Effects of dried fairy shrimp *Streptocephalus sirindhornae* meal on pigmentation and carotenoid deposition in flowerhorn cichlid; *Amphilophus citrinellus* (Günther, 1864) × *Cichlasoma trimaculatum* (Günther, 1867)

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**Abstract**

We investigated the effects of dried fairy shrimp *Streptocephalus sirindhornae* meal (FS) on skin pigmentation and carotenoid deposition in flowerhorn cichlid. Six experimental diets including three treatments of FS at 10% (FS10), 20% (FS20) and 30% (FS30), two dried *Spirulina* sp. meal (SP) at 6% (SP6) and 12% (SP12), and a control diet (a basal diet without FS or SP) were offered for 90 days. The results demonstrate an increase in the flowerhorn cichlid skin pigmentation from alternative carotenoid feeding. Fish fed the FS diet displayed higher ($P < 0.05$) chroma and redness values than those fed with a SP diet. The hue value (measure for skin pigmentation) was high when fish were fed with FS20 for 30 and 60 days ($P < 0.01$). However, fish also showed high hue values when fed for 90 days with FS10 ($P < 0.01$). The FS20 treatment gave better results than other treatments in terms of total carotenoid, canthaxanthin, astaxanthin and β-carotene concentration in the skin and musculature. The optimum level of FS in flowerhorn cichlid diets for achieving the highest skin pigmentation was 20%.

**Keywords:** astaxanthin, β-carotene, canthaxanthin, ornamental fish, *Spirulina* sp., colouration

**Introduction**

Pigmentation in ornamental fish is one of the most important quality criteria that determines their market value. The flowerhorn cichlid, *Amphilophus citrinellus* (Günther, 1864) × *Cichlasoma trimaculatum* (Günther, 1867) (Fig. 1) is an artificially selected crossbred fish that does not occur in natural habitats. It was first interbred between *C. trimaculatum* and other cichlid species in Malaysia. These fish varieties became popular ornamental fish among aquarium keepers in Thailand due to their beautiful colour and ability to grow under a wide range of environmental conditions (Silarudee & Kongchum 2008). Although the red colour of flowerhorn cichlids can be genetically inherited, they require pigment-supplemented foods for the enhancement of their skin colour (Sugie, Terai, Ota & Okada 2004). As fish cannot synthesize certain pigments, they rely on a dietary supply of carotenoids to achieve their natural skin pigmentation. Various synthetic carotenoids (β-carotene, canthaxanthin and astaxanthin) have been used as dietary supplements to enhance pigmentation of fish (Sawanboonchun, Roy, Robertson & Bell 2008). The main sources of natural carotenoids for aquatic animals are red yeast (Bjerkerø, Peisker, Von Schwartzzenberg, Ytrestøy & Åsgård 2007), bacteria *Streptomyces* sp. (Dharmaraj,
Ashokkumar & Dhevendaran 2009), microalgae Chlorella vulgaris (Gouveia, Rema, Pereira & Empis 2003) and crustacean shells (Babu, Chakrabarti & Surya Sambasivarao 2008). In recent years, the nutritional value of fairy shrimps (Munuswamy, Mertens, Walsche & Dumont 1992; Velu & Munuswamy 2007) as a food source for fish and crustaceans has been highlighted (Dumont & Munuswamy 1997). In Thailand, three species of fairy shrimps occur naturally (Sanoamuang, Murugan, Weekers & Dumont 2000; Sanoamuang, Saengphan & Murugan 2002; Sanoamuang & Saengphan 2006). These species have been continuously studied in terms of their life history (Dararat, Starkweather & Sanoamuang 2011), reproductive cycle (Plodsomboon, Maeda-Martinez, Obregon-Barboza & Sanoamuang 2012), egg hatching method and systems that allow them to be mass cultured (Saengphan, Shiel & Sanoamuang 2005) and their diseases (Saejung, Hatai, Wada, Kurata & Sanoamuang 2011). Some studies have been performed to culture freshwater fairy shrimp to use them as live feed for freshwater aquatic animals such as ornamental fish (Sanoamuang, Pakmaluk & Sirisan 2006) and giant freshwater prawn (Sripunthorn & Sanoamuang 2011; Sornsupharp, Dahms & Sanoamuang 2012). Nutritional analysis revealed that all three species of fairy shrimps in Thailand contained high protein levels of 50.24–74.41%. Similarly, carotenoid content analysis showed the presence of dominant groups consisting of \( \beta \)-carotene, canthaxanthin, astaxanthin and lutein (Dararat, Lomthaisong & Sanoamuang 2012). Although many crustacean meals have low protein digestibility due to the high levels of non-protein nitrogen in the exoskeletons of the animals, the growth of red tilapia (Oreochromis niloticus) was stimulated by shrimp head meal (Boonyaratparin & Unprasert 1989). This diet was also given to rainbow trout (Salmo gairdneri) and it provided a reddish pigmentation to the skin and muscle (Choubert & Luquet 1983). Even though two species of fairy shrimp (Streptocephalus sirindhornae and Branchinella thailandensis) have been commercially harvested as food for ornamental fish and freshwater shrimp in Thailand since 2004 (Sripunthorn & Sanoamuang 2011; Saejung, Hatai & Sanoamuang 2013), no fairy shrimp meal is available in the market yet. However, the use of fairy shrimp meal in aquaculture is expected to be implemented in the future. The objective of this study was to optimize feeding amendments to enhance pigmentation in flowerhorn cichlids: Amphilophus citrinellus (Günther, 1864) × Cichlasoma trimaculatum (Günther, 1867).

