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Color Enhancement of Zebra Malawi Cichlid (*Pseudotropheus zebra*) Using Carrot (*Daucus carota*) as Feed Additive

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ABSTRACT

The effect of carrot (*Daucus carota*) (in form of steamed carrot) mixed in basal wet diet at 10, 15, 20, 25 and 30% and a control group without any carotenoid supplementation, on skin pigmentation of zebra Malawi cichlid (*Pseudotropheus zebra*) was evaluated. Totally 180 zebra Malawi cichlid (15 g mean weight) were stocked in 18 aquariums (70 L) each containing 10 fish (3 replicates for each treatment). After 35 days of treatment fish skin colour was evaluated using colour parameters L, a, b, Chroma and Hue° described by CIE. The findings of the present study showed that the optimum carrot utilization in diet is 20% and at this level all examined factors except H° are significantly different from the control group (0%).

Key words: *Pseudotropheus zebra*, carotenoid diet, carrot, skin pigmentation

INTRODUCTION

Foods in the natural environment are the main sources of color ingredients in aquatic creatures. Brilliant appearance is one of the most important characteristics of ornamental fish. The ornamental fish industry has experienced the problem of faded coloration in fish, especially those grown in clear water, leads to subsequent difficulties in marketing. Low consumer demands for fish with faint color results in reduced price and profitability. However, some producers use artificial colorants and hormones for color enhancement, the acquired color is not stable and may lose after a while. Many animal species including most fishes cannot synthesize carotenoids and must obtain them via food (Sommer *et al.*, 1991; Storebakken and Nome, 1992).

For this reason, the effects of carrot as a source of natural carotenoids on color enhancement in zebra Malawi cichlide (*Pseudotropheus zebra*) were examined.

MATERIALS AND METHODS

In this study 180 Malawi cichlids *Pseudotropheus zebra* which were produced in aquarium at Arang Mahi Co., Bushehr, Iran, were used. The fish were treated in an indoor closed experimental system. Glass aquaria were filled (working volume of 70 L) with clear water, each aquarium contained an airstone that provided constant aeration and equipped with biofilter before experiments. All fish were in mean body weight of 15 g and were stocked randomly in 18 experimental aquaria at the density of ten per aquarium with three replicates for each treatment. Their sex was not taken into consideration. Oxygen, temperature, ammonia, nitrite, nitrate and other water quality factors were kept at the optimum range for Malawi cichlid during experiments.

Temperature maintained at $28 \pm 0.5^\circ\text{C}$ using a heating element connected to a thermostat. The photoperiod was provided by a florescent light with light at an intensity of 1200 lux during daylight hours. Cleaning and removing of dead fish (if present) was done daily. The feeding diet of cichlid fish included 61% Crude Protein (CP), 7% Crude Fat (CF), 16% hydrocarbons and 7% total ash. Five levels of water boiled (30 min) carrot content (10, 15, 20, 25 and 30%) were added to diet of each treatment group and control fish received no carrot in their diet. A pallet machine was used in preparing feed. Fish were feed daily at 8 am *ad libitum*. Sampling was performed at the end of the day 35 and samples were prepared for colour measurement. The fish were anesthetized using 30 mg L^{-1} of clove (*Caryophyllium aromaticus*) powder and photography was performed using Canon 4200F scanner with resolution of 10000×14000 dpi and further analysis was performed using Photoshop 8. software. Five millimeters below the first ray of the first dorsal fin was selected as sampling point in all samples. Five replicates was performed in each colorimetry.

The CIE 1976 system (L, a, b), recommended by international Commission on Illumination (CIE) Hue° and Chroma were chosen as color scales. “L measures lightness (from zero for black to 100 for perfect white). The two chromaticity parameters a and b measure, the first redness when positive, grey when zero and greenness when negative and the second yellowness when positive, grey when zero and blueness when negative” (HunterLab MiniScan™XE User’s Guide, 10-2, 10/99). Hue (H°ab) is the name of a color as found in its pure state in the spectrum and is determined by the dominant wavelength. Chroma refers to the saturation of a colour and it is a measure of how much grey and white light is mixed in with the pure focal colour. Oriana 300 software was used to measure hue-angle and statistical analysis of obtained data carried out using Excell 2007 and SPSS 15.

