XANTHOPHYLL IN CATFISH:
RESPONSE UNDER CONTROLLED AND OUTDOOR CONDITIONS

by

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ABSTRACT

Catfish industry is the largest component of aquaculture in the United States of America. It is a mature industry with more than 50 years of successful production, yet it faces various challenges while it adapts to shifting inputs and market demands. Dramatic increases in the price of feed ingredients and requests for more consistent fillet color are two core concerns of the industry. To help meet these challenges, a series of studies were conducted to evaluate lower cost feeds and the response of fish to xanthophyll levels of the feed.

To help reduce the cost of diets, a pond study was conducted to evaluate the utilization of two alternative feeds including 20% corn gluten feed and two traditional feeds, with 28% or 32% protein, for the production of channel-blue hybrid catfish. After a summer growing season, no significant differences were found in growth performance, feed utilization, hematology, and immune response indices among the four treatments. However, intraperitoneal fat ratio was significantly lower in fish fed the alternative feeds and hepatosomatic index was higher in fish fed 28% protein feeds. Xanthophyll levels in fish fed alternative diets were significant higher, but without discernible yellow color. Xanthophyll levels in diets and in fillet were linearly related. These data generally indicated that channel-blue hybrids could efficiently utilize all the four experimental diets with satisfying production result.

To help the catfish industry better understand the yellow coloration of catfish fillet, a color standard was developed with a digital camera and computer software. CIE LAB B value was used to indicate the yellowness. Lutein, zeaxanthin and alloxanthin were detected in the catfish fillet. A linear correlation was found between the B values and xanthophyll levels in

catfish fillets. This color standard provides a means to sort fillets into different categories for different markets and can furthermore help with development of color management practice in catfish industry.

An indoor aquarium trial was conducted to evaluate the deposition and depletion rate of xanthophyll in channel catfish fillet under various dietary treatments. The deposition experiment indicated that xanthophyll accumulated in fish flesh first and then trended to level off around a certain level related to the dietary xanthophyll level. Xanthophyll levels in fish fillets and in diets were linearly correlated after 16 weeks of culture. The dissipation experiment indicated that xanthophyll levels decreased linearly over 8 weeks. No difference was found in the dilution rate between diets with xanthophyll levels of 4.1ppm and 9.0ppm. Fish with higher initial xanthophyll level appeared to have a faster reduction in xanthophyll levels.

To better understand the contribution of natural foods to xanthophyll level in catfish, a study was conducted to identify if there were correlations of xanthophyll in catfish fillet and in natural productivity under pond conditions. Catfish fillet and field samples (plankton, shad and snail) were collected and analyzed quantitatively for xanthophyll levels through HPLC. A correlation between xanthophyll level in catfish fillets and in microorganisms (>75 microns) was found. Natural productivity consumed by gizzard shad also contributed to the xanthophyll level in catfish fillet.

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CHAPTER I

INTRODUCTION

1. Catfish industry

Catfish industry is developing rapidly in many countries around the world resulting in an expansion of product availability and species cultured. Thai walking catfish (*Clarias batrachus*), African catfish (*Clarias gariepinus*) and Striped catfish (*Pangasius hypophthalmus*), are extensively cultured in Africa and Asia. In North America, channel catfish (*Ictalurus punctatus*) is the major cultivated catfish species but it is also produced in China, Brazil and Russia. Channel catfish is a highly productive catfish species with a long history of culture. The global aquaculture production of channel catfish increased from 271,309 tons in 2000 to 462,416 tons in 2008, while the aquaculture value of channel catfish increased from $450,964,000 in 2000 to $688,800, 000 in 2008 (FAO, 2010). In the United States of America, catfish farmers produced a total of 478 million pounds of food size fish and ranked sixth in terms of consumption in the U.S. during 2010 (Hanson and Sites, 2011a). In the U.S., the catfish industry is a mature industry with a long history of success. However, the industry faces numerous challenges while it evolves and adapts to shifting markets, such as disease and survival issues, increasing feed costs, and quality concern. Efforts are currently under way to help improve the catfish industry using a wide range of technologies, such as application of new hybrid species, modification of feed formulations and improved quality control of catfish fillets.

Hybrids between different species of *Ictalurid*s have been studied for more than forty years. The channel catfish (*Ictalurus punctatus*) x blue catfish (*Ictalurus furcatus*) hybrid catfish is a newly developed line which has started to displace channel catfish on many farms. Of all these interspecific catfish hybrids, channel-blue hybrid catfish is the only hybrid that has been accepted for commercial application (Masser and Dunham, 1998). Compared to most commercially cultured strains of channel catfish, the channel-blue hybrid exhibits superior characteristics for the following traits: increased resistance to many diseases and higher survival (Dunham et al., 2008; Li et al., 2004; Masser and Dunham, 1998; Wolters et al., 1996), better tolerance of low oxygen (Dunham et al., 1983; Li et al., 2004), tolerance to crowded growth conditions in ponds, faster growth (Dunham et al., 1990; Yant et al., 1976), higher dressout percentage and fillet yield (Argue et al., 2003; Li et al., 2004), and increased vulnerability to angling and seining (Tave et al., 1981). Though channel-blue hybrid has many advantages, little information is available on its nutrition and no research has been conducted on its fillet coloration. As commercial culture of this line expands, more studies are required to improve culture technologies for this line.

In addition to species improvement, considerable effort has been invested in modifying the catfish feed. The catfish industry is facing financial challenges as the prices of core ingredients used in traditional catfish feed are increasing dramatically. For example, the price of 48% soybean meal increased from $174.17 in 2005 to $280-310 per ton in 2010 (NASS, 2011a), and the farm price of corn increased from $2.00 in 2005 to $5.15-5.65 per bushel in 2010 (NASS, 2011b) in the United States. Correspondingly, the price of catfish feed increased. Catfish feed price (32% protein) in 2010 averaged $354/ton, up $121/ton over the 2005 average feed price $233/ton (Hanson and Sites, 2012). But at the same time, the average price of fresh whole

catfish fluctuates slightly with a marginal decreasing trend from $1.69 per pound in 2006 to $1.58 per pound in 2010 (Hanson and Sites, 2011b). The increasing price of core feed ingredients and the marginal change of catfish price leads to difficulties in maintaining profit margins.