**Materials and methods**

**Fish preparation**

About 500 juvenile flowerhorn cichlids (Fig. 1) were transferred from the hatcheries to 500-L tanks for acclimation to the laboratory conditions for 2 months. During acclimatization, fish were fed a floating tilapia commercial diet two times a day to equalize their body carotenoid content. After acclimation, individual flowerhorn cichlids with an initial weight in the range of 10.97–13.49 g were transferred to 18 aquaria (70-L each) to receive six experimental diets with three replicate tanks and stocking densities of three fish per aquarium. Four groups of 18 aquaria (=72 aquaria) were used as the fish were sacrificed after days 1, 30, 60 and 90. Fish were adapted to accept the sinking pellets as a food by feeding a control diet at 5% body weight for 7 days. Two days before the experiment started, the fish were starved. Subsequently, the fish received an experimental diet at 5% body weight, two times a day at 09:00 and 15:00 hours. The flowerhorn cichlids were maintained under a light period set to 12 h. Dechlorinated tap water was used as culture water and dissolved oxygen was maintained at saturation by constant aeration. Water was exchanged daily at 1/3 volume and faeces were siphoned out. The fish were reared for 90 days and collected at the beginning and after 30, 60 and 90 days for weight, length and colour measurement, as well as

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**Figure 1** Habitus of flowerhorn cichlid; Amphilophus citrinellus (Günther, 1864) × Cichlasoma trimaculatum (Günther, 1867).
sampling of musculature. The feeding experiment was carried out at the Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

Feed preparation

Six experimental diets were used in this feeding trial. A basal or control diet was formulated without FS and SP substitutes. Three diets were formulated with dry fairy shrimp meal (FS) concentrations at 10% (FS10), 20% (FS20) and 30% (FS30), two diets were formulated with dry Spirulina meal (SP) at 6% (SP6) and 12% (SP12) in inclusion levels into the basal diet. Feed ingredients and chemical composition of the experimental diets are given in Table 1. The dried ingredients of each diet were mixed in a Hobart mixer (Tong Hor Machine Lex Product model L.N.K. 532, Taipei, Taiwan) for 10 min. The whole food was mixed thoroughly for another 5 min to ensure homogeneity. Water was added to this mixture to form a dough, which was then pressed through a 2-mm-diameter die press. The pellet feeds were dried in a hot air oven at 60°C for 12 h. The feed was then crushed, sieved to obtain a particle size of 0.9–1.2 mm and kept in an aluminium foil bag, which was stored at −20°C to avoid oxidation of the carotenoids.

