Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*

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Abstract

This study aimed to find out if dietary carotenoid (CD) supplement could make differences in survival, growth, pigmentation, and antioxidant capacity of characins *Hyphessobrycon callistus*, an ornamental fish. Two types of CD and its combination (AX — astaxanthin, BC — β-carotene, MX — 1:1 combination of AX and BC) at three concentrations (10, 20, and 40 mg/kg) were used resulting in nine pigmented diets. A diet without CD supplement served as control. No differences in growth and survival of the fish among treatments were found after 8 weeks rearing. Disregarding the types of dietary CD, AX dominated (>98%) the body CD, indicating that this fish converted most dietary BC into body AX for storage. Body AX and BC content increased with increasing dietary CD concentration. Body AX in BC-fed fish was lower than that in both AX- and MX-fed fish. No difference in body AX was found between AX- and MX-fed fish, and in body BC in all pigmented fish. Serum total antioxidant status [TAS], serum antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidases [GPx]) and serum transaminases (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) were chosen as indices of fish antioxidant capacity. Antioxidant activities changed with dietary CD type and concentration. Pigmented fish had lower SOD, GPx and ALT than control fish; dietary CD types only affected SOD and ALT in fish. AX-fed fish had the lowest SOD. Dietary AX had more numbers of negative correlations with antioxidant parameters in fish than BC.

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Keywords: Carotenoid; Astaxanthin; Pigmentation; Antioxidant capacity; Superoxide dismutase; Total antioxidant status; Beta-carotene

1. Introduction

Ornamental fish’s pigment is one of the most important quality criteria dictating their market value. Like other animals, fish are unable to perform de novo synthesis of carotenoids (CD) (Goodwin, 1984) and therefore rely on dietary supply to achieve their natural pigmentation. Under intensive farming conditions and aquarium rearing, ornamental fish are fed exclusively on compound feeds, which must therefore be supplemented with CD. Various synthetic CD (β-carotene (BC), canthaxanthin, zeaxanthin, and astaxanthin (AX)) and natural sources (yeast, bacteria, algae, higher plants, and crustacean meal) have been used as dietary supplement to enhance the
pigmentation of fish and crustacean (Shahidi et al., 1998; Kalinowski et al., 2005). Natural CD is usually composed of several CD in various forms and varies in terms of digestibility making their pigmentation efficiency complicated to interpret. On the contrary, synthetic CD (which is always a single CD) allows research results to clearly differentiate pigmentation efficiency. However, few comparative studies have been conducted on the pigmentation of ornamental fish by single or mix synthetic CD. Dietary supplementation of synthetic AX to enhance pigmentation of penaeids has been proven highly effective and superior to the other CD (BC, canthaxanthin, etc.) and natural sources (yeast, algae) (Chien and Jeng, 1992; Chien and Shiau, 2005). However, some studies with microalgal biomass supplementation have shown that Chlorella vulgaris is as efficient as synthetic pigments in the pigmentation of rainbow trout Oncorhynchus mykiss (Gouveia et al., 1996), gilthead seabream Sparus aurata (Gouveia et al., 2002), koi carp Cyprinus carpio and gold fish Carassius auratus (Gouveia et al., 2003; Gouveia and Rema, 2005).

The reactive oxygen species, which are produced under usual oxidative damage can be eliminated by the body’s antioxidant system (Halliwell and Gutteridge, 1989). CD belong to the non-enzymatic group of the antioxidant system and provides two mechanisms to protect against oxidative damage: (1) quenching of singlet oxygen, and (2) scavenging of radicals. Therefore, besides the function to improve pigmentation, CD have multi-functions as antioxidants. A relationship between dietary AX concentration and antioxidant status in liver and muscle was observed in Atlantic salmon Salmo salar (Christiansen et al., 1995), indicating possible health improvement in fish by CD (Torrissen and Christiansen, 1995; Christiansen and Torrissen, 1996). Dietary AX has shown to enhance antioxidant status in penaeid postlarvae and increase their resistance to various environmental stresses (Chien et al., 2003; Pan et al., 2003; Chien and Shiau, 2005).

This study was therefore undertaken to find out if synthetic AX’s and BC’s and their combination at various dietary concentrations would affect survival, growth, and antioxidant capacity of characins, Hypsesobrycon callistus.

