

FLESH PIGMENTATION OF *Clarias gariepinus* (Burchell 1822):  
UPTAKE AND DEPOSITION OF DIETARY CAROTENOIDS

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**Abstract**

Flesh colour enhancement by inclusion of carotenoids in the diet of the African sharp-tooth catfish, *Clarias gariepinus* (Burchell 1822), was studied. Fish were fed diets supplemented with either beta carotene, carophyll red (canthaxanthin), carophyll pink (astaxanthin) or an algal source of astaxanthin (*Hematococcus pluvialis*). Catfish were fed for 29 days at an active ingredient level of 100 mg/kg. After samples of muscle revealed some but not distinct colour uptake, levels in the feed were increased to 250 mg/kg and feeding continued for another 31 days. After 60 days of feeding there were significant visual differences between carotenoid fed fish and control fish fed a diet without colouring agents. Spectrophotometric measurements of acetone extract from muscle samples confirmed visual observations and showed that the flesh of catfish fed carophyll pink contained the most colour, about 4 times that of control fish, and double that of fish fed on beta carotene, carophyll red and *H. pluvialis*.

**Introduction**

Fish are not capable of synthesizing their own carotenoid pigments, and therefore depends on introduction of pigments via the food (Schiedt *et al.* 1985; Simpson and Kamata 1979). The technique of introducing pigments into flesh and skin of many cultured fish via their food is in use worldwide and is considered obligatory to maintain product value and consumer acceptance (Ellis 1979). For example, skin colour of hobby fish determines the

value of the product and salmon farmers are forced to add pigments to fish diets so their product is similar to wild caught salmon (Smith *et al.* 1992, Ellis 1979; Simpson and Kamata 1979). However, the naturally uncoloured flesh of other species such as the channel catfish is preferred by the consumer, eliminating the need for colour enhancement (Lovell 1984). African catfish, *Clarias gariepinus*, is considered a medium quality fish and is generally marketed as a grey or pale fleshed product. In the present work, an attempt has been made to achieve a pinkish flesh, aiming at improving catfish quality. The pinkish meat could be marketed smoked or as fresh fillet.

The purpose of this experiment was to study the effects of the carotenoids beta carotene, astaxanthin and canthaxanthin, supplied in the diet, on flesh colouration of the African sharptooth catfish.

### Materials and Methods

Groups of 7 juvenile catfish (avg weight 225 g), reared from eggs, were stocked into one of 4 experimental tanks and left for one week to acclimate. The experimental tanks (84 L; 50x70x24 cm) were within a 1200 L recirculation system which included a water purification unit and received 100 mls min<sup>-1</sup> of fresh water. Tanks were provided with aeration and water temperature was 27°C.

Diets were prepared by fine-grinding (AEG, Germany) pigment-free, commercial fish feed pellets, mixing in colouring agent, adding water, and extruding (WLS Loser, GmbH Co., Germany) to make 2 mm (dia) x 5 mm (length) pellets which were subsequently air dried. Three colouring agents, carophyll pink (astaxanthin), beta-carotene, and carophyll red (canthaxanthin; all Hoffman La Roche) were added at concentrations of 100 mg·Kg<sup>-1</sup> (active ingredient concentration) and a fourth colour free diet was prepared (control). Fish were fed at a rate of 4% of biomass four times a day.

After 31 days of feeding, one fish from each group was killed and samples of muscle and skin collected. Samples were compared visually, for differences in colouration, by a panel of 4 people. Observations at this time indicated that some pigment had been deposited in the flesh, but differences were not distinct. Subsequently, feeding of experimental diets continued and the concentration of pigment was increased to 250 mg·Kg<sup>-1</sup>. At this time a fourth experimental group was introduced and fed on a diet containing extract of the microalgae *Haematococcus pluvialis* (Fan *et al.* 1995; Boussiba and Vonshak 1991). The *H. pluvialis* contained 4% astaxanthin and cell walls had been disrupted prior to addition into the feed, which was prepared to give a final astaxanthin concentration of 250 mg·Kg<sup>-1</sup>. Feeding of the

4 experimental diets continued for another 29 days, after which 3 fish were sampled from each treatment. Subsequently, all fish were fed the control feed and muscle and skin samples of one fish were collected after 10, 20 and 30 days.

Before sampling, fish were moved from the recirculating system to a fresh water flow through system and food was withheld. After 2 days in fresh water, they were placed in a slurry of ice water for 1 hr, then removed, quickly decapitated, gutted and flesh samples collected from the dorsal musculature and stored at -30°C for subsequent analysis. At the time of sampling, there was also a visual comparison of the extent of colouration in muscle samples from different treatments.