Protein is one of the most expensive components in commercial feeds. Hence, lowering the protein content in feed is one way to help reduce feed cost. The traditional catfish feed contains 32% protein. Due to cost savings, the use of 28% protein catfish feed has been promoted. The protein requirement of channel catfish ranges from 25% to 36% (Robinson and Li, 1996). During the growout season, 28% and 32% protein feeds are typically used (Robinson and Li, 1996). Studies have shown that 28% protein feed can provide similar growth rate and feed conversion ratio if catfish are fed to apparent satiation (Robison et al., 1997, 1999; Li et al., 2000; Li and Robinson, 2007). Another way to reduce feed cost is to use low-cost alternative feed ingredients. Corn processing byproducts such as corn gluten meal and corn gluten feed, cottonseed meal and distiller's dried grains are currently being proposed as suitable alternatives to reduce cost. Some studies have demonstrated these feeds with appropriate supplemental amino acids can result in equivalent production of catfish as compared to results with traditional feed formulations (Robison and Li, 1994; Robison et al., 2001; Robison and Li, 2008; Zhou, 2010).

While the use of corn products and distiller's dried grains helps reduce the feed cost, they may bring the catfish industry to face another challenge, a yellow coloration to the fillet, due to their high concentration of yellow pigments. The proportion of fillets with a yellow color has been identified as a problem for consumer acceptance. Generally, customers expect catfish fillet with good quality to have a white color (Lovell, 1984). Yellow color of the catfish fillet is perceived as different and consequently undesirable. The price of catfish fillet with yellow color

is reduced in certain markets (Sørensen, 2005). Some markets even refuse catfish fillet with visible yellow color resulting in unwanted product that must be disposed of. From an industry perspective, though the alternative feed can result in a good production, it is applicable only if the quality of catfish is consistent with an acceptable white color. Thus, the control of catfish quality through catfish fillet color is important for catfish industry. Alternative low-cost feed needs to be studied and formulated to keep the catfish production without causing a yellow fillet problem.

Carotenoids are the pigments responsible for the yellow color in catfish fillets, and xanthophyll is the main class of carotenoids found in catfish fillets (Lee, 1987; Tsushima, 2002; Li et al., 2007). Catfish cannot synthesis xanthophyll; hence, food is the only source of xanthophyll in catfish fillets. Both the formulated feed and natural food play important roles in the yellow pigment content in fish fillets. Many of the ingredients commonly used in catfish feeds contain carotenoids at varying concentrations. Carotenoid contents in whole corn, corn gluten feed, corn gluten meal and distiller's dried grains solubles are about 20 mg/kg (Moros et al., 2002), 17-30 mg/kg (Lovell, 1989; Robinson et al., 2001), 145-350 mg/kg (Lovell, 1989; Moros et al., 2002) and 4-34 mg/kg (NRC, 2011), respectively. According to Lovell's (1989) demonstration, carotenoid (lutein and zeaxanthin) level of 11 mg/kg or above in feed would impart yellow color in channel catfish flesh. Li et al. (2009) proposed a threshold of 7 mg/kg for xanthophyll in catfish feed to avoid yellow fillet problem. The combination of these high-xanthophyll ingredients may cause a carotenoid level above 7-11 mg/kg in catfish feed and lead to a yellow fillet problem. Commercially produced feeds are assumed to be the primary source of carotenoids. However, in nature, prey fish, snails and filamentous algae are concentrated sources

of xanthophyll. If these natural foods are consumed by catfish, it could lead to the accumulation of yellow pigments in catfish (Lovell, 1984; Li et al., 2009).

Because of costumer expectation of catfish fillets with a consistent color, variation of fillet color has become an important topic in the catfish industry. Finding a way to address, prevent or solve the yellow fillet problem has become an important issue for the industry.

2 Carotenoids

Structure

Carotenoids are a group of isoprenoid polyene pigments with yellow to red coloration. The structure is formed by joining eight C_5– isoprene units in a regular head-to-tail manner with a symmetrical molecule center where the order is tail-to-tail (Shahidi et al., 1998). The oxygenated carotenoids are called xanthophyll, while those do not contain oxygen are called carotene. β-carotene is the most well-known carotene. Many important xanthophylls are oxygenated derivatives of β-carotene, such as lutein, zeaxanthin, astaxanthin, canthaxanthin and echinenone. Thus, these xanthophylls have similar chemical structures to each other.

Distribution

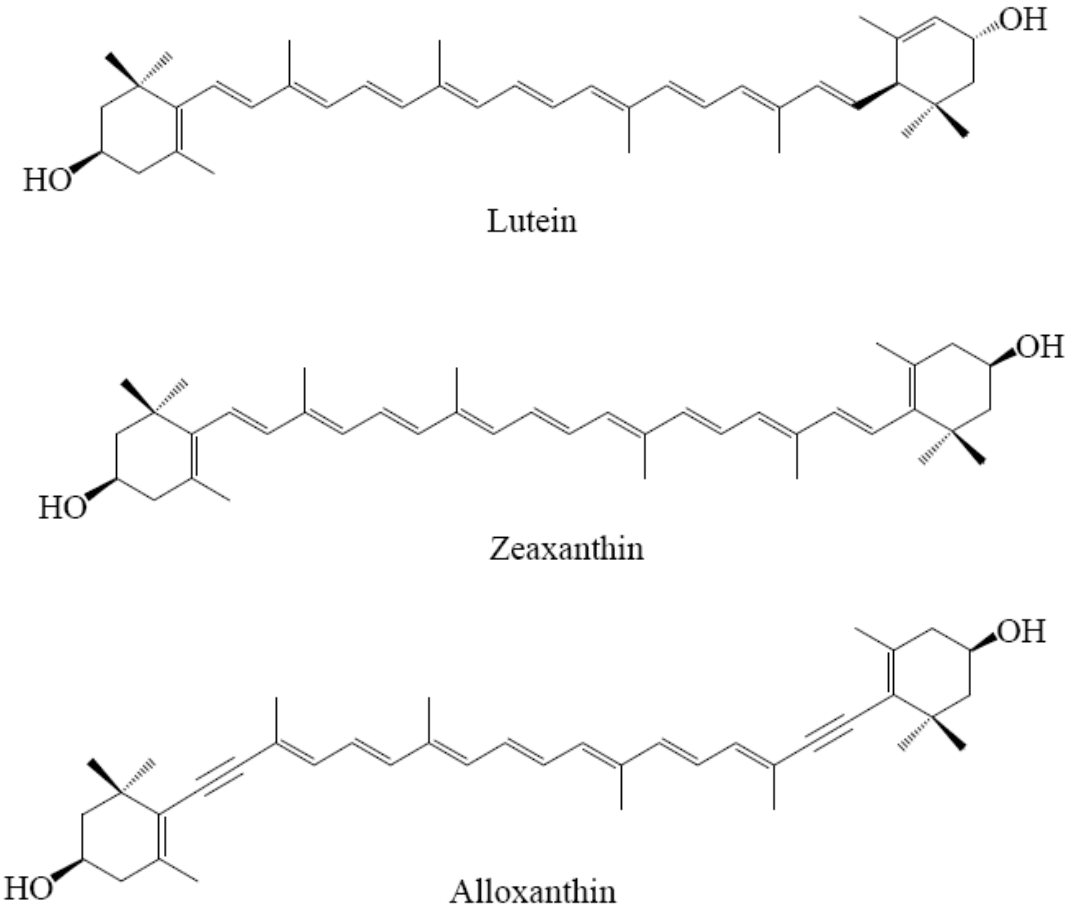
Carotenoids are the most widespread pigments found in nature (Shahidi et al., 1998). Carotenoids exist widely in bacteria, fungi, plants and animals, but can only be synthesized by microorganisms and plants. Biosynthetically, carotenoids are derived from the acyclic lycopene through hydrogenation, dehydrogenation, cyclization, and oxygenation reactions. In the animal kingdom, astaxanthin is the most widely distributed xanthophyll, followed by lutein and