Skin colour measurements

Data on skin colour were obtained and analysed as described by Ross and Ross (1999). In brief, the whole-body weight and total length were recorded in fish anaesthetized with 2-phenoxyethanol, at the beginning of the feeding trials, and after 30, 60 and 90 days. Skin pigmentation was determined from the first dorsal spine vertically to the ventral slightly below the lateral line, on both sides of the fish separately (method modified after Kalinowski, Robaina, Fernandez-Palacios, Schuchardt & Izquierdo 2005). The measurements were taken from the skin zones on both lateral body sides using a tristimulus colorimeter (Minolta Chroma Meter CR-300; Minolta, Osaka, Japan).

Analysis of carotenoids in skin and musculature

For the extraction of carotenoids, the method of Britton (1995) was adopted with some modifications. All materials were dried in an oven at a

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>FS10</th>
<th>FS20</th>
<th>FS30</th>
<th>SP6</th>
<th>SP12</th>
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<tbody>
<tr>
<td>Fish meal</td>
<td>320</td>
<td>260</td>
<td>180</td>
<td>80</td>
<td>265</td>
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<td>Soybean meal</td>
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<td>Wheat flour</td>
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<td>Squid liver meal</td>
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<td>Squid liver oil</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Premix*</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<td>Dry fairy shrimp</td>
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<td>100</td>
<td>200</td>
<td>300</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dry Spirulina sp.</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>120</td>
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<td>Analysed composition (% dry wt.)</td>
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<tr>
<td>Crude protein</td>
<td>35.38</td>
<td>35.09</td>
<td>35.85</td>
<td>35.95</td>
<td>35.90</td>
<td>35.97</td>
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<tr>
<td>Total carotenoid (mg kg⁻¹)</td>
<td>6.62</td>
<td>15.74</td>
<td>27.59</td>
<td>39.58</td>
<td>21.13</td>
<td>43.51</td>
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<tr>
<td>Lutein (µg kg⁻¹)</td>
<td>181.55</td>
<td>178.93</td>
<td>132.81</td>
<td>56.19</td>
<td>102.48</td>
<td>144.97</td>
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<tr>
<td>Canthaxanthin (µg kg⁻¹)</td>
<td>16.73</td>
<td>196.15</td>
<td>482.50</td>
<td>489.94</td>
<td>24.91</td>
<td>16.72</td>
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<td>Astaxanthin (µg kg⁻¹)</td>
<td>17.29</td>
<td>46.01</td>
<td>75.06</td>
<td>130.70</td>
<td>39.29</td>
<td>79.43</td>
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<tr>
<td>1-carotene (µg kg⁻¹)</td>
<td>15.16</td>
<td>17.09</td>
<td>26.73</td>
<td>41.03</td>
<td>17.29</td>
<td>28.21</td>
</tr>
</tbody>
</table>

*Premix contained the following diluted in cellulose (g kg⁻¹ mix): vitamin A (5 000 000 IU g⁻¹), 1.5; vitamin D3 (1 000 000 IU g⁻¹), 1.5; vitamin E (3000 IU g⁻¹), 6; vitamin K3, 0.50; thiamin, 0.30; riboflavin, 1.00; pyridoxine, 0.65; nicotinic acid, 5.50; vitamin C, 5.20; folie acid, 0.20; vitamin B12 (3000 mg kg⁻¹), 2.5; biotin (2 g 100 g⁻¹), 0.025; magnesium, 2.05; potassium, 3.05; sodium, 8.60; calcium carbonate, 23.82; magnesium hydroxide, 6; KCl, 9; ferric citrate, 1.5; KI, 0.4; NaCl, 2.2; calcium hydrogen phosphate (CaHPO4), 500; copper sulphate, 1; zinc sulphate, 2; cobalt sulphate, 0.15; manganese sulphate, 0.24; iodine, 0.007; selenium, 0.002.
Table 2 Effect of dietary carotenoid supplements on growth performance of flowerhorn cichlid. FS10, FS20 and FS30 mean the diets that were substituted with dried fairy shrimp meal at 10%, 20% and 30% respectively. SP6 and SP12 mean the diets that were substituted with dried Spirulina sp. meal at 6% and 12% respectively