RESULTS

The results of the experiments revealed, fish fed with carrot received significantly different values than control fish fed no carrot (Table 1). The L value results shows significant more desirable (lower lightness or higher darkness) values than control group at 10, 15 and 20% carrot treatments. Factor A (redness to greenness) was significantly different at 20% carrot level ($p < 0.05$). About 10 and 15% groups were not significantly higher than control group. In 30% level of carrot this factor was neutral (0) an the best results was achieved using 20% carrot which the colour tend to red ($1.92 \text{ } p < 0.05$), however, this change can not visually be observed apparently due to blue background of the fish skin.

Factor B (yellowness to blueness) did not change significantly at 10 and 15% carrot fed fish in contrast to control fish but there is an increase in 20% treatment was observed ($p < 0.05$).

Table 1: Colour parameters (L*, a*, b*) in fish skin for varieties of zebra Malawi cichlid (*Pseudotropheus zebra*) fed the experimental diets

Factors	Carrot (%)					
	0	10	15	20*	25	30
L	40.29±6.86 ^a	29.530±6.19 ^{d*}	35.150±7.65 ^{bc}	31.210±8.67 ^{dc*}	38.620±9.03 ^{ab}	42.72±8.61 ^a
A	0.43±1.73 ^{be}	0.610±1.75 ^{bcd}	0.070±1.41 ^{cef}	1.920±1.92 ^{a*}	1.170±0.94 ^{ab}	0.00±2.23 ^{df}
B	-7.51±7.12 ^{cd}	-7.920±6.95 ^{bc}	-7.890±5.86 ^{bd}	-15.330±4.89 ^{e*}	-11.760±4.65 ^{a*}	-9.31±5.56 ^{abd}
C	9.87±5.64 ^{abd}	8.600±6.29 ^b	9.140±4.06 ^{ab}	15.930±4.84 ^{c*}	11.730±4.86 ^{ab}	11.49±6.16 ^{bd}
H°	293.98±66.0 ^{abc}	316.668±70.05 ^{abc}	318.954±94.91 ^{abc}	287.947±65.80 ^a	289.988±64.08 ^{abc}	334.212±93.32 ^b
Survival rate	100.0%	83.0%	91.0%	98.0%	99.0%	95.0%

Values in the same row with different superscript letters are significantly different ($p < 0.05$), Asterick values shows significantly desirable values in contrast to control group

Factor C (chroma) was constant between 0, 10 and 15% treatments but in 20% carrot treatment this value represents an increase in colour saturation. In 25 and 30% carrot fed fish chroma level did not change significantly in contrast to control fish ($p < 0.05$). The H° factor measures didn't show significant different from control, however, hue was tend to blue in 20% treatment and to red in 30% treatment.

DISCUSSION

Assigning the optimal proportion of food additives in fish diet has economic importance as determining the minimum amount of additive which still produce high desirable results will decreases farming costs.

The results show that the best percent of carrot in zebra Malawi cichlid which can more significantly change colour factors in contrast to control fish was 20%. In fish fed with 20% of carrot in daily diet, all assessed colour factors except H° were in significant difference than control fish.

Several studies has been demonstrated the role of natural pigments as colorants in ornamental fishes (Ghyasvand and Shapouri, 2008; Baron *et al.*, 2008).

Different results using carrot as colorant has been related to different digestive physiology among species and/or preparing process of this carotenoids containing food (Shang, 2006). Long digestive tract of zebra Malawi cichlid allows more effective assimilation of plants and algae (Walisiewicz *et al.*, 2005). It seemed digestive efficiency for carrot in this fish has been increased up to 20% per diet, as colour enhancement was decreased using more than 20% of carrot in daily diet.

Carrot (*Daucus arota*) is a good source of β carotene ($10800 \mu\text{g g}^{-1}$ wet weight) and α carotene ($3610 \mu\text{g g}^{-1}$ wet weight) which is higher than many fruits, uncooked vegetables and seeds (Scott and Hart, 1994). In addition due to its low price and easy access, carrot is a good source of food additive in fish culture (Sommer *et al.*, 1991).

It is possible to use the extracts of carrot as a natural source of carotenoids for colour enhancement in this fish.

In addition to their use as colorant in fish, carotenoids are excellent antioxidants (Matsufuji *et al.*, 1998), thus the better growth or health might be the additional effect of the enrichment of diet with carotenoids (Harpaz and Padowicz, 2007). However, in this study no such effects could be detected but recommended for further studies.

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