2. Materials and methods

2.1. Diet preparation

Control diet was composed of white fishmeal 50%, wheat flour 15%, dextrin 27%, fish oil 3%, vitamin mix 2%, and mineral mix 3%. Pigmented diets, which CD was supplemented, had the same composition as the control diet (except for dextrin which was adjusted depending on CD levels used) but supplemented with synthetic AX (Carophyll Pink, 8%AX) and BC (Rovimix β-carotene, 10%BC) (DSM Nutritional Products Ltd, Basel, Switzerland). Water was added to the ingredients to form a dough, which was extruded through a 2-mm-diameter die press. The extruded feed was air dried in the dark to prevent the degradation of CD. The feed was then crushed, sieved to attain particle size of 0.9–1.2 mm, and stored at −20 °C to avoid oxidation of the CD. There were nine pigmented diets composed of 3×3 factorial combinations of CD type (AX, BC and 1:1 mixture of AX and BC) and concentrations (10, 20, and 40 mg/kg). Proximate analysis of these diets are listed in Table 1.

2.2. Fish rearing, feeding and sampling

The experimental characins were bought from an ornamental fish farm. During acclimatization in the laboratory in a 0.5-ton tank, fish were fed control diet for two weeks to equalize their body CD content. Fish were

<table>
<thead>
<tr>
<th>Carotenoid type</th>
<th>None</th>
<th>Astaxanthin (AX)</th>
<th>β-carotene (BC)</th>
<th>1/2 AX+1/2 BC (MX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoid concentration (mg/kg)</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Diet notation</td>
<td>Control</td>
<td>AX-10</td>
<td>AX-20</td>
<td>AX-40</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>30.35</td>
<td>30.76</td>
<td>30.44</td>
<td>30.81</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>5.4</td>
<td>5.68</td>
<td>5.44</td>
<td>5.54</td>
</tr>
<tr>
<td>NFE+CF (%)</td>
<td>40.93</td>
<td>39.85</td>
<td>39.75</td>
<td>40.25</td>
</tr>
<tr>
<td>Astaxanthin (mg/kg)</td>
<td>1.6</td>
<td>10.86</td>
<td>22.55</td>
<td>41.67</td>
</tr>
<tr>
<td>β-carotene (mg/kg)</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*a Nitrogen-free extracts and crude fiber.*
then transferred to 30 aquaria (44 cm × 33 cm × 21.5 cm) to receive their respective treatments (three replicates per treatment) at stocking density of 30 fish/aquarium. Mean fish weight was 0.41 ± 0.09 g. Culture water was passed through a 1-μm filter and sterilized by ultraviolet light to eliminate microalgae, a possible source of CD. Moreover, all aquaria were covered with black screen to discourage algal growth for the same precaution. Fish were fed twice daily at 08.00 and 15.00 at 5% body weight. The ration was adjusted at each biweekly sampling. Dissolved oxygen was maintained at 6–7 ppm by constant aeration, temperature at 26–28 °C, pH of 7.5–8 and NH₃ of 0.1–0.2 ppm. Feces and uneaten feeds were siphoned out daily and 1/3 of the water was exchanged. The fish were reared for 8 weeks. At the end of the experiment, all fish were counted and weighed. Six fish were randomly sampled from each aquarium and blood samples were collected through the branchial artery for the analysis of serum TAS (total antioxidant status), SOD (superoxide dismutase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and GPx (glutathione peroxidases). These parameters were chosen as indices of fish antioxidant capacity. These same fish samples were then freeze dried at −70 °C for carotenoid content analysis.