zeaxanthin which are also widespread in the plant kingdom (Shahidi et al., 1998). In fish, different carotenoids dominate in different species: tunaxanthins in yellow and blue-green fish; astaxanthins in red marine fish; zeaxanthins in anchovies, some flatfishes, sharks and rays; tunaxanthins, luteins and zeaxanthins in brackish water fish; and luteins and alloxanthins in freshwater fish (Matsuno, 2001). Carotenoids occur in a wide array of forms, such as free forms, esters, glycosides, sulfates and carotenoproteins (Matsuno, 2001). In fish, carotenoids are normally concentrated in the skin in the form of esters (Goodwin, 1984). Considerable amounts of carotenoids are also found in the muscle tissue (especially in salmonids), the ovaries and liver (Tacon, 1981). In channel catfish, lutein and zeaxanthin were found in skin, flesh, abdominal fat and liver (Lee, 1987). Liu et al. (2012) found alloxanthin in catfish flesh (Figure 1).

Absorption

Animals selectively absorb carotenoids. Rat, pig and sheep could not accumulate carotenoids (Goodwin, 1986). Cattle are excellent β-carotene accumulator. Poultry mainly deposit xanthophylls leading by lutein and zeaxanthin (Goodwin, 1986). In chickens, the absorption of zeaxanthin was three times of astaxanthin (Schiedt et al., 1985). In contrast, the absorption of lutein and zeaxanthin is 10 to 20 times less efficient than astaxanthin and canthaxanthin in Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* (Schiedt et al., 1985). Channel catfish also selectively deposit carotenoids. When fed with equal amount of lutein, zeaxanthin and β-carotene, catfish accumulated lutein and zeaxanthin better than β-carotene in flesh, skin, liver and abdominal fat. Little β-carotene was accumulated in muscle and skin, but considerable amount of β-carotene was found in liver and in abdominal fat (Lee, 1987).

Figure 1. Chemical structures of xanthophylls found in catfish: lutein, zeaxanthin and alloxanthin.



The percent absorption of xanthophyll was negatively correlated with the dietary xanthophyll levels in chickens (Dua et al., 1967).

Carotenoids also have interactions with each other. It was reported that dietary zeaxanthin could enhance lutein absorption in quail while lutein did not influence zeaxanthin absorption (Toyoda et al., 2002). In contrast, zeaxanthin supplementation did not influence plasma lutein in spider monkeys *Saimiri sciureus* (Snodderly et al., 1997). When lutein was given simultaneously with β-carotene to humans, an inhibitory effect of lutein on absorption of β-carotene was found (Van den Berg, 1998).

Metabolism

The mechanism of metabolism of xanthophyll in fish has not been clarified yet, though both reductive and oxidative metabolism pathways have been proposed in many fishes (Matsuno, 2001). Fish can modify one carotenoid to get another carotene. In *Salmoniformes* and *Silurifiormes* fish, reductive pathways of astaxanthin to zeaxanthin via β-carotene triols and tetrols were proposed (Tsushima et al., 1999). In brook trout *Salvelinus fontinalis*, different pathways of astaxanthin to zeaxanthin and lutein were proposed in muscle and in ovary (Ando et al., 1990). Reductive pathways of astaxanthin to tunaxanthin were proposed in yellowtail *Seriola quinqueradiata* (Miki et al., 1985), flying fish *Prognichthys* agoo and dolphin *Coryphaena hipppurus* (Matsuno et al., 1985). Astaxanthin was converted to β-carotene in *H. fossilis* (Goswami, 1984). Canthaxanthin was converted to β-carotene via echinenone in rainbow trout (Guillou et al., 1989). Oxidative pathway of zeaxanthin to astaxanthin was proposed in goldfish *Carassius auratus* and common carp *Cyprinus carpio L.* (Hata and Hata, 1972). In crustacean, pathways of β-carotene and zeaxanthin to astaxanthin have been proposed (Tanaka, 1976).

