daily at about 7.00 hours and 18.00 hours. They were fed 5% of their body weight per day which was divided into two equal feeding rations. The fish were sampled every two weeks and feed was adjusted to the new body weight. Water quality parameters were measured and monitored once every week and water was changed periodically. The following Water quality parameters were measured; dissolved oxygen, temperature, and pH. The proximate composition analysis of the experiment diet and feed ingredients were carried out according to AOAC, (2000).

Data obtained were used to calculate the following growth parameters: specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), percentage weight gain, (Ridha and Cruz, 2001), and percentage survival.

**Carotenoid Extraction**

The fish underwent some treatment before extraction to enhance the successful extraction of oil. These include washing the fish thoroughly to remove dirt that might get stuck to the body. Then the moisture content of the fish was reduced by oven drying since water is immiscible in oil. The samples were further reduced in size and then blended into a finer form after undergoing the moisture content elimination in the oven. The weights of the samples were taken accordingly, noting the differences in weight due to weight loss through evaporation.

For estimation of total carotenoid content in the skin and muscle of the test fish, the following procedures were adopted.

Soxhlet extractor and n-Hexane were employed as solvents for fish oil extraction. The sample was placed in a porous thimble which was covered with cotton wool and weighed. It was then placed in the inner tube of the apparatus and later fitted to a round bottom flash that contains the solvent. The heat was applied to the solvent until it reaches a boiling point for 1 hour. As the heating continued, the solvent in the flask start boiling just within 5 minutes of heating, and the water began to drop from the top to the sample in the thimble. When the solvent reached the top of the tube, it was siphoned over into the flask and the portion of the oil which has been extracted in the process of refluxing was removed. It was noticed that 18 minutes later, after boiling has started, there was refluxing and this continues at 2 minutes intervals. The solvent used was later recovered by applying heat and collected above the round bottom flask into the soxhlet apparatus while the oil extracted was collected and measured.

Carotene (vitamin A) content was determined using the spectrophotometric method. One (1) g of the sample was dissolved with 10ml acetone in a 50ml conical flask, and allowed to stand for 20 minutes, stirred gently at 4 minutes intervals to extract the colour substance in the sample. About 10ml of water was added after agitation and allowed to settle. The upper layer was separated and the solution cleared into a test tube, 5ml of hexane was added and allowed to settle in 2 layers, which were then separated using a separating funnel. The upper layer was used for beta carotene analysis. About 2ml of the supernatant was pipette into a cuvette and read in the spectrophotometer at 453nm and carotenoid content was calculated as follows;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{weight of a sample}}$$

A sensory evaluation test was carried out by two trained panelist groups according to the method outlined by Poste *et al*. (1991). Questionnaires were used by both panels. The questionnaires were prepared to have a hedonic value of 1-9 which were transformed into scale. The quality attribute assessed was appearance (colour).

Data were analyzed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test was used to determine significant differences among treatment means (Steel and Torrie, 1990).

**RESULTS AND DISCUSSION**

Table 3 is the result of the physicochemical parameters of the water. There were no significant differences ($p>0.05$) in temperature, pH, and dissolved oxygen throughout the experimental period. The range of values measured of the various parameters (5.88 – 6.02 for pH, 4.58 – 4.84mg/l for dissolved oxygen, and 26.94 – 27.23 $^0$C for temperature), in the various treatments fall within the range recommended by Boyd and Lichtopher (1990); Boyd and Tucker (1998) for warm water fishes. Generally, water quality monitoring revealed that any differences in catfish yield between the three treatments were not influenced by differences in the physicochemical parameters amongst the treatments.

Table 4, is the result of the growth performance for the period of study. There were no significant differences ($p>0.05$) in all the growth parameters measured. However, treatment 2 had the best FCR, SGR, and weight gain values, when compared to the rest of the treatments in terms of the average higher values. Treatment 3 had a betterPER. There was no significant difference ($p>0.05$) in the percentage survival of the fish across the treatments.

Table 5 shows the result of the organoleptic test on colour. There was no significant difference ($p>0.05$) amongst the colour treatments. On the other hand, there was a significant difference ($p<0.01$) between the colour treatments. Treatment 2 had a better panelist score than the other two treatments, followed by treatment 1 with treatment 3 having the least.

Table 6 is the result of the carotenoid content amongst the treatments. There was no significant difference ($p>0.05$) for carotenoid content in fish at the beginning of the experiment amongst the treatment, however, the fish at the end of the trial showed a significant difference ($p<0.05$) in carotenoid content between the treatments. β –carotene, alpha-carotene, β –cryptoxanthin are carotenes that are converted into vitamin A or retinol in the body, and β –carotene is the most widely studied carotenoid (Alam and Sultan, 2004). Vitamin A concentration had the highest value in feed with the inclusion of carrot (0.13±0.001) and

the lowest value recorded in the control diet (0.009±0.001).