2.3. Analysis of fish body carotenoids

The dried sample was grounded with porcelain mortar and pestle, and placed into a 50-ml polypropylene centrifuge tube. Then 20 ml of acetone (0.05% butylated hydroxytoluene, BHT) was added as solvent (Schwartz and Patroni-Killam, 1985; Khachik et al., 1986), and the mixture was homogenized (Polytron PT-MR-3000) at 8000 rpm for 1 min. The contents of each tube was centrifuged (Hitachi 18 PR-52) under 4 °C at 12,700 × g for 15 min. The pellet was resuspended and again centrifuged with additional 20 ml aliquots of acetone until the acetone extract was clear. The pooled acetone extracts were transferred to a 250-ml separatory funnel, partitioned with 30 ml n-hexane, which was washed three times with 10% NaCl to remove residual acetone. The epiphase was washed repeatedly to remove acetone. Water was removed by adding 5–10 g Na₂SO₄/100 ml (Simpson et al., 1985). Extract volume was reduced to 10 ml using a rotary evaporator and then filtered through a 0.2 μm Millipore filter and stored in 4 ml brown vials. Identification of CD (BC and AX) was accomplished by visible spectra, co-TLC and co-HPLC with standards. Precoated aluminium silica gel plates (Merck, 20×20 cm, 0.25 mm) and a solvent system of 30% acetone in hexane were used for TLC. The volume of the solution was recorded and at 450 and 470 nm measured by HPLC to calculate the BC and AX concentration.

2.4. Analysis of antioxidant parameters and blood protein

Blood samples were prepared by mixing 200 μl isotonic NaCl solution containing 0.94 m mol/l EDTA with 50 μl blood immediately after withdrawing the blood. The samples were chilled if not immediately used for determination of TAS, SOD, GPx, AST, ALT and blood protein.

The different antioxidant parameters were analyzed using Randox Laboratories kits (Crumlin, Co. Atrim, UK) by spectrophotometry (U-2000; Hitachi Ltd., Japan). The volumes of serum samples used were 20 μl for TAS and GPx, 25 μl for SOD and 100 μl for AST and ALT. Activities were expressed in international enzyme units (U/l).

Blood protein was determined using a protein assay kit (No. 500-0006, Bio-rad laboratories, Richmond, CA., USA) with BAS (bovine serum albumin, 66 Kda, Sigma) as standard. The method used was based on Bradford (1976) using 200 μl of serum sample.

2.5. Statistical analysis

A two-way ANOVA was performed to find out the main effects of CD types and concentrations on survival,
growth, body AX and BC content, ratio of body AX to BC, TAS, SOD, GPx, ALT, and AST. Duncan’s multiple range test was then used to compare those parameters among various levels within each main effect. Correlation analysis was conducted to find out the relationships between body AX and BC and antioxidant parameters.

3. Results

3.1. Survival and growth

No mortality occurred throughout the experiment. There was also no observed effect of dietary CD supplementation on growth of the experimental fish (Table 2).

3.2. Pigmentation

Initial overall average body BC and AX contents were 0.58±0.14 mg/kg and 12.8±2.49 mg/kg, respectively. After 8 weeks of feeding, body BC content of control fish was 0.06 mg/kg, 1.23% of body AX content (4.86 mg/kg) (Table 2). Both body AX and BC contents of control fish were significantly lower than the fish fed pigmented diets (Tables 2 and 3). Body AX content significantly increased with increasing dietary CD concentrations, regardless of the type of CD. Body BC content did not increase with increasing dietary CD between 10 mg/kg and 40 mg/kg (Table 2). Body AX contents of BC-fed fish were lower than those of AX-fed fish and MX-fed fish; however, no difference in body AX content was found between AX-fed fish and MX-fed fish (Table 3). Dietary carotenoid type did not affect body BC content.

3.3. Ratio of body AX to BC content

The initial overall average ratio of body AX to BC content of 22 increased to 59 at the end of the 8-week rearing period (Tables 2 and 3). The final ratio was higher than initial ratio for all fish. No differences in this ratio were found between control fish and pigmented fish. Various ratios were also observed among the different treatments with no clear trend with respect to dietary CD types and concentrations.

3.4. Antioxidant capacity

There were no significant effects of dietary CD concentration (Table 4) and type (Table 5) on fish serum TAS.

Table 3
Main effects of dietary carotenoid type on final total weight, body astaxanthin and β-carotene, and the ratio between them of characins, Hyphessobrycon callistus fed with diets supplemented with combinations of various types and concentration of carotenoid for 8 weeks

<table>
<thead>
<tr>
<th>Parameters in fish</th>
<th>Dietary carotenoid typea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Final total weight (g)</td>
<td>22.87 (5.03)</td>
</tr>
<tr>
<td>Body astaxanthin (AX) (mg/kg)</td>
<td>4.86a (1.22)</td>
</tr>
<tr>
<td>Body β-carotene (BC) (mg/kg)</td>
<td>0.06b (0.03)</td>
</tr>
<tr>
<td>Ratio of body AX to BC (R)</td>
<td>86.10 (44.05)</td>
</tr>
<tr>
<td>Initial R</td>
<td>22.33 (1.15)</td>
</tr>
</tbody>
</table>

Values in table are averages and standard deviations (in parentheses). No significant (p > 0.05) difference between means with common superscript(s). AX—Astaxanthin, BC—β-carotene, and MX—Mix of half of AX and BC.

