Clearance of Yellow Pigments Lutein and Zeathanxin in Channel Catfish, Ictalurus punctatus, Reared at Different Water Temperatures

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Abstract

A study was conducted to determine the clearance time of yellow pigments lutein and zeaxanthin in channel catfish at various temperatures. Fish of initial weight of 13.4 g were stocked into flow-through aquaria and fed daily with a pigment-enhanced diet for 11 wk when yellow color became visible in the flesh. All fish were then transferred into tanks in three recirculating systems that were assigned one of the three temperatures (10, 20, and 30 °C). During the pigment clearance period, fish were fed a control diet without added pigments daily to satiation for 12 wk. Every 4 wk, fish from three randomly chosen tanks per temperature were euthanized and fillets were analyzed for yellow color intensity (Commission Internationale de l’Eclairage [CIE] \(b^*\)) and lutein and zeaxanthin concentrations. The \(b^*\) values of fillets of fish reared at 20 and 30 °C decreased linearly as time progressed. There was no significant linear regression of \(b^*\) value against time for fish raised at 10 °C. The rate of pigment clearance was similar for fish reared at 20 and 30 °C. Results demonstrate that about 8 wk were needed for catfish to “purge” most of yellow pigment at warm temperatures (20 and 30 °C). A longer period of time (>12 wk) was required at 10 °C.

Recently there have been increasing cases of yellow colored, commercially processed channel catfish, Ictalurus punctatus fillets in the southeastern United States. Pigmentation is desirable in some fish, such as salmon and ornamental fish, but consumers in the United States generally prefer catfish flesh to be white (Lovell 1989). Because catfish cannot synthesize the pigment, it has to come from either commercial feeds or natural foods found in the pond. The most common pigment seen in catfish is yellow, which comes mainly from the carotenoids lutein and zeaxanthin (Lee 1987; Li et al. 2007). The pigment is not evenly distributed within the catfish fillet but appears to be concentrated along the front, dorsal part of the fillet; it can also be found on the bottom part of the fillet. However, it does not affect the flavor, keeping quality, or safety of the catfish product (Lovell 1989). There are some indications that consumption of catfish containing the yellow pigment may be beneficial to human health. Yellow pigments lutein and zeaxanthin are found in high concentrations in the retina of the human eye and are thought to reduce the risk of blindness (age-related macular degeneration) and protect the eye from intensive light (Snodderly 1995). Their potent antioxidant activity (Young and Lowe 2001) may improve human health by helping protect tissues from free radicals that have a role in various diseases. However, unless consumer perception is changed, yellow pigment in catfish fillets will continue to be an undesirable trait.

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The increasing cases of yellow colored catfish noted recently are thought to be mainly caused by fish consuming pigment-rich natural food because of restricted feeding regimens applied on some farms. Commercial feeds do not contribute to the problem if lutein and zeaxanthin levels are maintained below 7 mg/kg (Li et al. 2009). Because yellow colored catfish products are not marketable at the present time, it is important to know how long it takes for the fish to clear the color to better manage the problem. Research has shown that once channel catfish accumulate enough of the pigment to be visible, the pigment levels in the flesh remain unchanged for up to 6 mo when fish are fed during the winter at low water temperatures (Lee 1987). Color clearance time is not known for catfish at warmer temperatures; therefore, a laboratory study was conducted to determine the clearance time of yellow pigment in channel catfish reared at various temperatures.

### Materials and Methods

Two 28%-protein, practical diets (Table 1) were formulated to contain 0 or 100 mg added lutein plus zeaxanthin (1:1 ratio) per kilogram of diet. The pigments were supplied by DSM Nutrition Products, Inc. (Parsippany, NJ, USA). All known nutrient requirements of channel catfish were satisfied (NRC 1993). The pigment-enhanced diet contained more added fat than the control diet (3 vs. 1.5%) to better facilitate pigment absorption by the fish. The diets were prepared as sinking pellets according to procedures described previously (Li et al. 1993) and stored at −20 C until used.

To obtain pigment-enhanced fish for the pigment clearance experiment, 225 juvenile channel catfish were stocked into fifteen 110-L flow-through aquaria (15 fish/aquaria). Water temperature was maintained at 30 ± 1 C. A diel light : dark cycle was set at 1400 : 1000 h. Fish were acclimated to the aquaria conditions for 2 wk during which time they were fed a control diet (Table 1) once daily to apparent satiation. After acclimation, fish were weighed and initial weight was determined (13.4 g/fish). Fish were fed once daily with a yellow pigment-enhanced diet for 11 wk when the yellow color became visible in the flesh. All fish were then pooled to be used for the subsequent pigment clearance experiment, of which five fish were euthanized with an overdose (500 mg/L) of tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA) to determine the initial yellow color intensity of fish fillets. Digital pictures were taken of one fillet from each fish using an EOS ID Mark II digital SLR camera (Cannon USA, Inc., Lake Success, NY, USA). The yellow intensity values (Commission Internationale de l’Eclairage [CIE]) \( b^* \) (negative: blueness; positive: yellowness) were determined from the digital picture of the fillet at three locations (Fig. 1) along the dorsal line of the fillet using an Adobe Photoshop CS3 image editing software (Adobe Systems, Inc., San Jose, CA, USA). The CIE color system has been widely used to determine the color intensity of fish (Wathne et al. 1998; Gouveia et al. 2003).