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Parameter*</th>
<th>Control</th>
<th>FS10</th>
<th>FS20</th>
<th>FS30</th>
<th>SP6</th>
<th>SP12</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IW (g fish⁻¹)</td>
<td>10.97 ± 0.84a</td>
<td>11.67 ± 0.47a</td>
<td>11.87 ± 1.20a</td>
<td>13.42 ± 1.30a</td>
<td>12.89 ± 1.48a</td>
<td>11.74 ± 1.41a</td>
<td>0.6355</td>
</tr>
<tr>
<td></td>
<td>FW (g fish⁻¹)</td>
<td>41.69 ± 1.84ab</td>
<td>59.96 ± 2.55ab</td>
<td>55.34 ± 1.56ab</td>
<td>59.19 ± 1.26ab</td>
<td>46.85 ± 1.04ab</td>
<td>39.44 ± 2.39a</td>
<td>0.0442</td>
</tr>
<tr>
<td></td>
<td>IL (cm)</td>
<td>8.76 ± 1.42a</td>
<td>8.83 ± 1.67a</td>
<td>8.81 ± 1.64a</td>
<td>9.34 ± 1.44b</td>
<td>9.16 ± 1.39a</td>
<td>8.74 ± 1.16a</td>
<td>0.2346</td>
</tr>
<tr>
<td></td>
<td>FL (cm)</td>
<td>12.01 ± 1.88b</td>
<td>12.63 ± 1.09b</td>
<td>13.59 ± 1.97a</td>
<td>14.14 ± 1.46a</td>
<td>13.22 ± 1.48b</td>
<td>12.69 ± 1.01b</td>
<td>0.0373</td>
</tr>
<tr>
<td></td>
<td>Fl (g day⁻¹)</td>
<td>6.77 ± 0.48a</td>
<td>6.81 ± 0.90a</td>
<td>6.21 ± 0.11b</td>
<td>6.17 ± 0.97b</td>
<td>6.35 ± 0.55b</td>
<td>6.18 ± 0.76b</td>
<td>0.0399</td>
</tr>
<tr>
<td></td>
<td>GP (% day⁻¹)</td>
<td>3.11 ± 0.44a</td>
<td>3.70 ± 0.48ab</td>
<td>4.07 ± 0.51b</td>
<td>3.76 ± 0.37ab</td>
<td>2.75 ± 0.31a</td>
<td>2.62 ± 0.53a</td>
<td>0.0450</td>
</tr>
<tr>
<td></td>
<td>SGR (% day⁻¹)</td>
<td>1.48 ± 0.52ab</td>
<td>1.63 ± 0.36ab</td>
<td>1.71 ± 0.27a</td>
<td>1.64 ± 0.62ab</td>
<td>1.38 ± 0.56b</td>
<td>1.35 ± 0.24b</td>
<td>0.0358</td>
</tr>
</tbody>
</table>

*a = 9, means in the same row, sharing the same letter is not significantly different according to Duncan’s new multiple range test. IW, Initial body weight; FW, Final body weight; IL, Initial body length; FL, Final body length; GP, Growth percentage; SGR, Specific growth rate.

Results

Effect of dietary carotenoids on growth performance

The growth performance of the experimental fish is shown in Table 2. There was a significant difference in body weights at the time of the experiment (P ≤ 0.05). The body weights of the fish fed diets with FS10, FS20, FS30 and SP6 were significantly higher than those of the control and SP12 during the whole experiment (P ≤ 0.05). The highest body weight at the end of the experiment was found in the fish fed the FS10 diet. The body length of the fish fed with FS30 was longer than those of the other treatment groups. There was a significant difference (P ≤ 0.05) in feed intake of the fish when fed the FS10 supplemented diets and control as compared with those fed the SP diets. The growth percentages decreased significantly (P ≤ 0.05) when fish were fed the SP-supplemented diets compared with those fed FS and control diets alone (P ≤ 0.05). The highest specific growth rate was found in the fish fed the diets FS20, FS30 and FS10.
Effect of diets on skin colour

After 30 days of the feeding trials, the skin lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$) did not seem to be influenced by the different diets tested (Table 3). On the other hand, hue and chroma values of the fish had higher values. Both average hue and chroma values were higher in the FS than in the control and SP diets ($P \leq 0.05$). Hue average values of the FS, control and SP diets were 69.02, 50.49 and 27.76 respectively. Chroma average values of the FS, control and SP diets were 10.33, 10.24 and 9.93 respectively. In addition, hue and chroma values also influenced each other positively in the FS diet; the FS20 and FS30 treatment groups showed significantly higher ($P \leq 0.05$) than the other treatments.