Table 4
Main effects of dietary carotenoid concentration on activity of 5 antioxidants in characins, Hyphessobrycon callistus fed diets supplemented with combinations of various types and concentrations of carotenoid for 8 weeks

<table>
<thead>
<tr>
<th>Antioxidant parameter in fisha</th>
<th>Dietary carotenoid concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TAS (m mol/l)</td>
<td>1.14 (0.03)</td>
</tr>
<tr>
<td>SOD (unit/mg protein)</td>
<td>0.59a (0.10)</td>
</tr>
<tr>
<td>GPx (unit/mg protein)</td>
<td>43.50a (1.98)</td>
</tr>
<tr>
<td>ALT (unit/mg protein)</td>
<td>7.75a (0.49)</td>
</tr>
<tr>
<td>AST (unit/mg protein)</td>
<td>8.70a (0.85)</td>
</tr>
</tbody>
</table>

Values in table are averages and standard deviations (in parentheses). No significant (p > 0.05) difference between means with common superscript(s). TAS—total antioxidant status, SOD—superoxide dismutase, GPx—glutathione peroxidase, ALT—alanine transaminase, AST—aspartate transaminase.
The SOD of control fish was higher than that of pigmented fish (Tables 4 and 5). SOD decreased with increasing dietary CD concentrations (Table 4). AX-fed fish had the lowest SOD among fish fed various types of dietary CD (Table 5).

Control fish had the highest GPx and ALT activities (Tables 4 and 5). The GPx and ALT of fish fed with 40 mg/kg CD were lower than those of the fish fed with 10 mg/kg and 20 mg/kg CD. No difference in GPx and ALT were found between those fed with 10 mg/kg and 20 mg/kg CD (Table 4). Dietary CD type had no effects on GPx activity (Table 5). MX-fed fish had lower ALT than AX-fed fish. No difference in ALT was found between MX-fed fish and BC-fed fish and between BC-fed fish and AX-fed fish (Table 5).

Control fish had higher AST than all fish fed pigmented diet (Tables 4 and 5). AST decreased with increasing dietary CD concentration (Table 4). Various types of dietary CD had no effects on AST (Table 5).

4. Discussion

4.1. Survival and growth

There have been no reports that dietary CD deficiency results in negative effect on survival and growth of fish. Our results showed that various sources of dietary CD did not affect growth and survival of fish. This agrees with the study of Bell et al. (2000), in which no effects of dietary supplement of AX 70 mg/kg were found on the growth of Atlantic salmon (*Salmo salar*) reared for 22 weeks. Amar et al. (2001) also found that there were no differences in growth and feeding rates among rainbow trout fed AX and BC. Neither growth nor feed efficiency of gilthead seabream were affected by diets supplemented with synthetic AX or *Haematococcus pluvialis* or no CD (Gomes et al., 2002). In the study by Gouveia et al. (2003), growth and feed efficiency of koi carp and gold fish were not different when fed with diets supplement with *C. vulgaris*, *H. pluvialis*, and cyanobacterium *Arthrospira maxima* (*Spirulina*). Main CD in *H. pluvialis* are esterified AX, canthaxanthin, BC, lutein, and echinenone (Czygan, 1968; Choubert and Heinrich, 1993). No data on CD composition on *A. maxima* was available, but one species of Spirulina (*S. pacifica*) contains mainly zeaxanthin and BC (Soejima et al., 1980; Liao et al., 1993). Amar et al. (2004) fed rainbow trout with *Dunaliella salina* which contains BC, and *Phaffia rhodozyma* which contains astaxanthin and found no difference in growth rate between the treatment groups and the control group. However, in the study by Kim et al. (1999), the Korean rose bitterling (*Rhodeus uyekii*) fed AX supplemented diet showed higher growth rates than those fed lutein or BC and the control.