Function

Carotenoids have a wide array of functions. Carotenoids can serve as precursor to vitamin A. β-carotene is the most important vitamin A precursor and possesses full vitamin A activity. Xanthophylls are not vitamin A precursors in mammals, but can be converted to vitamin A in lower animals because they can metabolize xanthophylls to β-carotene and then convert it to vitamin A. Astaxanthin and canthaxanthin are precursors of vitamin A in guppies *Lebistes reticulatus*, platies *Xiphophorus variatus*, rainbow trout, and Atlantic halibut, *Hippoglossus hippoglossus L.* (Gross and Budowski, 1966; Moren et al., 2002; Schiedt et al., 1985). Lutein is precursors of vitamin A_2 in goldfish, freshwater fishes *Saccobranchus fossilis* and *Clarias batrachus* (Barua et al., 1973; Goswami and Bhattacharjee, 1982; Benjamin and Tito, 1983). Zeaxanthin can be converted to vitamin A in both rainbow trout and Nile tilapia *Oreochromis niloticus* (Katsuyama and Matsuno, 1988; Schiedt et al., 1985). Carotenoids have various protective functions, such as the ability to absorb and reflect damaging radiation, to quench singlet oxygen, and to act as an antioxidant protecting tissues from oxidation (Matsuno, 2001; Siefermann-Harms, 1987; Tacon, 1981). In aquatic animals, carotenoids assist in communication, especially associated with courtship and to sexual dichromatism (Hubbs and Stavenhagen, 1958; Tacon, 1981). Carotenoid can enhance general performance as well as specific functions in reproduction and metabolism of fish (Torrissen and Christiansen, 1994). The mobilization of carotenoids into gonads during sexual reproductive activity also indicates a possible role of carotenoids in aquatic animal reproduction (Goodwin, 1950). Recently, carotenoids have been found to play a role in immunology, and can act as antitumor agents (Matsuno, 2001). It has been observed that fish with a high level of carotenoids are more

resistant to bacterial and fungal diseases (Czeczuga, 1979a, 1979b). Studies showed that dietary intake of carotenoids can enhance the immunity of rainbow trout (Amar et al., 2001, 2004).

Carotenoids and pigmentation

Color is an important factor indicating the quality of food and affecting the acceptance of consumers. Consumers expect a certain color on food product, such as pink-red for salmon and trout, yellow for poultry skin and orange for egg yolk. In many cases, the grade or price of food is directly related to their color, such as shrimp, salmon, rockfish, and snapper (Sacton, 1986; Ostrander et al., 1976). Synthetic carotenoids and natural source carotenoids are added into food product and animal feed to ensure the pleasing color of animal product. β-carotene is a widely used non-toxic additive to many foods such as butter, ice cream, orange juice and candies (Kläui and Bauernfeind, 1981). In poultry feed, the major natural source of xanthophyll are yellow corn, corn gluten meal and dehydrated alfalfa meal (Dua et al., 1967). Dua et al. (1967) reported that xanthophylls from yellow corn can be better utilized by the chick for skin deposition of pigments than those from dehydrated alfalfa meal and corn gluten meal. Hinton et al. (1976) reported corn gluten meal and yellow corn had better xanthophyll utilization than alfalfa in broiler skin pigmentation. In fish feed, shrimp meal, crab meal, crayfish meal, extracts from paprika or marigold, and pure carotenoid preparation such as canthaxanthin, astaxanthin, zeaxanthin and lutein are commonly used for pigmentation (Lee, 1987). The ability of pigmentation varies among different carotenoids in different species. In an absorption experiment, it was found that the visual yellow color intensity score was highest for catfish fed lutein, following by zeaxanthin, astaxanthin, and canthaxanthin, and lowest for fish fed basal and β-carotene diets (Li et al., 2007). Brockmann and Völker (1934) fed laying hens with various purified carotenoids, and

found that lutein and zeaxanthin gave the most intense color to the egg yolks, while carotene, lycopene and vioolaxanthin only gave very little color. In rainbow trout, the individual optical isomers of astaxanthin are more efficient than canthaxanthin in pigmenting the flesh (Foss et al., 1984). Astaxanthin could be utilized more efficiently than canthaxanthin, followed by astaxanthin dipalmitate in Atlantic salmon (Storebakken et al., 1987). Astaxanthin performed better than lutein for pigmentation of fancy carp (Iwahashi and Wakui, 1976).

In catfish industry, coloration of catfish fillet is not accepted by costumers. This is because catfish is considered as white-fillet fish, and the color shift is considered of lower quality by customers. Although the yellow coloration does not affect the flavor, keeping quality, or safety of the catfish product, it is still considered undesirable as long as customer perception is not changed (Lovell, 1989). The yellow coloration is concentrated along the front, dorsal part of catfish fillet, and also can be found on the bottom of the fillet (Lovell, 1984; Li et al., 2011). Lee (1987) reported that a concentration above 0.6 µg carotenoid/g of catfish flesh would cause discernible yellow color in the fillet. Li et al. (2009) reported a CIE (International Commission on Illumination) B^* value above 30 was considered unsuitable for marketing. The B^* value was determined by averaging B^* values of three locations along the dorsal line of catfish fillet. Once the yellow color becomes visible, it can be removed by feeding catfish low-pigment diet. In a yellow pigment clearance study, it was found that 8 weeks were needed for catfish to washout most of yellow pigment at 20˚C and 30˚C, and 12 weeks at 10˚C (Li et al., 2011).

3. Objectives of Study

The overall objective of this study was to help the catfish industry better understand xanthophyll in the catfish fillet. Four specific objectives were included as followed:

1. Evaluate two dietary protein levels and the use of corn gluten feed in feed formulations for xanthophyll concentration and culture of hybrid catfish under pond conditions.

2. Develop a standardized color grading scale, and correlate “yellow color” to tissue levels of xanthophyll.

3. Determine the deposition rate and dissipation rate of xanthophyll in channel catfish fillet under controlled conditions.

4. Evaluate the contribution of natural food to the yellow color in catfish fillet under pond conditions.

CHAPTER II

EVALUATION OF TRADITIONAL DIETS AND CORN GLUTEN FEED SUBSTITUTED ALTERNATIVE DIETS FOR POND-RAISED HYBRID CATFISH ON PRODUCTION AND XANTHOPHYLL LEVEL

Abstract

In the United States, catfish industry is a mature industry with a long history of success. However, the industry faces numerous challenges while it evolves and adapts to shifting inputs and market demands, such as the dramatic increase in the price of feed ingredients and requests for more consistent fillet color. There is considerable interest in reducing the cost of production diets either by reducing the protein content or by using alternative ingredients such as corn gluten feed. Towards this goal, a pond study was conducted to evaluate the utilization of alternative feed containing 20% corn gluten feed compared to traditional feed without corn gluten feed for the production of channel-blue hybrid catfish. Both alternative and traditional feeds were formulated with two protein contents (28% and 32%). In addition, the xanthophyll contents on the fish fillets were determined. Upon initiation of the pond trial, 600 hybrid catfish fingerlings were stocked in each of 20 0.04-ha ponds, with a mean weight of 34.5g/fish. Fish were fed four experimental diets once daily to apparent satiation over a summer growing season. No significant differences were found in growth performance and feed utilization among the four treatments, including gross and net yield, total amount of feed fed, estimated feed conversion

ratio and survival. No significant differences were found in mean individual fish weight and mean total body length at harvest, relative weight index and dressout (headed and gutted). However, intraperitoneal fat ratio was significantly lower in fish fed the alternative feeds indicating lower energy content of the diet. No significant differences were observed in hematology and immune response indices. Xanthophyll levels in diets and in fillet were linearly related, but without discernible yellow color. These data generally indicate that channel-blue hybrids can efficiently utilize all the four experimental diets with satisfying production result.