The colour variables $a^*$, hue and chroma had the highest values ($P \leq 0.05$) after 60 days of experimentation in the FS, control and SP diets respectively. The $b^*$ value of the fish fed the SP6 and SP12 diets had significantly higher values ($P \leq 0.05$) than the other treatments and showed a yellowness value. On the other hand, fish fed FS and control diets showed blueness. The hue values were higher in the FS diet compared with the control and SP diets with averages of 141.79, 108.05 and 85.97 respectively. Similarly, chroma values of the fish were higher in the FS diet compared with the control and SP diets with averages of 10.96, 10.31 and 9.93 respectively.

By the end of the trial, the $L^*$ values between the different dietary treatments were significantly different ($P \leq 0.05$). These results contrasted with the results after 30 and 60 days. The $L^*$ values were higher in the control compared with the SP and FS diets, with values of 93.77, 93.00 and 91.05 respectively. Redness values of the FS, SP and control diets were 3.84, 2.54 and 2.43, respectively, and these groups showed a significant effect on red hue ($P \leq 0.01$). The $b^*$ value of fish fed SP6 and SP12 diets showed similar results after 60 days. Those fish had significantly higher $b^*$ values ($P \leq 0.01$) and showed a yellowness value that the fish from the other treatments did not have. Other fish fed with FS and control diets developed a blueness value. The hue and chroma values between the different dietary treatments were higher in the FS diet compared with the control and SP diets. Average hue values in FS, control and SP diets were 179.19, 121.56 and 100.84 respectively. Similarly, average chroma values in FS, control and SP diets were 4.54, 3.14 and 2.74 respectively.

Skin and musculature carotenoid deposition

After the flowerhorn fish were fed with carotenoid-supplemented diets for the three periods (30, 60 and 90 days), they showed marked carotenoid depositions in the skin and musculature ($P \leq 0.05$). Total carotenoid content in fish skin and muscle of each experimental group showed significant differences ($P \leq 0.05$) in the total carotenoid contents for each fish (Fig. 2a). The diet supplemented with FS had a higher total carotenoid content than SP and the control. This holds particularly for the FS10 diets at 30 days (2.68 ± 0.30 g kg$^{-1}$), the FS30 diets at 60 days (6.12 ± 0.66 g kg$^{-1}$) and the FS20 diets at 90 days (6.88 ± 0.30 g kg$^{-1}$). No significant difference was found in the lutein deposition of the fish fed different diets after 30 days (Fig. 2b). Highly significant differences ($P \leq 0.05$) in lutein after 60 and 90 days were found in the SP diet group, with average values of 1.85 and 0.89 µg kg$^{-1}$ respectively. Significantly higher ($P \leq 0.01$) canthaxanthin deposition was shown for carotenoids in diets supplemented with FS than in the SP and control groups (Fig. 2c). After 30, 60 and 90 days of feeding, there was a tendency among all dietary treatments to provide high canthaxanthin levels when fed with FS20, FS30 and FS10 diets, respectively, differing significantly ($P \leq 0.01$) from the remaining groups.

Skin and musculature of fish fed FS, SP and control diets showed highly significant differences ($P \leq 0.01$) in astaxanthin levels (Fig. 2d). After 30 days, the fish fed FS diets had a higher astaxanthin concentration than SP and control diets, with values of 0.22, 0.02 and 0.01 µg kg$^{-1}$ respectively. Correspondingly, mean values of astaxanthin in musculature of fish fed FS, SP and control diets after 60 and 90 days were 0.46, 0.21, 0.01 µg kg$^{-1}$ and 0.01, 25, 0.46, 0.07 µg kg$^{-1}$ respectively. Similarly for canthaxanthin, the fish showed significantly different ($P \leq 0.05$) β-carotene levels in all treatments after 30, 60 and 90 days (Fig. 2e). β-carotene concentrations in musculature after 30 days of FS, SP and control diets were 0.12, 0.03 and 0.02 µg kg$^{-1}$ respectively. Comparable to the results after 30 days, β-carotene levels after 60 days of FS, SP and control diets were on average 0.033, 0.021 and 0.002 µg kg$^{-1}$, respectively.
and after 90 days were 0.074, 0.043 and 0.004 μg kg⁻¹ respectively.