Carotenoids have a role to play in the intermediary metabolism of fish (Tacon, 1981; Segner *et al*. 1989) and this could enhance nutrient utilisation and may therefore result in improved growth (Alma*et al*., 2013). In this study, the fish fed the carotenoid included diets did not differ from the control fish in terms of growth and Survival. This result is following a work carried out with red porgy juveniles fed krill meal, for 75 days, as a source of astaxanthin (Chatzifotis *et al*., 2005) and agrees with the work of Gomes *et al*. (2002) whose work did not record growth or feed efficiency enhancement when gilthead sea bream were fed different carotenoids for 9 weeks. Also, a study with rainbow trout found no significant differences in growth (Nickell and Bromage, 1998). Chow *et al.* (2016) in an experiment with micro emulsified and non-micro emulsified carotenoids diets in a feed of hybrid catfish observed that the result showed no significant difference in fish growth. The result, however, disagrees with the findings of Sinha and Asimi, (2007), who reported an increased growth rate (in terms of weight) and high carotenoid value (4.01µg/g) in the group of goldfish fed with China rose petal. In the same vein, Arulvasu *et al*. (2013) showed that carotenoid pigments extract from *Rosa rubiginosa* petals which is a natural source have a positive role in the survival, growth, and colour development of ornamental swordtail *Xiphophorus helleri* fry. Observed significance in growth may be directly proportional to the length of time the fish is subjected to the carotenoid-containing diets.

The fish fed with carrot had the highest carotenoid deposition (vitamin A concentration) and this result shows that the feed with carrot was well utilized. Sheriff and Mathew (1996) observed colour enhancement in goldfish using different ingredients such as carrot meal, beetroot meal, and red grape skin meal. Yanar and Tekelioglu (1999) suggested that carrot has been used as a carotene source of diets in aquarium fish. In this research, allexperimental diets were equally accepted by thefish. The findings are also in line with Ramamoorthy*et al,* (2010) who reported that *D. carota* increased the pigmentation of *Amphiprion ocellaris* cultured for 60 days in an experiment where feed was prepared with natural carotenoid sources such as carrot, marigold petal, china rose petal and rose petalat a rate of 15g/100g feed. At the end of the experiment, carotenoid concentration in control averaged 2.687±0.287 mg/kg, Carrot 7.681±0.462mg/kg. Marigoldn7.235±0.438mg/kg, Hibiscus 5.236±0.314mg/kg and Rose petal 4.254±0.252mg/kg. He observed that pigmentation was highest in *D. carota* (7.681 mg/kg),

The feed with turmeric inclusion did have a positive impact or increase in the carotenoid level (vitamin A concentration) in the fish but not as high as the carrot contained diet. Boonyaratpalin and Unprasert, (1989) reported that the percentage of inclusion of turmeric in his diet inhibited the proper performance of the red tilapia. He stated that an 8-week feeding trial indicated that spirulina, marigold petal meal, and shrimp head meal had an appreciable influence on the pigmentation in red tilapia whereas those fed turmeric had the same colour as those on the basal diet. Turmeric, however, has been shown to contain little or no vitamin A and measurable amounts of phytochemicals.

The control diet had a decrease in the final carotenoid level from the initial. This is in agreement with the work of Chow *et al.* (2016) who is an experiment with micro emulsified and non-micro emulsified carotenoids diets in a feed of hybrid catfish, noted a non-pigmentation in the control diet and an increase in the total carotenoid deposition in the catfish muscle and enhanced yellowness in the skin of the carotenoid containing diets. Jebaraja *et al.* (2013) stated that the total carotenoids were significantly highest in fish fed with red chili powder (2.07 µg/g) containing diet and very low in fish fed with the diet containing no pigment supplement. Boonyaratpalin and Unprasert, (1989) reported that captivity affects the carotenoid composition of a fish without access to any source of carotenoid supplemented artificially. This might probably be the reason for the decrease as fish do not synthesize carotenoids.

## CONCLUSION

From a commercial view of point, the maintenance of natural skin pigmentation is of great importance in aquaculture practice as it has a direct impact on consumer acceptance or rejection as well as product market price. Natural carotenoid sources are usually cheap when compared to synthetic carotenoid sources and readily available with a variety in use. In this study, carrot was found to be more effective in increasing the carotenoid pigmentation in the fish while turmeric had little effect on the carotenoid level increase, this work also confirms the fact that fishes left in captivity without access to supplementary carotenoid sources will be affected as a result of their carotenoid level decrease because this fish is not able to synthesize carotenoid but needs it to be supplemented through their diet. The feed containing carotenoid, however, induced a bright reddish to yellowish colouration on the fish while the one without carotenoid containing feed (control) exhibited a dull fading colour.