1. Introduction

In the United States of America, the channel catfish is a primary aquaculture species and channel-blue hybrid catfish has been adapted by this industry in recent years. In 2010, catfish farmers produced a total of 478,850 pounds of food size catfish (Hanson and Sites, 2011a). As a mature industry, it faces numerous challenges which require modification of production practices and adaptation to new markets. One big challenge is that the price of core ingredients in traditional catfish feed has increased dramatically while the price of fresh whole catfish has remained relatively stable at around $1.6 per pound (Hanson and Sites, 2011b). For example, the price of 48%-protein soybean meal increased from $205.44 in 2006 to $280-310 per ton in 2010 (NASS, 2011a), and the farm price of corn increased from $2.00 per bushel in 2005 to $5.15-5.65 in 2010 (NASS, 2011b). The increasing price of core feed ingredients and the marginal changes of whole catfish price, have led to the decrease of the catfish farmers' profits. In order to minimize feed price increases, low-cost alternative feed ingredients need to be evaluated. Although some studies have indicated that appropriate formulated catfish feeds with alternative ingredients can result in a statistically no different production from traditional feed (Robison et al., 2001; Zhou, 2009), more research is still needed to evaluate feed formulations with alternative feed ingredients.

Corn processing byproducts, such as corn gluten meal and corn gluten feed, are under study to be used as ingredient substitutes due to the lower price and rich nutrient content (Robinson et al., 2001). The use of corn byproducts with high concentrations of yellow pigments as alternative ingredients may reduce costs but they may also lead to increased pigment in the feed and consequently incidences of yellow fillets. Yellow fillets have become a quality control and consumer problem. In the U.S., yellow fillets often appear in catfish produced in the

southeast (Lovell, 1984; Li et al., 2011). Generally, customers expect catfish fillet with good quality to have a white color. Yellow color of the catfish fillet is considered as one criterion of poor quality. Some markets even refuse catfish fillet with visible yellow color resulting in unwanted product that must be disposed of (personal communication). Inconsistencies of fillet color that result in lower consumer acceptance are consequently a concern to the catfish industry.

Carotenoids are the pigments responsible for the yellow color in catfish fillet, and xanthophylls (mainly lutein and zeaxanthin) are the main class of carotenoids existing in catfish fillet (Lee, 1987; Li et al., 2007b; Tsushima, 2002). Carotenoids in catfish fillet are accumulated from dietary source as catfish cannot synthesize xanthophyll (Lovell, 1998). It was suggested that xanthophyll (lutein and zeaxanthin) levels of 11 mg/kg or above in feed would impart yellow color in catfish flesh (Lee, 1987). Corn products are the primary source of xanthophyll in the feed. Yellow corn, corn gluten feed and corn gluten meal are all high xanthophyll content ingredients with xanthophyll levels of 17 mg/kg, 17-30 mg/kg and 145-290 mg/kg, respectively (Moros et al., 2002; NCR, 2011). The use of these ingredients may lead to incidence of yellow fillet. Additionally, under pond conditions, some natural organisms containing high levels of xanthophyll may also contribute to the yellow color in catfish fillet. Though some contributing factors are known, all factors contributing to yellow fillet are still not clear. The influence of catfish feed and natural food on fillet color needs to be further studied.

For this research, the channel-blue hybrid catfish is used as experimental species as it exhibits superior characteristics for many traits (Masser and Dunham, 1998) and has been widely adapted by the industry recently. Though channel-blue hybrid has many advantages, little information is available on its nutrition and levels of xanthophyll in the fillet.

2. Materials and Methods

In the early spring, 600 channel-blue hybrid catfish fingerlings were stocked in each of the 20 0.1-acre (0.04ha) ponds in E.W. Shell Fisheries Center, North Auburn, Alabama. The overall mean weight of the fingerlings was 34.5 g. Fish were fed four experimental diets (Table 1) over a summer growing season from late April to late October, with 5 replicates for each diet. Two of the four experimental diets contained 20% corn gluten feed as a protein and energy source at two levels of protein (28% and 32%). The other two experimental diets used the traditional catfish feed formulae with porcine meat and bone meal, more corn and wheat middlings, at two levels of protein (28% and 32%) as well. Fish were fed once daily in the morning with floating feeds to apparent satiation. Water temperature and dissolved oxygen level were monitored twice daily. Nightly aeration was provided when needed. Water quality was monitored20 at least every two weeks to maintain an optimum water quality for channel catfish (Table 2). Fish from all ponds were harvested at the end of October. The total fish number and yield were recorded. Thirty fish from each pond were randomly collected to obtain individual weights and total body lengths. These fish were then processed to determine dressout (headed and gutted) weight. Ten fish out of the thirty fish were processed to collect the visceral fat weight and liver weight. Another three fish out of the thirty fish were randomly taken for hematology and immune response analyses (Yildirim-Aksoy et al., 2009). Five fish out of the thirty fish were filleted and frozen at -20°C for further pigment analysis (Liu et al., 2012).

Table 1. Composition (percentage, as-fed) of test diets. All diets were formulated to meet or exceed nutrient and energy requirement of channel catfish, and manufactured by a commercial feed mill (Dad's Products Company Inc., Meadville, PA).