4.2. Pigmentation

AX dominates the body CD in characins, regardless of the types and concentrations of dietary CD. The higher final ratio of body AX to BC indicated that this fish did not store BC as its major CD but converted most dietary BC into body AX for storage. This was especially evidenced in two cases. First, the ratio of control fish with no dietary CD supplement still increased from 22.33 to 86.10 after 8-week rearing (Table 2). Second, those fed with BC only had the ratio increased from 21.97 to 63.66 (Table 3).

The SOD of control fish was higher than that of pigmented fish (Tables 4 and 5). SOD decreased with increasing dietary CD concentrations (Table 4). AX-fed fish had the lowest SOD among fish fed various types of dietary CD (Table 5).

Control fish had the highest GPx and ALT activities (Tables 4 and 5). The GPx and ALT of fish fed with 40 mg/kg CD were lower than those of the fish fed with 10 mg/kg and 20 mg/kg CD. No difference in GPx and ALT were found between those fed with 10 mg/kg and 20 mg/kg CD (Table 4). Dietary CD type had no effects on GPx activity (Table 5). MX-fed fish had lower ALT than AX-fed fish. No difference in ALT was found between MX-fed fish and BC-fed fish and between BC-fed fish and AX-fed fish (Table 5).

Control fish had higher AST than all fish fed pigmented diet (Tables 4 and 5). AST decreased with increasing dietary CD concentration (Table 4). Various types of dietary CD had no effects on AST (Table 5).

### Table 5

Main effects of dietary carotenoid type on activity of 5 antioxidants in characins, *Hyphessobrycon callistus* fed diets supplemented with combinations of various types and concentration of carotenoid for 8 weeks

<table>
<thead>
<tr>
<th>Antioxidant parameter in fish</th>
<th>Dietary carotenoid type&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>TAS (m mol/l)</td>
<td>1.14 (0.03)</td>
</tr>
<tr>
<td>SOD (unit/mg protein)</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt; (0.10)</td>
</tr>
<tr>
<td>GPx (unit/mg protein)</td>
<td>43.50&lt;sup&gt;a&lt;/sup&gt; (1.98)</td>
</tr>
<tr>
<td>ALT (unit/mg protein)</td>
<td>7.75&lt;sup&gt;a&lt;/sup&gt; (0.49)</td>
</tr>
<tr>
<td>AST (unit/mg protein)</td>
<td>8.70&lt;sup&gt;a&lt;/sup&gt; (0.85)</td>
</tr>
</tbody>
</table>

Values in table are averages and standard deviations (in parentheses). No significant (<i>p</i> > 0.05) difference between means with common superscript(s).<sup>a</sup> AX—Astaxanthin, BC—β-carotene, and MX—Mix of half of AX and BC.<sup>b</sup> TAS—total antioxidant status, SOD—superoxide dismutase, GPx—glutathione peroxidase, ALT—alanine transaminase, AST—aspartate transaminase.
were converted to AX so that BC-fed fish had lower body AX than AX- and MX-fed fish. The fish could reach its full efficiency in AX deposition when dietary CD concentration was at 40 mg/kg. Body AX at this level was significantly higher than those fish fed with 10 mg/kg, but not significantly different from those fed with 20 mg/kg.

Meyers and Chen (1982) classify the aquatic animals into 3 categories by their ability of converting CD into AX. Type I: salmonoids or sea bream type, which cannot oxidize β-ionone of the CD and can only use the oxidized CD. Type II: carp type, which can use and convert zeaxanthin into AX and store AX. Type III: crustacean type, which can convert BC, zeaxanthin, canthaxanthin, and echinenone into AX. Results from this study show that characins belong to Type III.

 Dietary AX was more effective than BC in deposition of body AX in this fish (Table 3). Since body AX and BC in MX-fed fish were not different from those in AX-fed fish, replacing half of dietary AX with BC in Characins diet should be more cost-effective. Synthetic AX is much more expensive than synthetic BC. Since AX dominated body CD in this fish and for the highest body AX deposition the dietary CD was best at 40 mg/kg, MX-diet containing 20 mg/kg each of AX and BC is the most efficient and cost-effective CD formulation.