**Discussion**

**Effect of dietary carotenoid on growth performance**

Growth performance, in terms of growth percentage or specific growth rate, is an important parameter for the estimation of fish production. This study found that feeding fish with 200 g kg⁻¹ FS resulted in high body weight and growth. On the other hand, the body weight and specific growth rate of fish fed with SP decreased when the amount of SP increased in the formula. These results showed that fish were digesting protein from FS better than from SP. Similar to our previous studies, we showed that a decreased growth rate was observed, as in common carp exposed to *Microcystis* by feeding with boom scum (Li & Chung 2004) and in planktivorous fish by natural ingestion of *M. aeruginosa* (Rai 2000). In contrast, Zhao, Xie, Zhu, Yang, Gan and Song (2006) reported that Nile tilapia that constantly consumed the dietary cyanobacterium *Microcystis* increased their growth and it had no impact on feed conversion efficiencies. Moreover, Dong, Zhu, Han, Yang, Song and Xie (2009) reported that hybrid tilapia *O. niloticus × O. aureus* fed cyanobacteria from Lake Taihu showed a high specific growth rate. All fish performed well with acceptable weight increments and specific growth rates in all treatment groups in our experiments. Results of weight gain and specific growth rate after a 90-day growth trial indicated that the experimental diets sustained a good growth performance, similar to the results of Rodehuts cord, Becker, Pack and Pfeffer (1997). The average growth percentage of fish was enhanced with DFSM supplementation (368.03%). Similarly, Ahmad and Abdel-Tawwab (2011) reported that fingerling Tilapia (*O. niloticus*) showed an average growth increase between 583.3% and 797.2% when fed with caraway seed meal. Specific differences in final weight and specific growth rate among the different fish groups at the end of the period were larger at the lower ration level indicating that feed restrictions and ration size had an effect on the growth of the fish. The specific growth rates for fish fed during this period ranged from 1.33 to 1.77 and were similar to the observations reported for *C. carpio* (Desai & Singh 2009), but were higher than those for tilapia (Xie, Cui, Yang & Liu 1997).

**Effect of diets on skin colour**

The colouration level of flowerhorn fish had not been investigated as yet. However, it is generally accepted that the market value of flowerhorn fish increases with increasing degrees of its skin pigmentation. Webber, Webber Barlow and Brush (1973) demonstrated that the pigments in the cichlid *Cichlasoma citrinellum* responsible for the carotenoid colouration were β-carotene and canthaxanthin. The carotenoids are dietary in origin, but their distribution and intensity were presumably under genetic control. Normally, the chromatophores in fish include melanophores, xanthophores, erythrophores, leucophores and iridophores that are responsible for the revelation of skin pigmentation. However, pigment patterns in fish predominantly result from the positioning of different coloured chromatophores. Theoretically, pigment cell patterning might result from long-range patterning mechanisms, from local environmental cues or from interactions between neighbouring chromatophores (Kelsh 2004). This study shows that skin lightness (L*) of the fish at
30 and 60 days of the food trials was not influenced by the different treatment diets. Carotenoid supplementation did not influence lightness of red porgy skin neither as reported by Kalinowski et al. (2005). The results of this study are similar to Kalinowski, Izquierdo, Schuchardt and Robaina (2007) who investigated dietary supplementation times with shrimp shell meal on red porgy (Pagrus pagrus) skin pigmentation and carotenoid concentration. In contrast, the flame-red dwarf gourami, Colosa idia, fed with carotenoid diets showed a lightening in skin colouration following group activities and social interaction (Baron, Davies, Alexander, Snellgrove & Sloman 2008). Pavlidis, Papandroulakis and Divanach (2006) described the colouration pattern of red-skinned representatives of Sparidae. Their results showed that all sparid species have a dorsoventral gradient in common, with the ventral area being brighter than the dorsal skin area. However, the Pagrus species were lighter in the dorsal area than were the Dentex species.

