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Comparison on inclusion of potent and expired Astaxanthin in the diet of ANH7 African catfish *Clarias gariepinus* fingerlings for skin and flesh pigmentation

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Abstract

Background and Objective: The aim of this study seeks to Compare the effects of Inclusion levels of Potent and Expired Astaxanthin in the Diet of African catfish *Clarias gariepinus* Fingerlings for Skin and Flesh Pigmentation.

Materials and Methods: A 120 fingerlings *Clarias gariepinus* was used which was procured from a reputable farm in Jos, Plateau State, Nigeria. They were then taken to the Hydrobiology and Fisheries Laboratory of the University of Jos, Nigeria and allowed to acclimatize for three weeks before the feeding started. The experiment involved the use of 19 fibre glass tanks having average capacity of 95 litres. It was run under the flow-through system at 100 ml/min in order to avoid pollution.

Results: After a feeding period of eight weeks, increasing the level of astaxanthin (potent and expired) in the feed of *Clarias gariepinus* fingerlings from 100 (T1) to 150 (T2) to 200g/kg (T3), the concentration of carotene in the skin increased significantly ($p< 0.05$) between the treatments except for the control. The effect of pigmentation was given in the skin and flesh of *Clarias gariepinus* fingerlings fed both potent and expired astaxanthin, except that, the potent astaxanthin gave higher effect of pigmentation on both skin and flesh than the expired astaxanthin.

Conclusion: It was however, found that, the concentration of astaxanthin in the skin was higher than that in the flesh under every treatment for both potent and expired astaxanthin.

Keywords: Potent Astaxanthin; expired Astaxanthin; African catfish; *Clarias gariepinus;* Fingerlings; Skin and Flesh Pigmentation

1 Introduction

Carotenoids are highly conjugated polyprenoids found in a variety of natural sources. They are classified into two major groups, carotenes and xanthophylls. They are the main pigments of many aquatic animals. Fish skin colors primarily depend on the presence of chromatophore (xanthophores and erythrophores) containing carotenoids (e.g. astaxanthin, canthaxanthin, lutein and zeaxanthin[1]. Astaxanthin is a keto-carotenoid responsible for the pinkish color of some fish, crustaceans and birds and is used as a pigmentation agent in aquaculture and as a potent antioxidant for human health[2,3,4].

Astaxanthin is the main carotenoid pigment of red-pink colored aquatic animals, being widely used in aquacultural processes because it is a standardized and chemically stable product with a high carotenoid concentration[5].

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Generally, animals lack the capability to synthesize carotenoids and, hence, they need a dietary source for these pigments that act as antioxidants and, more importantly, as precursors of vitamin A (retinol) and its derivatives retinal and retinoic acid[6,7]. Pigmentation is one of the important quality attributes of the fish for consumer acceptability[8]. Ornamental fish's pigment is the first parameter dictating their market value[9]. Of practical importance to the trade is that skin color in fishes originates and is obtained principally from colored chemicals or pigments they eat[10]. Carotenoids are responsible for pigmentation of muscle in food fish and skin color in ornamental fish[11]. Fish contain various kinds of carotenoids, the dominant of which is peculiar to the species concerned. Carrot is natural beta carotene source and red pepper is dark red color due to its capsantine in its content, being used for flesh pigmentation of salmonids given capsorobin in it [5,11]. Both of which are cheaply available considering their high level of carotene[8]. Carotenoids play a main role in healthy growth, metabolism, and reproduction, too[13]. Also, carotenoids are potent biological antioxidants that can absorb the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule but preventing other molecules or tissues from being damage (Arous, *et al.*, 2014). To achieve or enhance certain colors of the fish, specific pigments and amounts must be added to their diet. These pigments are principally carotenoids of which some 600 types have been described[10]. When carotenoids bind to proteins or lipoproteins they also form complexes of carotenoproteins and carotenolipoproteins[10]. Carotenoids and their complexes produce biological pigments that can be used to display the visible spectral colors from red and orange, to yellow, green, blue and violet[10]. Carotenoids are synthesized only by algae, plants, and some microbes but are accumulated and become available in the natural foods fish eat such as plankton, worms and shellfish[10]. Lutein, found naturally in marigold meal, is also an effective pigment for inducing the orange coloration of goldfish[14] and is now used commercially in ornamental fish food[15].