4.3. Antioxidant capacity

Few studies were conducted on the effects of dietary CD on oxidation status in fish. It was found that diseased coho salmon had lower plasma AX level but higher erythrocytic SOD activities than control fish (Sakai et al., 1994). Lygren et al. (1999) showed that in diet with high levels of fat-soluble antioxidants, such as AX and vitamin E, there was a reduced need for endogenous antioxidant enzymes, such as CAT and total SOD, in protection against H2O2 and O2, respectively. In juvenile tiger prawn Penaeus monodon, AX-fed ones had lower SOD and AST than the control (Chien et al., 2003). It appears that since body AX can already provide protection against reactive oxygen species then there is less induction of the production of endogenous antioxidant enzymes.

The antioxidant capacity that TAS expresses includes enzymatic and non-enzymatic antioxidant activities. The higher TAS value, the higher antioxidant capacity it has. In this study, all types and concentrations of dietary CD used have no effects on TAS in this fish. This could be attributed to the insensitive response of TAS towards reduction–oxidation status in this fish.

SOD, a cytosolic enzyme that is specific for scavenging superoxide radicals, is involved in protective mechanisms within tissue injury following oxidative process and phagocytosis. The higher the SOD activity, the more superoxide radicals need to be reacted. In this study, the decrease of SOD with increasing dietary CD concentration indicated that dietary CD effectively reduced SOD in fish. Our results showed that AX-fed fish had the lowest SOD. Di Mascio et al. (1991) found that AX had twice the ability as BC and 80 times as vitamin E in inhibiting the generation of singlet oxygen. Terao (1989) pointed out that AX was 50% stronger in eliminating free radicals than BC. In inhibiting the formation of lipid superoxide, AX was also more effective than BC (Lim et al., 1992).

GPx exists in blood, liver, mitochondria, and cytoplasm and involves in the reaction of removal of H2O2 and is recognized as one of the most important antioxidant defenses against oxygen toxicity in organisms (Kappus and Sies, 1981; Cohen and Doherty, 1987). The lower the GPx value, the higher protection that cell have already been provided. Much lower GPx in fish fed pigmented diets than that in control fish and the decreasing trend of GPx with increasing dietary CD concentration all showed that CD could reduce peroxide in cells and concomitantly the GPx. However, there was no difference in the effectiveness between AX and BC as our results showed.

Both ALT and AST are key enzymes for interconversion of amino acids and other intermediary metabolites and are detected in liver, muscle and gill. The higher value AST and ALT are, the more amino acids are transformed or metabolized into waste in tissue, and therefore become indicators for liver function (Ozaki, 1978; Yamamoto, 1981) or for liver damage (Oda, 1990). In this study, dietary supplement of CD demonstrated its effects in lowering ALT and AST, suggesting its function in

<table>
<thead>
<tr>
<th>Antioxidant parameterb</th>
<th>TAS</th>
<th>SOD</th>
<th>GPx</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astaxanthin</td>
<td>0.48</td>
<td>−0.78</td>
<td>−0.87</td>
<td>−0.69</td>
<td>−0.66</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>**</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.25</td>
<td>−0.76</td>
<td>−0.61</td>
<td>−0.54</td>
<td>−0.48</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ns: not significant (p>0.05), *: significant (0.01<p≤0.05), **: highly significant (p ≤0.01).

b TAS—total antioxidant status, SOD—superoxide dismutase, GPx—glutathione peroxidase, ALT—alanine transaminase, AST—aspartate transaminase.
improving liver protection. Our results are in accordance with Nakano et al. (1995, 1999) in which dietary AX significantly decreased levels of lipid peroxides in the liver and lowered ALT and AST of rainbow trout. A recent study showed that CD treatment significantly altered the total lipid profile and hepatic mucopolysaccharide contents of livers of rainbow trout (Page et al., 2005).

In this study, fish fed CD supplement have lower SOD, GPx, ALT, and AST activities than control fish, showing that dietary CD could increase antioxidant capacity and protection of the liver. However, the effects of various CD on the activity of these enzymes varied. In general, body AX had more influence on antioxidant capacity than BC since the former had negative correlations with SOD and GPx capacity than BC since the former had negative correlations with SOD and GPx.

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Acknowledgements

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