Tejera, Cejas, Rodríguez, Bjerkeng, Jerez, Bolaños and Lorenzo (2007) demonstrated that astaxanthin and tunaxanthin esters most probably reflect the characteristics of skin carotenoids, skin redness ($a^*$) and yellowness ($b^*$). Both xanthophylls are known as the main carotenoids in red porgy integuments. Therefore, the increase in skin redness with increasing supplementation time could be due to direct skin deposition of esterified astaxanthin, the main carotenoid found in shrimp shell meal (Pu, Bechtel & Sathivel 2010). Consequently, the skin yellowness of our result decreased after 30 days of supplementation with the FS diet. Kalinowski et al. (2005) reported a similar decrease in skin yellowness of red porgy after 120 days of supplementation with a SM diet. The authors suggested that this reflected a limitation in the synthesis of tunaxanthin from astaxanthin. Meanwhile, Choubert, Cravedi and Laurentie (2009) found that fish muscle colour parameters reacted differently to fish diet: the lightness ($L^*$) value decreased while chroma ($C^*$), $a^*$ and $b^*$ values increased over time as fish consumed the pigmented diets for 42 days.

We found that an increase in the redness value ($a^*$) showed an average amount of carotenoid and $a^*$ value in the skin of fish being fed with dietary carotenoids from FS (300 mg kg$^{-1}$) after 90 days. This study showed a higher $a^*$ value when the fish was fed a FS diet rather than the other diets. Similarly, Choubert, Mendes-Pinto and Morais (2006) reported that $a^*$ data of rainbow trout, Oncorhynchus mykiss, fed synthetic astaxanthin were higher than those of trout fed green microalgae (Haematococcus pluvialis).

However, the effect of dried fairy shrimp as a feed ingredient for the enhancement of pigmentation in flowerhorn fish has not been reported as yet. There are related studies on other fish species with variable results. Juangsoi, Jintaataporn, Areechon and Tabthipwon (2011) demonstrated that pigmentation response of skin redness of fancy carp fed with diets combined with lutein and β-carotene at 25:25, 50:50 mg kg$^{-1}$ and lutein 50 mg kg$^{-1}$ was higher than in other treatments ($P \leq 0.05$), but they were similar to fish fed with a 25 mg kg$^{-1}$ astaxanthin diet. Correspondingly, Yagiz, Kristinsson, Balaban, Welt, Raghavan and Marshall (2010) reported that the colour ($a^*$ value) of Atlantic salmon (Salmo salar) muscle was related to the content of astaxanthin in skin and muscle. Similarly, Wathne, Bjerkeng, Storebakken, Vassvik and Olland (1998) reported that the redness $a^*$ of Atlantic salmon (S. salar) flesh obtained from feeds that were supplemented with astaxanthin in alternating meals was higher than for fish fed a...
mixture of pigmented and unpigmented feeds. In our experiment, fish skin colour parameters, such as the hue ($H_{ab}$) value, varied with diets. Fish fed the FS diet had higher hue values than the control and SP diets. In addition, FS20 had higher hue values in each group. This may indicate that the carotenoid uptake or transportation to the tissue was saturated due to the FS diet inclusion levels in flowerhorn fish. Our study showed that chroma ($C_{ab}$) increased over time as fish consumed pigmented diets. It was higher in fish fed the FS diet than in the control and SP diets. In particular, fish fed the FS30 diet had higher chroma values than with the other feeds, due to high carotenoids.

Figure 2 Effect of dietary carotenoid supplementation on total carotenoid (a), lutein (b), canthaxanthin (c), astaxanthin (d) and β-carotene (e) in skin and musculature of flowerhorn cichlid. FS10, FS20 and FS30 are the diets in which dried fairy shrimp meal was substituted at 10%, 20% and 30% respectively. SP6 and SP12 are the diets in which dried *Spirulina* sp. meal was substituted at 6% and 12% respectively.
in this formulated diet. This is in agreement with previous findings in rainbow trout, *O. mykiss*, which was fed with different dietary and lipid sources (Choubert *et al.* 2006). In this study, the dietary pigment that enhanced the reddish hue and chroma values tended to be reduced after 60 days of feeding. This behaviour from hue and chroma suggests certain skin colour saturation. In agreement, an apparent colour saturation point was found in red porgy (*P. pagrus*) fed with shrimp meal diets. These diets enhanced the reddish hue and chroma values. Nevertheless, hue values at days 75 and 105 did not show a marked difference and chroma levels attained after 75 days tended to be reduced at day 105, suggesting skin colour saturation after a certain feeding time (Kalinowski *et al.* 2005).