Astaxanthin is a pigment that belongs to the family of the Xanthophylls, the oxygenated derivatives of caroteniods whose synthesis in plants derives from lycopene[17]. Schiedt[18] observed that astaxanthin deposition efficiency is larger than other carotenoids followed by adonirubin, canthaxanthin, zeaxanthin, lutein and finally β-carotene. It is one of the main pigments included in crustacean, salmonids, and other farmed fish feeds. Its main role is to provide the desirable reddish-orange colour in these organisms as they do not have access to natural sources of caroteniods[17]. The use of astaxanthin in the aquaculture industry is important from the standpoint of pigmentation, consumer appeal as well as growth and reproduction[17]. In addition to its effect on colour, one of the most important properties of astaxanthin is its antioxidant properties, which have been reported to surpass those of β-carotene or even α-tocopherol[19]. Due to its outstanding antioxidant activity, astaxanthin has been shown to have extraordinary potential for protecting the organism against a wide range of ailments such as cardiovascular problems, different types of cancer and some diseases of the immunological system[17].

The use of astaxanthin as pigment agent in aquaculture species has been well documented for more than two decades[4,19,20,21,22,23,24,25,26,27,28,29]. In spite of the fact that astaxanthin is widely used with the sole purpose of attaining a given pigmentation, it has many other important functions in fish, such as reproduction, acceleration of sexual maturity, increasing fertilization and egg survival and a better embryo development[22]. It has also been demonstrated that astaxanthin improves liver function, increases the defense potential against oxidative stress[30] and has a significant influence on biodefense mechanisms[31]. Red cherry shrimp fed on diets with astaxanthin had greater weight gain, SGR (Specific Growth Rate), pigmentation and total carotenoid than those of shrimp fed diets without astaxanthin[32].

Farmed fishes and crustaceans do not have access to natural sources of astaxanthin, hence the total astaxanthin intake must be derived from their feed. Synthetic astaxanthin is an identical molecule to that produced in living organisms and it consists of a mixture 1:2:1 of isomers (3S, 3S'), (3R, 3S') and (3R, 3R) respectively. It is the main carotenoid used worldwide in the aquaculture industry[16]. This therefore, means that, synthetic astaxanthin will be incorporated in the experimental fish diet to achieve flesh pigmentation in *Clarias gariepinus*.

2 Material and methods

2.1 Experiment site

Hydrobiology and Fisheries Laboratory, Department of Zoology, Faculty of Natural science, University of Jos, Nigeria.

2.2 Experimental procedure

A 120 fingerlings *Clarias gariepinus* (4-9g) was procure from a reputable farm in Jos, Plateau State, Nigeria. They were then taken to the Hydrobiology and Fisheries Laboratory of the University of Jos, Nigeria and allowed to acclimatize for three weeks before the feeding started. The experiment involved the use of 19 fibre glass tanks having average capacity of 95 litres. It was run under the flow-through system at 100 ml/min in order to avoid pollution[33]. The darkness of the system was maintained by properly covering the top of the fibre glass tank by lid and black colour polythene[34]. The temperature and photoperiod combination that were use were, 35°C and 0 hour light (total darkness. This work adopted the aforementioned environmental conditions to investigate the effect of astaxanthin (Potend/Expired) on flesh and skin pigmentation of *Clarias gariepinus* fingerlings. It should be noted that, the astaxanthin had expired for two years before inclusion in the diet. The fingerlings were then placed 15 per tank. Commercial Floating Fish feed (Vital Fish Feed, Grand Cereals and Oil Mills Ltd., Jos.) was used and Astaxanthin (Potent/Expired) at (100, 150 and 200 mg/kg) were added to the treatment diet. Approximately 60% distilled water per kg was added, and diet thoroughly blended. Pelleted diet was dried in hot air oven at 60°C. Diet was stored in plastic bags[32].

Table 1 Level of Incorporation of Astaxanthin in Feed of *Clarias gariepinus* Fingerlings

Astaxanthin	**Level of Incorporation (mg)**			
	(T1)	**(T2)**	**(T3)**	**Control**
Potent	100	150	200	0
Expired (2 years)	100	150	200	0

Key: T1:100mg incorporation of astaxanthin/kg of fish feed; T2-:150mg incorporation of astaxanthin/kg of fish feed; T3: 200mg incorporation of astaxanthin/kg of fish feed

The Statistical analysis was conducted on the data obtained using SPSS version 23. The full model included astaxanthin (potent and expired) as explanatory fixed factors and weeks included in the model as a continuous covariate in order to test if the rate of skin and flesh pigmentation, feed intake weight, and total length changed significantly over time. A two-way interaction term between temperature and photoperiod was also included in the model. The interaction term was included to test if one of the variables modulated the effect of the other variable on the response variables (skin and flesh pigmentation, feed intake, weight, and total lengths). The data were analyzed using one-way analysis of variance (ANOVA). The variant means were separated using Duncan multiple range (DMR) tests were done and the probability value ($p \leq 0.05$) was considered significant. Results obtained were reported as mean ± SE of triplicate (n=3) measurements.

