Original Paper

The effect of astaxanthin and β-carotene on the colour of Kissing gourami (*Helostoma temminckii*)

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In this study, we have investigated the effects of astaxanthin and β-carotene on the skin colour of fish Kissing Gourami (*Helostoma temminckii*). Altogether 80 fish were randomly selected into 2 groups (40 each group). The control group fish were fed with standard flake feed. Astaxanthin (100 mg kg) and β-carotene (150 mg kg) were added to the standard diet for the experimental group. Fish skin colour was compared with colour scale. Colour change was recorded weekly for the 12 weeks of experiment. The first colour change was recorded in 6th week in the control group. No other change of colour was determinated. In experimental group, the first colour change was recorded in 4th week and the maximal influence of astaxanthin and β-carotene was recorded in 10th week (light red). Therefore, it was determined that these pigment sources have an effect on the colour of gourami fish.

Keywords: *Helostoma temminckii*, kissing gourami, astaxanthin, β-carotene, colour scale

1. Introduction

Microalgae, which are important in the production of larval fish because of their nutritive ingredients, can be used as a natural pigment source in fish feeds. The use of microalgal biomass has been recently investigated with regard to its potential as a colouring agent (Gouveia et al. 1997; Raymundo et al. 2005). But the use of synthetic pigment sources is more common because they are easy to obtain (Sales and Janssens 2003). Astaxanthin is another high-value carotenoid produced from microalgae which has been achieving commercial success. Astaxanthin is ubiquitous in nature, especially in the marine environment, and is probably best known for eliciting the pinkish-red hue to the flesh of salmonids, as well as shrimp, lobsters and crayfish. Because these animals are unable to synthesize astaxanthin de novo, carotenoid pigments must be supplied in their diet (Steven, 1948; Goodwin, 1984). Dietary carotenoids play an important role in regulating fish colour because fish, like other animals, are unable to synthesize carotenoids and their skin colour is highly dependent on carotenoids from the diet (Torrissen et al. 1990). Ornamental fish obtain carotenoids from feeding upon algae, corals or prey that have accumulated these pigments. One of the greatest challenges in the tropical marine ornamental industry is to accurately replicate the natural colour of fish that are maintained in captivity. Numerous operations that have mastered the art and science of propagation have failed to successfully market their fish as a result of the loss of pigmentation. Various products have been introduced to alleviate this problem, but none has performed as effectively and consistently as natural astaxanthin from Haematococcus. On a commercial scale, an inclusion rate of 30 ppm astaxanthin is used to supplement live and flaked foods resulting in a significant colour improvement in most species of tetras, cichlids, gouramis, goldfish, koi and danios (Ako and Tamaru, 1999). The focus of this study was to manipulate the type of carotenoids (astaxanthin, β-carotene) in the diet of kissing gourami (Helostoma temminckii) and to examine if the fish colour expression is related to pigment intake, to investigate the effect of carotenoids on fish colouration and to record the chronological colour change of fish for 12 weeks.

2. Materials and methods

2.1 Fish, feed and rearing conditions

Trial was undertaken with Kissing Gourami (*Helostoma temminckii*) in 8 weeks of age for the period of 12 weeks. As much as 80 homogeneous fish were randomly distributed in 2 tanks. Their sex was not taken into consideration. The first tank (the control group) fish were fed standard flake feed, and the second tank (the experimental group) fish were fed standard flake feed with astaxanthin and β-carotene (Table 1). In both groups, fish were fed by hand *ad libitum* three times a day (6 am, 2 pm and 10 pm). The water conditions of the tanks are shown in Table 2.

Two air pumps and one sponge filter were used in the aquariums for filtration and airflow. The aquariums were placed side by side in two lines. Natural photoperiod was used during the experiment.

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Table 1 Composition of the control and experimental diets

Composition	Control group	Experimental group
Crude protein	48%	48%
Crude fat	6%	6%
Ash	8%	8%
Astaxanthin	-	100 mg kg
β-carotene	-	150 mg kg

Table 2 Water conditions in control and experimental group

Water temperature	26 °C
pH	6.5
NO_2	max. 0.1 mg l
Water hardness	15 °dGH

2.2 Colour determination

Fish skin colour was compared with help of the scale designed by us, shown in Figure 1. The comparisons were performed on both sides of the dorsal fish skin weekly, and colour changes were recorded. The colour scale presents 5 colour described by degrees (Table 3).