	32 % protein		28% protein	
Ingredient	Diet 1 (T)	Diet 2 (A)	Diet 3 (T)	Diet 4 (A)
Meat/bone/blood meal, pork (65%)	5.00	-	5.00	-
Soybean meal (48%)	44.40	46.45	32.60	34.20
Cottonseed meal (41%)	10.00	10.00	10.00	10.00
Corn gluten feed	-	20.00	-	20.00
Corn	27.78	20.10	29.545	20.00
Wheat middlings	10.00	-	20.00	12.37
Lysine-HCl	-	0.13	0.085	0.21
Dicalcium phosphate	0.60	1.10	0.55	1.00
C-free vitamin premix (2 lbs/ton)[a]	0.10	0.10	0.10	0.10
Vitamin C (Stay-C 35)[b]	0.02	0.02	0.02	0.02
Trace mineral premix (2 lbs/ton)[c]	0.10	0.10	0.10	0.10
Poultry fat[d]	2.00	2.00	2.00	2.00
As is basis analysis result				
Fiber (%)	4.337	5.163	4.687	5.560
Protein (%)	33.90	33.10	30.03	29.41
Fat (%)	5.43	5.66	6.81	5.97
Xanthophylls (mg/kg)	3.82	6.23	3.56	5.66

[a]Meet vitamin requirements of channel catfish.
[b]Provide active vitamin C level ≥ 50 ppm in finished diets.
[c]Meet trace mineral requirements of channel catfish.
[d]Sprayed on finished diets.
T: Traditional feed
A: Alternative feed

Table 2. Water quality of ponds where channel-blue hybrid catfish fed four experimental diets were cultured over a summer growing season.

	32 % protein		28% protein	
Water quality parameters	Diet 1 (T)	Diet 2 (A)	Diet 3 (T)	Diet 4 (A)
Morning Temperature (˚C)	24.4	24.3	24.6	24.5
Morning DO mg/L	5.97	6.01	6.08	6.03
Afternoon Temperature (˚C)	26.5	26.4	26.7	26.6
Afternoon DO mg/L	7.46	7.43	7.97	7.66
TAN	0.45	0.51	0.82	0.49
Nitrite	0.08	0.11	0.09	0.11
pH	7.98	8.54	8.61	8.16
Alkalinity	58	39.3	53.8	48.1

T: Traditional feed
A: Alternative feed

Carotenoid analysis

The extraction of carotenoid was modified from the general procedure described by Rodriguez-Amaya (2001). Five fish fillet from each pond was pooled by blended in a Black & Decker Quick N Easy Food processor to produce a homogenous sample. A 5-g sample of the ground fillet was mixed with 25 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole) in a test tube (16×150mm). The mixture was homogenized by a Brinkmann Instruments homogenizer and then filtered under light vacuum through 1.0 µm filter paper into another test tube. The test tube with remaining tissue and extract on the filter paper were washed three times with 5 ml acetone. Samples were then mixed with a secondary organic solvent for preliminary purification and concentration. For this, 10 ml hexane/ethyl ether mixture (1:1, v/v) was gently added into the test tube, followed by 20 ml distilled H_2O. The mixture was allowed to stand for 15 min to allow separation. The upper layer (containing pigments) was then removed using a Pasteur pipette and transferred to a separate test tube. Finally, the pigments were concentrated by evaporating the solvents under nitrogen flow. The collecting test tube containing extracted pigment samples was sealed and stored in a freezer for subsequent HPLC (High-performance Liquid Chromatography) analysis. A gradient elution program was developed for analysis of xanthophyll in Shimadzu LC-20 HPLC system (Moros et al., 2002).

Statistics

The collected data was analyzed for statistical differences using SAS 9.1(Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2008). Data was subjected to two-way analysis of variance (ANOVA) with protein level and feed type as main effects, followed by the Student–Neuman–Keuls multiple comparison test to determine if significance ($P < 0.05$) differences

existed between means (Montgomery, 1997). Correlation and regression analyses between xanthophyll levels in diets and in catfish fillet were conducted using the SAS regression module (Zar, 1999).

3. Results

In this study, three out of the twenty ponds were excluded from the study due to aeration failure or disease (ichthyobodo and/or trichophrya) related mortality, which was not related to nutrition status of the fish. The three ponds lost were under treatments 1, 2 and 4, respectively. Data from the other seventeen ponds were reported. No significant differences were found in growth performance and feed utilization among the four treatments, including survival, gross and net yield, total feed input and estimated feed conversion ratio (Table 3). Across all four treatments, the relative weight indices of hybrid catfish were above 100. No significant differences were observed in mean fish weight and mean total body length at harvest, relative weight index and dressout (Table 3).

There were no significant differences in hematology and immune response indices among the four treatments (Table 4 and 5). The fish fed alternative diets with 20% corn gluten feed had a significant higher level of yellow pigments in fillet, with regard to lutein, zeaxanthin and total xanthophyll (Table 6). There is a moderate linear relationship between xanthophyll levels in diets and those in fillet (Fig.1). The equation is $y = 10.62x + 6.61$, where x stands for xanthophyll content (ppm) in feed and y stands for xanthophyll content (ng/g) in catfish fillet, with an R-square value of 0.53.

Table 3. Growth performance and processing results of channel-blue hybrid catfish fed four experimental diets over a summer growing season in outdoor ponds. Test diets contained 28% or 32% protein, with or without 20% corn gluten feed included in the diet.

		32% protein		28% protein			P-values		
Parameter	Unit	T	A	T	A	PSE	Protein	Feed	Interaction
Survival	%	85.9	83.6	81.3	87.2	4.9	0.93	0.71	0.41
Gross yield	kg/ha	7680	8380.5	8378	8052	876.7	0.83	0.83	0.56
Net yield	kg/ha	7169	7860	7873	7543	874.8	0.82	0.84	0.56
Total feed fed	kg/ha	11189	12254	11073	11396	928	0.61	0.47	0.70
FCR		1.7	1.6	1.4	1.5	0.22	0.35	0.92	0.75
IFW	kg/fish	0.67	0.65	0.70	0.62	0.076	0.66	0.92	0.19
TL	cm	40.8	40.7	40.9	39.6	0.52	0.34	0.22	0.25
IPF ratio	%	4.81	4.10	4.86	4.15	0.17	0.79	<0.01	0.98
HIS	%	1.54	1.41	1.87	1.73	0.08	< 0.01	0.12	0.97
Dressout	%	67.0	67.8	68.8	67.9	1.08	0.40	0.94	0.44
Wr	%	103.1	100.4	104.9	104.4	2.02	0.18	0.44	0.58

FCR (Feed conversion ratio) = Total feed fed / net yield
PSE: Pooled standard Error = $\sqrt{MSE/n}$
IFW (individual fish weight): mean weight of the 30 fish taken out of each pond
TL: Total body length
IPF ratio (Intraperitoneal Fat ratio) = weight of visceral fat * 100 / fish weight
HSI (Hepatosomatic Index) = liver weight * 100 / fish weight
Dressout = headed and gutted fish weight / total fish weight
Wr (relative weight index) = (Fish weight in grams / Ws) x 100 (Anderson and Neumann, 1996), where the equation for Ws in channel catfish is: log10 (Ws) = -5.800 + 3.294 (log10 TL) (Brown et al., 1995)

Table 4. Hematology results of channel-blue hybrid catfish harvested after a summer growing season, fed with four experimental diets containing 28% or 32% levels of protein, with or without 20% corn gluten feed included in the diet.