3 Results

There was no significant ($p>0.05$) effect in feed intake after feeding *Clarias gariepinus* fingerlings astaxanthin incorporated (potent and expired) diet for a period of eight weeks across the groups. The result also reveals that, the mean feed intake increased as the weeks increased.

Table 2 Mean Vaues of Feed intake(g) of *Clarias gariepinus* Fingerlings Fed Astaxanthin Incorporated Diet for eight Weeks using potent and expired Astaxanthin across treatments

			Treatments		
Weeks	**Astaxathin**	**100mg**	**150mg**	**200mg**	**Control**
Wk 1	Potent	$1.35±0.03^{b}$	$1.23±0.03^{a}$	$1.22±0.03^{a}$	$1.28±0.00^{ab}$
	Expired	$1.27±0.05^{a}$	$1.31±0.03^{a}$	$1.32±0.10^{a}$	$1.28±0.00^{a}$
Wk 2	Potent	$1.79±0.12^{a}$	$1.76±0.07^{a}$	$1.89±0.03^{a}$	$1.77±0.00^{a}$
	Expired	$1.67±0.06^{a}$	$1.70±0.08^{a}$	$1.74±0.06^{a}$	$1.77±0.00^{a}$
Wk 3	Potent	$2.34±0.12^{a}$	$2.43±0.12^{a}$	$2.60±0.22^{a}$	$2.31±0.00^{a}$
	Expired	$2.25±0.08^{a}$	$2.35±0.11^{a}$	$2.51±0.08^{a}$	$2.31±0.00^{a}$
Wk 4	Potent	$3.05±0.10^{a}$	$3.18±0.10^{ab}$	$3.54±0.23^{b}$	$3.02±0.00^{ab}$
	Expired	$2.89±0.06^{a}$	$3.06±0.04^{ab}$	$3.18±0.08^{b}$	$3.02±0.00^{a}$
Wk 5	Potent	$3.47±0.08^{a}$	$3.74±0.18^{ab}$	$4.22±0.31^{b}$	$3.46±0.00^{ab}$
	Expired	$3.36±0.09^{a}$	$3.68±0.22^{ab}$	$3.88±0.18^{b}$	$3.46±0.00^{a}$

Wk 6	Potent	4.48±0.24[ab]	4.61±0.23[ab]	5.28±0.48[b]	4.06±0.00[a]
	Expired	4.17±0.23[a]	4.42±0.25[a]	4.63±0.22[a]	4.06±0.00[a]
Wk 7	Potent	5.56±0.36[a]	6.00±0.26[a]	6.62±0.62[a]	5.62±0.00[a]
	Expired	5.22±0.14[a]	5.70±0.29[ab]	6.00±0.29[b]	5.62±0.00[ab]
Wk 8	Potent	7.03±0.65[a]	7.37±0.59[a]	8.25±0.91[a]	6.80±0.00[a]
	Expired	6.05±0.03[a]	7.04±0.42[a]	7.33±0.34[b]	6.80±0.00[ab]

After a feeding period of eight weeks, increasing the level of astaxanthin (potent and expired) in the feed of *Clarias gariepinus* fingerlings from 100 (T1) to 150 (T2) to 200g/kg (T3), the concentration of carotene in the skin increased significantly ($p< 0.05$) between the treatments except for the control (Tables 2)

The effect of pigmentation was given in the skin and flesh of *Clarias gariepinus* fingerlings fed both potent and expired astaxanthin, except that, the potent astaxanthin gave higher effect of pigmentation on both skin and flesh than the expired astaxanthin (Figures 1 & 2. That is, after feeding *Clarias gariepinus* fingerlings for the same eight weeks, increasing the level of astaxanthin (potent and expired) in the diet of *Clarias gariepinus* fingerlings from 100 to 150 to 200g/kg, the level of carotene in the skin and flesh of *Clarias gariepinus* fingerlings increased significantly ($p< 0.05$) between treatments except for the control that gave no effect.It was however, found that, the concentration of astaxanthin in the skin was higher than that in the flesh under every treatment for both potent and expired astaxanthin.

Table 3 Carotene Concentration in Skin of Astaxanthin (Potent/Expired) Fed *Clarias gariepinus* Fingerlings

	Carotene concentration (μg/g)		
	100 mg	**150 mg**	**200 mg**
Potent Astaxanthin	163.00±1.73[a]	190.33±1.45[b]	215.00±2.89[c]
Expired Astaxanthin	140.00±1.15[a]	150.67±1.20[b]	160.33±1.45[c]

Different superscrips [a, b, c, d] varies significantly ($p<0.05$) across groups

The result indicates that the mean carotene concentration in skin of fish is better when fed with potent Astaxanthin compared to expired one across the groups.