Table 3 Colour scale

Degree	Colour
0	silver (unchanged)
1	light pink
2	pink
3	light red
4	red

3. Results and discussion

Colouration is controlled by the endocrine and nervous systems, but dietary sources of a pigment also play a role in determining the colour of fish. The effectiveness of carotenoid source in terms of deposition and pigmentation is species-specific. In addition, all fish species do not possess the same pathways for the metabolism of carotenoids, and therefore, there is no universal transformation of carotenoids in fish tissues (Chatzifotis et al. 2005). Farmed fish have no access to carotenoids from natural food; necessary carotenoids must be obtained from their diet to maintain their bright colouration (Winterhalter, 2002).

According to the results obtained from the experiment, it was observed that the gourami fish responded to colouration effected by the use of pigment sources.

Tables 4 and 5 show the number of fish and the colour change during the experiment. Significant colour change in the control group occurred at week 6, when 24 fish were coloured light pink. At 8 weeks, all fish were already coloured light pink. Further colour change was not observed. In the experimental group the first significant change took place in week 4, when 31 fish were coloured light pink. At 6 weeks, 30 fish were pink. At 10 weeks, 33 fish were light red. At the end of the experiment, all 40 fish turned red light.

Table 6 present chronological colour changes in both tanks (the control one and the experimental one) in time of 12 weeks. In the control group fed with standard flake

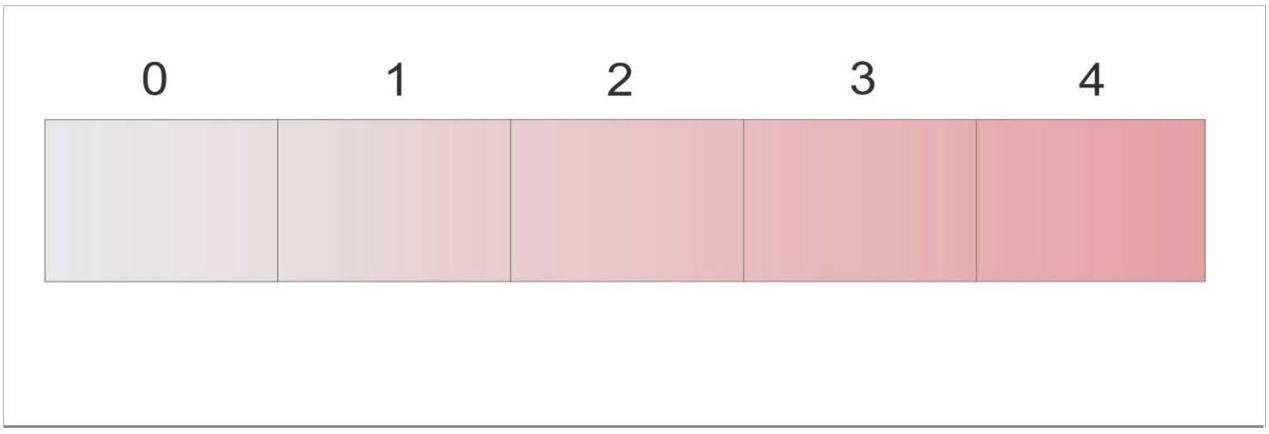

Figure 1 Colour scale

Table 4 Number of fish and the colour change in the control group

Degree	Weeks											
	1	2	3	4	5	6	7	8	9	10	11	12
0	40	40	38	9	0	0	0	0	0	0	0	0
1	0	0	2	31	40	10	5	0	0	0	0	0
2	0	0	0	0	0	30	35	40	38	7	1	0
3	0	0	0	0	0	0	0	0	2	33	39	40
4	0	0	0	0	0	0	0	0	0	0	0	0

Table 5 Number of fish and the colour change in the experimental group

Degree	Weeks											
	1	2	3	4	5	6	7	8	9	10	11	12
0	40	40	40	40	37	16	1	0	0	0	0	0
1	0	0	0	0	3	24	39	40	40	40	40	40
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0