		32% protein		28%		
Parameter	Unit	T	A	T	A	P-value
Total cell count	10^6/µL	3.17	2.75	2.57	2.80	0.0542
Red blood cell	10^6/µL	2.95	2.59	2.43	2.69	0.0887
White blood cell	10^5/µL	2.02	1.58	1.38	1.38	0.0728
Hemoglobin	g/dL	9.49	8.90	9.68	9.34	0.5624
Hematocrit	%	38.13	37.14	36.83	40.71	0.2146
Mean corpuscular volume	ft	134.30	145.83	155.60	153.38	0.1777
Mean corpuscular hemoglobin	pg	33.50	34.97	41.13	35.32	0.1268
MCHC	%	24.93	23.97	26.44	23.03	0.2706

MCHC: Mean corpuscular hemoglobin concentration

Table 5. Immune response of channel-blue hybrid catfish harvested after a summer growing season, fed with four experimental diets containing 28% or 32% levels of protein, with or without 20% corn gluten feed included in the diet.

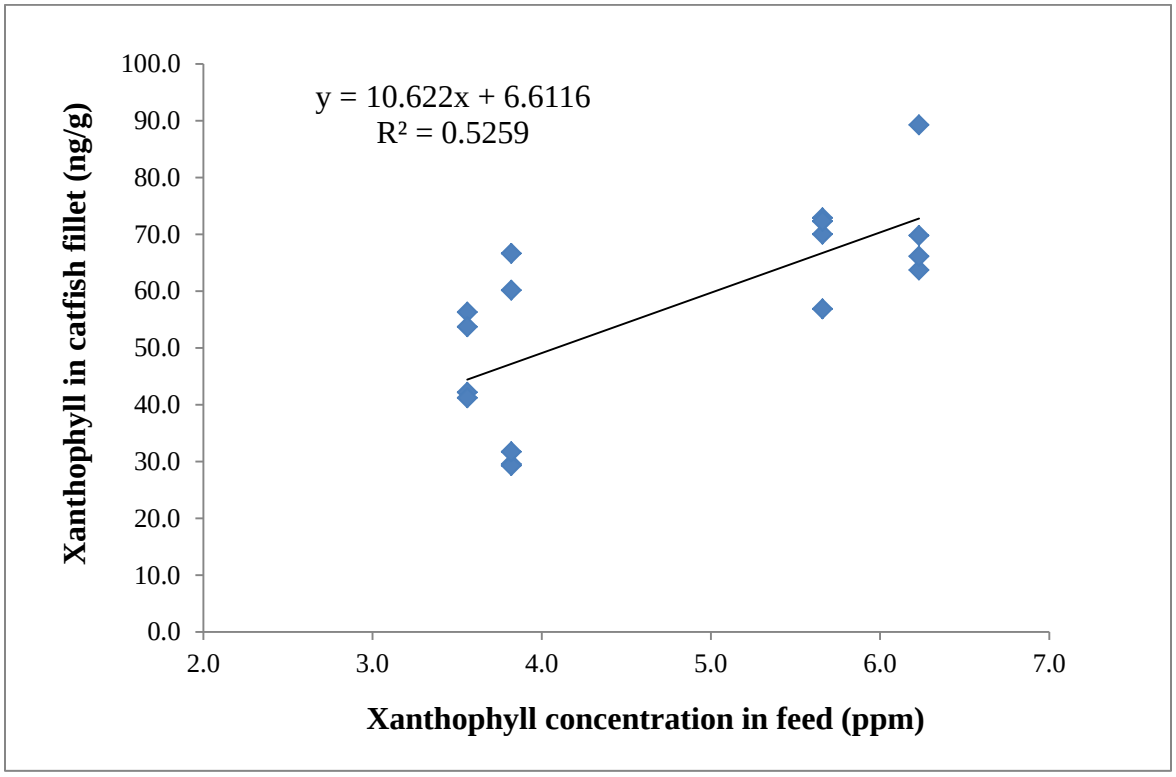
		32%		28% protein		
Parameter	Unit	T	A	T	A	P-value
Lysozyme activity	µg/mL	7.13	6.47	5.99	6.93	0.7791
Alternative complement activity	unit/mL	185.83	94.99	162.60	130.53	0.5121
Serum protein	mg/mL	62.35	67.59	55.10	61.02	0.4207
Total immunoglobulin	mg/mL	14.63	12.27	15.81	14.38	0.9093

Table 6. Xanthophyll content of channel-blue hybrid catfish fillets harvested after a summer growing season, fed with four experimental diets containing 28% or 32% levels of protein, with or without 20% corn gluten feed included in the diet.

		32% protein		28% protein			*P*-values		
Parameter	Unit	T	A	T	A	PSE	Protein	Feed	Interaction
Lutein	ng/g	28.4	48.8	32.2	43.8	2.3	0.89	0.01	0.36
Zeaxanthin	ng/g	15.1	23.4	16.2	24.2	1.0	0.62	<0.01	0.93
Total xanthophyll[a]	ng/g	43.5	72.2	48.3	68.1	3.1	0.95	<0.01	0.48

[a]Total xanthophyll = lutein + zeaxanthin

Figure 1. Relationship between xanthophyll concentrations in four experimental diets and the fillet from channel-blue hybrid catfish harvested after a summer growing season. The four experimental diets containing 28% or 32% protein, with or without 20% corn gluten feed included in the diet.