**Skin and musculature carotenoid deposition**

This study tested the effect of carotenoid concentration on the pigmentation of flowerhorn fish. Diet supplementation with carotenoids can enhance total carotenoid content and hue in the skin and muscle of flowerhorn fish. Total carotenoid content accumulated in this study showed a significant increase (*P* ≤ 0.01) with all diets. The total carotenoid content value was higher in the fish fed with FS diets than in the control and SP diets. The highest value of total carotenoid content was found in the FS20 diet. These results were similar to Lee, Pham and Lee (2010) who reported a high total carotenoid content in the skin and muscle of pale chub fed a diet with paprika supplementation. These authors reported that the highest total carotenoid content was observed in fish fed a mixed diet of 16% paprika and 8% lipids. Correspondingly, Kop, Durmaz and Hekimoglu (2010) found that the cichlid *C. severum* had a high carotenoid content in their skin when receiving dietary carotenoids from red pepper. Moreover, *C. severum* could accumulate total carotenoid content in their skin when fed diets containing 50 mg kg\(^{-1}\) astaxanthin and \(\beta\)-carotene and *P. cruentum* powder for 50 days (Kop & Durmaz 2008). Hynes, Egeland, Koppe, Baardsen and Kiron (2009) reported that carotenoid concentration in the muscle of Atlantic salmon was significantly affected by its source and dietary concentration. A positive relationship between the amount of supplemented feeds and deposition of carotenoids in muscle of Atlantic salmon was observed by Baker, Pfeiffer, Schöner and Smith-Lemmon (2002). Diler and Gokoglu (2004) demonstrated that carotenoid concentration in rainbow trout significantly increased by feeding diets that contained red pepper and astaxanthin. There seems to be a tendency for an overall improvement of colour parameters (*L*, *b*, *c*, *b*) in fish fed diets with high levels of FS in our study.

Although muscle lutein increased with dietary levels, there was a clear selection in favour of the deposition of astaxanthin, or alternatively, against lutein. In this study, lutein tended to decrease when fish were fed dietary carotenoids for a long time. The fish showed high values of lutein after 60 days, particularly those fed in the SP6 diet. This result was similar to early reports on Atlantic salmon, *S. salar* (Olsen & Baker 2006). In this study, canthaxanthin, astaxanthin and \(\beta\)-carotene concentrations were increasing significantly in skin and musculature during the experiment whatever the carotenoid feed content was. These results showed that canthaxanthin deposition was higher than astaxanthin and \(\beta\)-carotene. In contrast, astaxanthin deposition was higher than canthaxanthin in rainbow trout and Atlantic salmon flesh (Page & Davies 2006). Pan and Chien (2009) found that dietary synthetic astaxanthin provided a similar deposition of astaxanthin in skin and fin compared to natural astaxanthin, but a higher content in the gonad than from natural carotenoid sources. Variations in muscle astaxanthin concentrations may be explained by different factors, some of which reflect the nature of carotenoids using dietary carotenoid concentration, fish size or physiological state of fish (Torrissen 1989). Choubert *et al.* (2009) indicated that a feeding strategy based on astaxanthin feeding in alternate meals each day led to a higher astaxanthin muscle retention compared with continuous astaxanthin feeding. They also found that pigment retention values, both of astaxanthin and canthaxanthin, appear to be utilized to the same extent.

It can be concluded that carotenoid concentrations in feeds play a major role in skin colouration of flowerhorn cichlids. The results reported here provide additional evidence for the potential applicability of alternative carotenoid feeding on flowerhorn fish pigmentation, which could affect the quality and acceptance of fish colouration on the market. Food amendments with fairy shrimp inclusion of 20%, as here, provide one way to obtain better results in terms of total carotenoid content,
canthaxanthin, astaxanthin and β-carotene in skin and musculature.

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