Table 6 Colour change in the control and experimental group

Weeks	Colour scale			
	Control group		Experimental group	
	degree	colour	degree	colour
1	0	silver (unchanged)	0	silver (unchanged)
2	0	silver (unchanged)	0	silver (unchanged)
3	0	silver (unchanged)	0	silver (unchanged)
4	0	silver (unchanged)	1	light pink
5	0	silver (unchanged)	1	light pink
6	1	light pink	2	pink
7	1	light pink	2	pink
8	1	light pink	2	pink
9	1	light pink	2	pink
10	1	light pink	3	light red
11	1	light pink	3	light red
12	1	light pink	3	light red

feed from the beginning of the experiment to 5 weeks there was no record of the colour change. The first colour change was detected in 6th week of the experiment. From 6th week to the end of the experiment there was no other colour change recorded, and the colour of fish was similar to the degree 1 of the colour scale. Subsequently, 1% *Haematococcus algae* meal was used to augment the diet of red velvet swordtails, rainbowfish, 24K mollies, topaz cichlids, discus, rainbow sharks, pink kissing gouramis and rosy barbs. Within one week, in each of the species, there were significant improvements in pigmentation, and some species had faster growth rates. Although a relatively high concentration of *Haematococcus algae* was used in the feed, many commercial producers prefer to use an acute pigmentation treatment to prepare fish for the market. Much lower dosages could effectively be used to maintain pigmentation (Ako and Tamaru, 1999).

In our experiment, there was no record of the colour change in the experimental group in 3 weeks. In the 4th week, there was recorded the colour change equal to the degree 1 of the colour scale. The colour of fish was similar to the degree 2 of the scale in 6 weeks, during 9th week of the

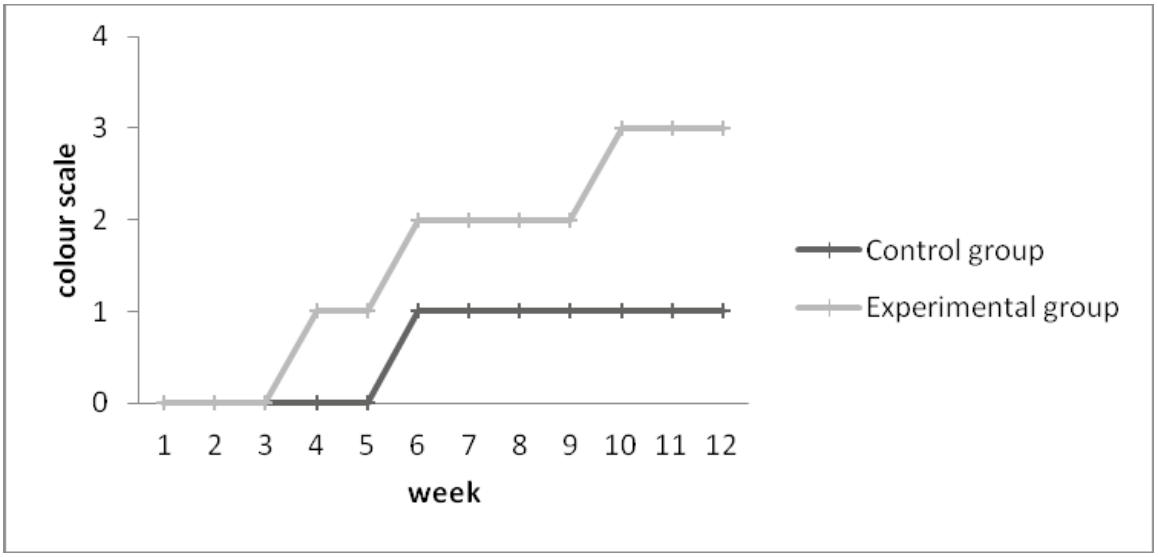

Figure 2 Schematic colour change in the control and the experimental group

experiment. The period most successful in inducing the red colouration in the skin of gouramis was after 10 weeks of feeding. Additionally, natural esterified forms of astaxanthin such as Naturose are known to increase skin redness and total carotenoid content in red porgy (Chatzifotis et al. 2005).

No other colour change was recorded until the end of the experiment. Maximum colouring of fish can be compared to the degree 3 of the scale and it was caused by astaxanthin and β-carotene.

4. Conclusions

The focus of this study was to manipulate the type of carotenoids (astaxanthin, β-carotene) in the diet of Kissing gourami (*Helostoma temminckii*) and to examine if fish colour expression is related to pigment intake. In the control group fed with standard flake feed there was no colour change recorded in the beginning of the experiment. The first colour change was detected in 6th week of the experiment. From 6th week to the end of the experiment there was no further colour change recorded, and the colour of fish was similar to the degree 1 of the colour scale. In the experimental group, there was no colour change recorded in first 3 weeks. In the 4th week there was recorded colour change equal to the degree 1 of the colour scale. The colour of fish was similar to the degree 2 of the scale in 6 weeks during 9th week of the experiment. The period most successful in inducing the red colouration in the skin of gouramis was after 10 weeks of feeding.

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