More emphasis should be made on the need for natural pigment colouring agents which will act as an alternative to synthetic chemicals which will pave the way for many aqua feed industries to promote their products, most especially from plant origins which will cause a drift away from synthetic products. More work, however, should be done on turmeric to be able to ascertain the best inclusion

level for it in feed and the incorporation of turmeric in fish feed, one should consider the percentage of inclusion because turmeric should be used sparingly to avoid it overpowering the other fish feed ingredient. Finally, a comparison should be made between ornamental fish and probably, a catfish given the same time frame or interval to see if carotenoids are only efficient in enhancing growth better in ornamental fish than in other fish.

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**Table 1: The proximate composition analysis of Carrots (*D. carota*) and Turmeric (*C. longa*)**

| Parameters | Carrot (%) | Tumeric (%) |
|---|---|---|
| Moisture | 8.78 | 9.50 |
| Crude Protein | 7.55 | 6.74 |
| Crude Fiber | 5.12 | 4.20 |
| Ether Extract | 2.45 | 6.35 |
| Ash | 8.50 | 5.95 |
| Nitrogen Free Extract | 67.60 | 67.26 |

**Table 2: Diet formulation of the three treatments**

| Feed Ingredients | Treatment 1 (%) | Treatment 2 (%) | Treatment 3 (%) |
|---|---|---|---|
| Fishmeal | 37.08 | 37.08 | 37.08 |
| Soyabean meal | 37.08 | 37.08 | 37.08 |
| Maize | 22.36 | 22.36 | 22.36 |
| Oil | 1 | 1 | 1 |
| Salt | 0.5 | 0.5 | 0.5 |
| Vitamin Premix | 2 | 2 | 2 |

| | | | |
|---|---|---|---|
| Carrot | - | 10 | - |
| Tumeric | 10 | - | - |

**Table 3; Physico-chemical parameters of water during the experimental period.**

| Parameters | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|
| Temperature ($^0$C) | 27.23 ± 0.67$^a$ | 26.94 ± 0.54 $^a$ | 27.08 ± 0.47 $^a$ |
| pH | 6.02 ± 0.31$^a$ | 5.90 ± 0.13$^a$ | 5.88 ± 0.22 $^a$ |
| Dissolved Oxygen (mg/l) | 4.69 ± 0.31$^a$ | 4.58 ± 0.25 $^a$ | 4.84 ± 0.16 $^a$ |

Values are expressed as mean ±SE of triplicate (n=3).
Values with different superscript across the rows are significantly different at ($p<0.05$)

**Table 4: Growth parameters for the various treatments**

| Parameters | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|
| Initial Weight (g) | 37.76±1.36$^a$ | 42.08±1.08$^a$ | 40.08±1.82$^a$ |
| Final weight (g) | 62.69±2.85$^a$ | 75.97±2.94$^a$ | 79.16±2.40$^a$ |
| Weight Gain (g) | 24.92 ± 2.26$^a$ | 33.89 ± 2.87$^a$ | 31.81 ± 2.40$^a$ |
| Feed Conversion Ratio | 6.64 ± 0.43$^a$ | 6.42 ± 1.09$^a$ | 6.57 ± 1.31$^a$ |
| Specific Growth Rate | 0.84 ± 0.59$^a$ | 0.94 ± 0.16$^a$ | 0.92 ± 0.17$^a$ |
| Protein Efficiency Ratio | 0.36 ± 0.24$^a$ | 0.38 ± 0.61$^a$ | 0.39 ± 0.67$^a$ |
| Survival (%) | 96.67 ± 3.34$^a$ | 83.34 ±3.34$^a$ | 93.34 ± 3.34$^a$ |

Values are expressed as mean ±SE of triplicate (n=3).
Values with different superscript across the rows are significantly different at ($p<0.05$)

**Table 5: The organoleptic properties (colour) of the test fish**

| Test | Treatment 1 | Treatment 2 | Treatment 3 | Significance Level |
|---|---|---|---|---|
| Colour | 3.60±0.24$^a$ | 2.20±0.50$^a$ | 4.40±0.90$^a$ | $\alpha$ =0.05 |
| Colour | 3.60±0.24$^b$ | 2.20±0.50$^a$ | 4.40±0.90$^{bc}$ | $\alpha$ =0.01 |

Values are expressed as mean ±SE of triplicate (n=3).
Values with different superscript across the rows are significantly different at ($p<0.05$) and ($p<0.01$), respectively

**Table 6: Vitamin A concentration of the experimental fish**

| Value | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|
| Initial | 0.035±0.00$^a$ | 0.034±0.00$^a$ | 0.031±0.00$^a$ |
| Final | 0.043±0.001$^b$ | 0.134±0.001$^c$ | 0.009±0.001$^a$ |
| Change in Carotene Level | 0.008±0.001$^b$ | 0.100±0.001$^c$ | -0.022±0.001$^a$ |

Values are expressed as mean ±SE of triplicate (n=3).
Values with different superscript across the rows are significantly different at ($p<0.05$)