For pigment clearance, the pigmented fish were transferred into three X-Rack Aquarium rack systems (Model XR3; Marine Biotech, Inc., Beverly, MA, USA) at the USDA-ARS Catfish Genetics Research Unit, Stoneville, MS, USA. The pigs were acclimated to the rack system for 1 wk. Fish were maintained at 30 ± 1 C and fed once daily with a pigment-enhanced diet for 13 wk to determine the yellow color intensity of the fish fillets. The pigments were maintained at 50 mg/kg. The visual yellow color intensity was determined using the CIE \( b^* \) color intensity. The visual yellow color intensity was determined by the CIE color system and was used to determine the initial yellow color intensity of fish fillets. Digital pictures were taken of one fillet from each fish using an EOS ID Mark II digital SLR camera (Cannon USA, Inc., Lake Success, NY, USA). The yellow intensity values (Commission Internationale de l’Eclairage [CIE]) \( b^* \) (negative: blueness; positive: yellowness) were determined from the digital picture of the fillet at three locations (Fig. 1) along the dorsal line of the fillet using an Adobe Photoshop CS3 image editing software (Adobe Systems, Inc., San Jose, CA, USA). The CIE color system has been widely used to determine the color intensity of fish (Wathne et al. 1998; Gouveia et al. 2003).

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### Table 1. Ingredient composition of experimental diets (percentage, as-fed)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>Pigment-enhanced diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td>46.80</td>
<td>47.15</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn grain (extrusion-cooked)</td>
<td>38.23</td>
<td>36.38</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.275</td>
<td>1.275</td>
</tr>
<tr>
<td>C-free vitamin mix</td>
<td>0.045</td>
<td>0.045</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Trace mineral mix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Carboxymethylcellulose</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Catfish offal oil</td>
<td>1.50</td>
<td>3.00</td>
</tr>
<tr>
<td>Lutein</td>
<td>50 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>50 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Pigment analysis</td>
<td>4.5</td>
<td>82.2 mg/kg</td>
</tr>
</tbody>
</table>

\( a \) Same as described by Robinson et al. (2001).

\( b \) Provided by L-ascorbyl-2-monophosphate (35% activity).
Mississippi, USA. Each system was assigned one of the three water temperatures (10, 20, and 30°C). Five fish were stocked in each of the nine 23-L clear polycarbonate tanks in each system. Each tank had a polycarbonate lid with access holes for feeding and water and air inlets. Each tank also contained individual water and air valves, airstones, and rear-side, mid-tank drains with removable mesh screens to prevent feed loss. The tanks were independently drained at a rate of >8.0 L/min via an adjustable flow regulator. Three-stage particulate filtration was used that included a 150-μm reusable polyester filter pad, chemical filtration via activated carbon, and a mechanical pleated cartridge filter. Biofiltration was accomplished by passing water through a submerged silica gravel bed in the reservoir tank. A thermostat-regulated water heater was installed in one rack system to maintain the water temperature at approximately 30°C. Two of the rack systems were connected to separate 90-kw air-cooled water chillers with a single six cylinder Copeland compressor (Copeland Corporation, Sidney, OH, USA). Fish were acclimated to the colder temperatures by reducing the temperature 2°C a day until the targeted temperatures (20 and 10°C) were obtained. Temperatures in the three rack systems averaged 10.5 ± 0.2, 20.4 ± 0.1, and 29.4 ± 0.1, respectively, during the 12-wk study period. Water quality tests for ammonia and nitrite were monitored twice a week, whereas dissolved oxygen (DO) was maintained above 6 mg/L.

During the pigment clearance period, fish were fed the control diet once daily to apparent satiation. Every 4 wk, all fish from three randomly chosen tanks per temperature treatment were euthanized by an overdose of MS-222 to determine the fillet yellow color intensity.

Diet and fillet samples were analyzed for lutein and zeaxanthin concentrations using high performance liquid chromatography (Moros et al. 2002).

Linear regressions were conducted on yellow color intensity value CIE b* and lutein plus zeaxanthin concentrations against time (wk) at each temperature (Zar 1999) using contrast statement of the Statistical Analysis System (SAS) version 8.0 (SAS Institute 2004). Slopes of the lines obtained for the three water temperatures were compared using the Tukey test (Zar 1999). Tanks were used as experimental units. A significant level of P ≤ 0.05 was used.