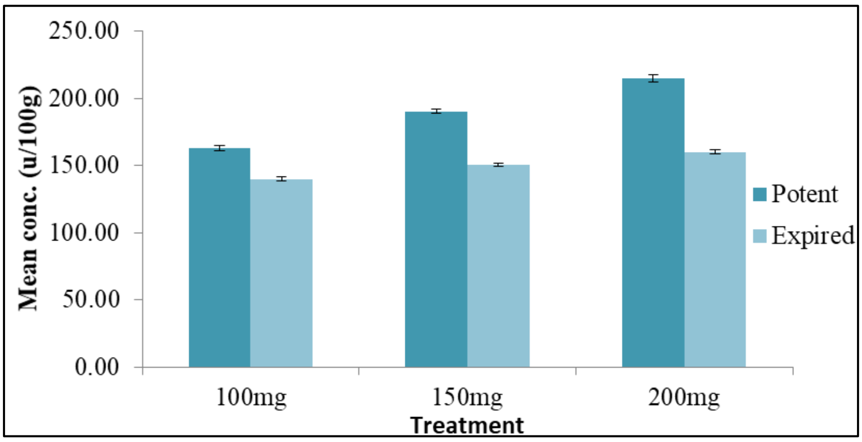

Figure 1 Mean variation of carotene concentration in skin of *Clarias gariepinus* fingerlings fed with Astaxanthin (Potent and Expired) one across treatments

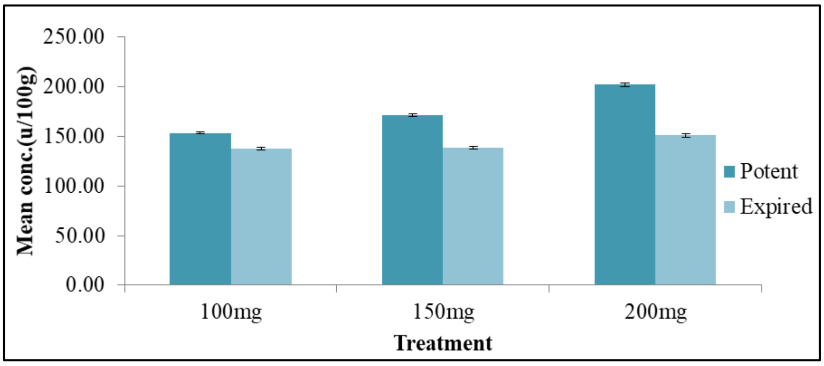

Figure 2 Mean variation of carotene concentration in flesh of *Clarias gariepinus* fingerlings fed with Astaxanthin (Potent and Expired) one across treatments or groups

A

B

Plate 1 ‘A’ Dorsal view of Astaxanthin Fed *Clarias gariepinus* Fingerling; ‘B’ Dorsal view of *Clarias gariepinus* Fingerling Fed Control Diet (without Astaxanthin) Diet, both for eight weeks

Plate 2a

Plate 2b

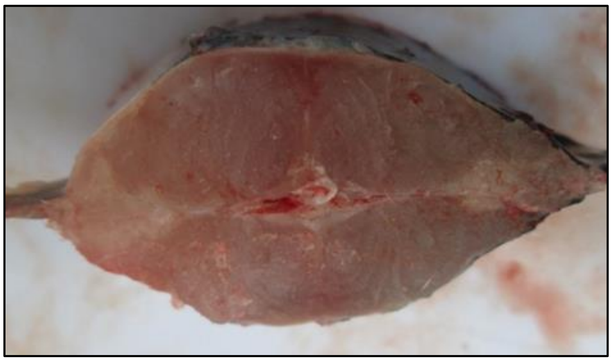

Plate 4c

Plate 2 ‘a’ Cross section of Clarias gariepinus fed diet with 200mg of potent astaxanthin; ‘b’ Cross section of Clarias gariepinus fed diet with 200mg of expired astaxanthin ‘c’ Cross Section of Clarias gariepinus Fingerling Fed Control Diet (without Astaxanthin) for eight weeks

Table 4 Carotene Concentration in Flesh of Astaxanthin (Potent/Expired) Fed *Clarias gariepinus* Fingerlings

	Carotene concentration (µg/g)			
	100mg	**150mg**	**200mg**	**Control**
Potent Astaxanthin	153.33±0.88[ab]	171.67±2.08[ab]	202.67±1.76[c]	
Expired Astaxanthin	137.33±1.45[a]	138.61±1.33[a]	151.00±2.08[a]	

Different superscripts [a, b, c] varies significantly (p<0.05) across groups.