Spectrophotometric quantification of colouration of muscle and skin was done by measuring the absorbency at 470  $\mu\text{m}$  following acetone extraction. Prior to extraction samples were freeze dried and ground to a fine powder with mortar and pestle. The relative amount of colour was expressed on a sample dry weight basis.

## Results

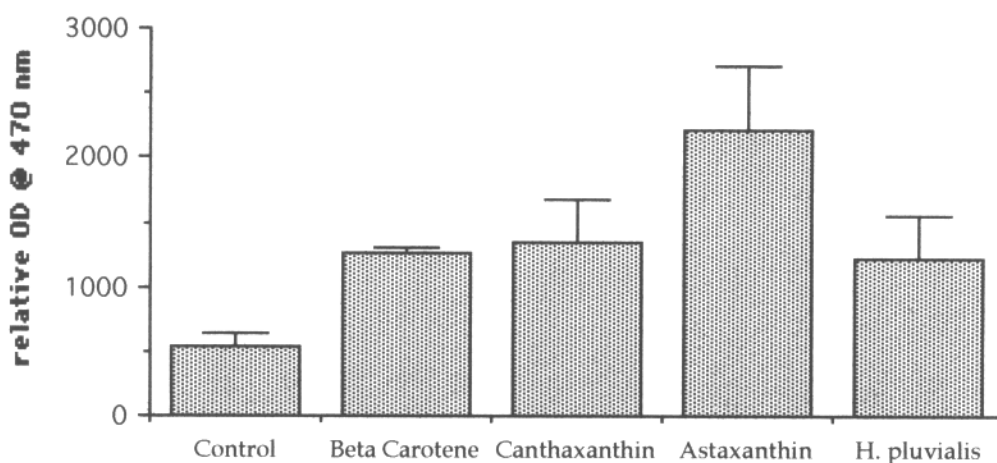
Fish in all groups grew during the experiment, indicating that food containing pigment was consumed and utilized. The specific growth rate and food conversion efficiency are shown in Table 1.

**Table 1.** Specific growth rate and food conversion efficiency of catfish fed different diets

Treatment	Specific Growth Rate (% / day)	Food Conversion Efficiency
Control	1.82	0.44
Carophyll pink	1.52	0.49
Carophyll red	0.83	0.20
Beta Carotene	1.18	0.34
<i>H. pluvialis</i>	0.89	0.33

Visual assessment of the muscle samples indicated that carotenoid pigments had been stored in the muscle but probably not the skin of catfish. The differences between treatments were still visually apparent up to 30 days after feeding of carotenoids had stopped.

Spectrophotometric measurements of the extract of muscle tissue confirmed visual observations with fish fed astaxanthin showing the greatest degree of flesh colouration (Figure 1).



**Figure 1.** Weight adjusted relative OD at 470 nm of muscle samples of *C. gariepinus* fed diets supplemented with carotenoids.

## Discussion

This study shows that carotenoid pigment, when supplied in the feed, is taken up and deposited in the flesh of *C. gariepinus*. This preliminary study suggests that the levels of pigment required to give a desirable muscle colouration may be higher than is required in trout or salmon (Torrissen 1985). Further studies manipulating dose and feeding duration are required before recommendations can be made to aquaculturists.

The uptake/deposition of the 3 different carotenoids in catfish are similar to the results of experiments on salmonids (Schiedt *et al.* 1985) as beta carotene did not result in as much flesh colouration as carophyll pink. Muscle colouration in catfish fed carophyll red was also less than those fed carophyll pink and these findings are similar to those of Bjerkeng *et. al.* (1990) showing canthaxanthin uptake less efficient than astaxanthin uptake in rainbow trout. Deposited carotenoids also appeared to be quite stable in catfish flesh with little depletion after 30 days, a finding similar to that of Choubert (1985) with rainbow trout.

The reason that fish fed on a natural source of astaxanthin (*H. pluvialis*) were less coloured than those fed synthetic astaxanthin (carophyll pink) may be the duration of carotenoid feeding as those on carophyll pink were fed for an additional month at 100 mg/Kg. It may also be that the naturally occurring astaxanthin in *H. pluvialis* is less available to catfish compared to carophyll pink because of digestibility differences or degradation of the

astaxanthin through oxidation (Bubrick, 1991). Less efficient uptake of astaxanthin from *H. pluvialis* compared to carophyll pink has been shown in rainbow trout (Sommer *et al.* 1991).

In conclusion, this study shows that *C. gariepinus* absorb dietary carotenoids, resulting in improved flesh colouration. A further study will quantify the specific feeding procedures (eg. dose and duration) required to produce a higher value product.

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