Results and Discussion

The CIE b* values of fillets of channel catfish reared at 20 and 30°C decreased linearly as the time progressed (Fig. 2). There was no significant linear regression of the b* value

![Figure 1. Locations on catfish fillets along the dorsal line where the yellow intensity value (b) was measured. The b values were taken from the same locations in each fillet.](image1)

![Figure 2. Yellow intensity value (Commission Internationale de l’Eclairage [CIE] b*) as a function of time at various water temperatures. At 20°C, Y = 35.47 − 1.088X, R² = 0.84, P < 0.01; at 30°C, Y = 36.42 − 1.511X, R² = 0.86, P < 0.01, where Y = CIE b* value and X = week.](image2)
against time for fish raised at 10 C. Slopes of the lines for 20 and 30 C were not significantly different, indicating that the rate of pigment clearance was similar. Li et al. (2009) reported that fillets of pond-raised channel catfish with *b* values of 30 and above were considered unsuitable for marketing because of the obvious yellow color. The mean initial *b* value was 35.5 for fillets of channel catfish that had been fed a pigment-rich diet for 11 wk. This value decreased to below 30 after 8 wk of feeding a low-pigment control diet at 20 and 30 C, whereas the value for fish reared at 10 C remained above 30 after 12 wk of feeding. Visual examination of fillets confirmed that yellow pigment became unnoticeable at Week 8 for fish reared at 20 and 30 C.

As the time progressed, total lutein plus zeaxanthin concentrations decreased in fillets of fish at all three temperatures (Fig. 3). This generally followed the same trend as the CIE *b* values, although the regression of total lutein plus zeaxanthin concentration against time was also significant for fish reared at 10 C. Results from this study generally agreed with results of a previous study with channel catfish conducted in outdoor tanks during the winter (Lee 1987). However, in a pond study, Lee (1987) reported that lutein plus zeaxanthin levels in fillets of channel catfish remained unchanged when fed and increased when not fed during the 6-mo overwintering period. Lee (1987) attributed the increased yellow pigment concentrations in fish not fed during the winter to reduced body fat in the muscle tissue. In addition, during the winter fish may obtain yellow pigments from consuming some natural foods present in the pond water, especially when fish are not fed.

In this study, there was no significant difference in linear regression slopes for 20 and 30 C; however, the slopes were significantly lower than that obtained for 10 C, indicating that the clearance of the yellow pigment was faster at high temperatures. Lee (1987) reported that a maximum of total lutein and zeaxanthin level without causing objectionable yellow pigmentation was 0.6 μg/g tissue. In this study, the total lutein and zeaxanthin concentration in catfish fillets decreased from 1.22 μg/g below 0.6 μg/g after 8 wk of feeding of the low-pigment control diet at 20 and 30 C, respectively, whereas the pigment level in fillets of catfish reared at 10 C remained above 0.6 μg/g after 12 wk of feeding (Fig. 3).

At the end of Week 12, fish reared at 20 and 30 C more than doubled their body weight (Table 2). This study did not attempt to compare the growth of fish at various temperatures because each temperature had only one temperature-controlled culture system. However, fish weights were approximately the same for 20 and 30 C. This response is not readily explained because fish reared at 30 C would
be expected to grow faster than those at 20 C because the optimum temperature for channel catfish is reported in the range of 28–32 C (Tucker and Hargreaves 2004). Nevertheless, because of the increased fish biomass there is likely a diluting effect on lutein and zeaxanthin concentrations in the fillets as fish grow larger, resulting in reduced pigment concentrations and the intensity of yellow color. However, at 10 C fish generally maintained their body weight during the 12-wk period and the pigment concentration also decreased, though at a slower rate. This suggests that part of the pigment is utilized or degraded in the muscle. The metabolism, degradation, or clearance of carotenoids, especially lutein and zeaxanthin, have not been well understood in animals including fish. These pigments have been considered antioxidants; therefore, they may be utilized to neutralize the free radicals released during the oxidation process within the living cells. It has been reported that lutein can be converted to vitamin A in several freshwater fish, such as goldfish, Carassius auratus (Benjamin and Tito 1983), crucian carp, Carassius carassius (Czeczuga and Czerpak 1976), and stinging catfish, Saccobranchus fossilis (Barua et al. 1973). However, channel catfish apparently lack the ability for such conversion (Lee 1987).

In summary, results from this study demonstrated that it would take about 8 wk for channel catfish to “purge” most of the yellow pigment at warm temperatures (20 and 30 C). A longer time was needed at 10 C (>12 wk). In practice, if a relatively large number of yellow pigmented catfish are found, lutein and zeaxanthin levels of the feed and the presence of pigment-rich natural foods should be examined. After corrective measures are taken, a minimum of 8 wk is needed when fish are actively feeding before fish can be harvested. Fish should be adequately fed during the growing season and over the winter to reduce the chances of consuming natural foods in the pond.

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