The result indicate that the mean carotene concentration in flesh of fish is better when fed with potent Astaxanthin compared to expired one across the groups.

4 Discussion

Carotenoids are known to have a positive role in the intermediary metabolism of fish[36,27]. Also, carotenoids could enhance nutrient utilization and may ultimately result in improved growth[31]. The carotenoid-supplemented diets did not appear to have any effect on jewel cichlid growth performance[16]. This is in disagreement with my findings. The absorption and accumulation of astaxanthin in the fish is higher than the other carotenoids[12]. Astaxanthin was efficiently utilized for deposition and coloration of the skin in cichlid, Oscar, red sea bream and Australian snapper[27,38,39,40,41]. Rate of retention of dietary carotenoids in fish depends on the efficiency of absorption from the digestive track, transportation capacity, deposition mechanisms in the various tissues, metabolism and rate of excretion[8]. Because astaxanthins contain a long conjugated double bond system, they are relatively unstable and usually scavange oxygen radicals in cells[42]. Many reports have demonstrated that skin colour change over time depended on the level of carotenoid in the diet and differed among species[43,44,45,46,47]. Level of astaxanthin in diet agrees with this work.

In carotenoid analysis, validation of methods has not been strongly advocated, even with the introduction of high-performance liquid chromatography, because the emphasis has been on chromatographic separation[1]. In more recent study of [48] found that the absorbtion of astaxanthin is species dependent. However, increasing levels of pigment deposit was found in the muscle and skin of *Clarias gariepinus* in this study. In agreement with this work is the fact that astaxanthin has a substantial effect on larval growth and survival[49,50.] This is because there was increase in the growth rate of the experimental fish with increase in the level of astaxanthin compared to those with lower levels of inclusion of the synthetic carotenoid. The least was the one without astaxanthin (the control diet). No mortality was even recorded while the eight week study lasted. Chien[51] proposed that astaxanthin is a "semi-essential" nutrient for tiger shrimp (*Penaeus monodon*) because the presence of this compound can be critical to the animal when it is physiologically stressed due to environmental changes. Astaxanthin in the aquaculture industry is important not only from their standpoint of pigmentation to increase consumer acceptance but also as a necessary nutrient for adequate growth and reproduction of commercially valuable species[16]. This is also in agreement with this study though not to reproductive stage. The reports of Bell[35] in Atlantic salmon[9] in Clownfish (*Amphiprion ocellaris*) indicated that dietary astaxanthin did not affect significantly their growth and survival, which disagrees with this work. Also carotenoid supplementation had no positive or negative effect on the growth, survival or apparent health of ornamental red zebra cichlid, *Maylandia estherae*[10]. These disagree with this study. Similar findings were also reported in other fishes and penaed shrimp supplemented with astaxanthin, β-carotene, or lutein[52,53].

Red cherry shrimp fed diets with astaxanthin had greater weight gain, specific growth rate, pigmentation and total carotenoid than those of shrimp fed diets without astaxanthin[32.] This also agrees with this study.

Chatzifotis[57] fed red porgies with diets supplemented with red (mainly astaxanthin esters) and yellow (mainly β-carotene, lutein and zeaxanthin) carotenoids that affected significantly the carotenoid deposition in the skin as well as the skin hue and chroma. Torrissen[54, 55] reported that increasing the dietary lipid level resulted in a higher deposition rate of carotenoids in the muscle of the rainbow trout, while [56], detected in turbot a significant subcutaneous fat accumulation. This agrees with my work also. Just like in my finding[57, 58] reported increased levels of carotenoids with increased levels of inclusion of astaxanthin in the fish diet. However, [59]did not observe any effects of different dietary lipid levels on the deposition of carotenoidsin the pale chub. Grigorakis [60,] reported that 4 weeks of carotenoid deprivation determined a discoloration of the fish skin in the dorsal area in red porgy. Bjerkeng[61] showed that the fish deposited small amounts of carotenoids in the flesh (<3 mg/kg up to 50 weeks. A minimum of 9 weeks of feeding with xanthophylls is necessary for proper pigmentation of the Arctic char. However, the maximum uptake of carotenoids occurs after 15 weeks of feeding with diets enriched in xanthophylls[62]. This goes to show that deposition of the pigment is species dependent.

5 Conclusion

In conclusion, it was found out that the concentration of astaxanthin in the skin was higher than that in the flesh under every treatment for both potent and expired astaxanthin. Making the skin to have more astaxanthin tan the flesh.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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