4. Discussion

Compared to the other reports regarding channel-blue hybrid catfish, the indices of production in this study are satisfying. Green and Rawles (2010) reported that hybrid catfish fed full ration of 32% protein had a FCR of 1.52. The FCR in this study ranging from 1.4 to 1.7 was comparable with Green's data. Argue et al. (2003) reported that hybrid catfish had a skinless dressout of 61.1% while in the present study the dressout with skin was higher, ranging from 67.0% to 68.8%. Relative weight indices across the four treatments were all above 100. These results indicate that the channel-blue hybrid catfish from the present study had a heavier weight compared with the average weight of channel catfish in the same length which is typical for cultured fish. There was no significant difference on any of the production parameters, indicating that 28% protein level and 20% corn gluten feed were applicable in hybrid catfish feed.

The production data generally indicate that channel-blue hybrids can efficiently utilize all the four experimental diets, regarding any combination of the two protein levels and two feed types. This result is supported by studies with hybrid catfish and channel catfish. Li and Lovell (1992) reported that a dietary protein concentration of 26% was adequate for optimum weight gain when channel catfish were fed as much as they would consume. Li and Robinson (2007) reported that dietary protein levels ranging from 28% to 36% did not affect feed consumption, weight gain, or FCR of channel-blue hybrid catfish fed to apparent satiation. Another pond study conducted on channel catfish indicated that channel catfish can efficiently utilize corn gluten feed up to 50% without adverse effect on feed palatability, weight gain or feed efficiency (Robison et al., 2001). It has also been reported that using 30% corn gluten feed with 10% cottonseed meal in diets would not cause significant difference in production for channel-blue hybrid catfish under pond conditions. However, the dressout of hybrid catfish would reduce if

corn gluten feed content is reduced and cottonseed meal content is increased to 20% or above (Li et al., 2012). In other species, it has been reported that an incorporation of up to 19% corn gluten feed would not result in production differences for cage-raised Nile tilapia (Wu et al., 1995).

Intraperitoneal fat ratio and hepatosomatic index are both indicators of the status of energy reserve in fish. Levels of intraperitoneal fat were significantly lower in fish fed the alternative feeds indicating that these diets contained a lower level of available energy. This corresponds with the lower digestible energy in alternative feeds due to high crude fiber content associated with corn gluten feed (Kitagima and Fracalossi 2011). Similar results have been reported in channel catfish that fish fed diets substituted by corn gluten feed had a lower intraperitoneal fat compared with those fed diets without corn gluten feed (Robison et al., 2001). Hepatosomatic index was higher in fish fed 28% protein feeds. The higher hepatosomatic index was associated with higher fat content in 28% protein feeds (Table.1). It was supported by research in hybrid bass (Nematipour et al., 1992), yellowtail (Shimeno et al., 1980), Atlantic cod (Grisdale-Helland et al., 2008) and red drum (Serrano et al., 1992) that hepatosomatic index increased with increased dietary lipid level. The higher energy to protein ratio in 28% protein feeds could cause more lipid accumulation in fish liver, which could also result in the higher hepatosomatic index in fish fed 28% protein feeds (Nematipour et al., 1992; Jantrarotai et al., 1998).

Hematological characteristics and immune response are closely related to the physiological condition of fish. In this study, all the indices tested on the four groups of hybrid catfish were not statistically different. Those data are comparable with data on channel catfish that have been reported (Yildirim-Aksoy et al., 2009; Klinger et al., 1996; Yildirim et al., 2003).

This indicates that the four diets can provide hybrid catfish similar nutrition that can satisfy its healthy growth.

Fillet color is an important parameter in food quality and xanthophyll are the pigments responsible for the unwanted yellow color in catfish fillet. These xanthophyll levels in fish fillet were moderate linearly correlated with those in the feeds (Fig.1). That was probably because that hybrid catfish consumed very little natural organisms as they were fed daily to satiation, or because the composition of xanthophyll in natural organisms consumed by catfish was similar to that in the feed. Lee (1987) reported that above 0.6 μg carotenoid/g of flesh would probably cause discernible yellow color in channel catfish fillet. None of the four groups of hybrid catfish fillet was considered as having problematic yellow color based on Lee's report. Several attendees' observation supported the conclusion that there was no incidence of yellow fillet from the cultured ponds. This result corresponded with multiple studies on channel catfish. Lovell (1998) demonstrated that catfish would have distinguished undesirable yellow color in fillet by feeding diets with a xanthophyll level of 11 mg/kg or more. None of the four xanthophyll concentrations in diets used in the present study exceeded 11mg/kg, which should not result in yellow fillet based on Lovell's recommendation. Robinson et al. (2001) also reported that diets containing up to 50% corn gluten feed didn't result in visible differences from those without corn gluten feed. All the four experimental diets should provide a satisfying production without causing yellow fillet problem.

In summary, all the four experimental diets can be efficiently utilized by channel-blue catfish resulting in satisfying production with analogous hematology and immune response and acceptable fillet color. More research needs to be done to evaluate different productive feeds and to learn more about the yellow coloration of catfish fillet.

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CHAPTER III

A COLOR STANDARD DEVELOPED FOR THE CATFISH INDUSTRY

Abstract

Catfish fillets with yellow coloration has shown up frequently and become a problem for the catfish industry. This problem is due to the unacceptability in the market because the shift in fillet color is considered of lower quality by the consumer. To help the catfish industry better understand the yellow coloration of catfish fillet, a digital photography measurement method was developed to evaluate the yellowness. Sixty catfish fillets were taken directly from the processing line. The fillets were photographed in a light box with a digital camera. The photos were calibrated with the X-Rite ColorChecker standardized color target. CIE LAB readings of the fillet photos were recorded and B value was used to indicate the yellowness of catfish fillets. Xanthophyll level of fillets was analyzed with High-Performance Liquid Chromatography. The actual xanthophyll level in catfish fillet was calculated as the sum of lutein, zeaxanthin and alloxanthin. A linear correlation was found between the LAB B values and xanthophyll levels of the sixty catfish fillets.

1. Introduction

Color, as one of the primary impressions to people, plays an important role in food quality. It is considered one of the most important sensory attribute of food because it can affect consumer judgment of other sensory and nonsensory characteristics (Clydesdale, 1991). Color is considered an important parameter of food quality in many food industries, because it can influence the acceptability and price of food to customers. In the seafood industry, skin and fillet color of fish together with texture, flavor and odor are used as sensory attributes dictating value in the market (Hardy and Lee, 2010). For example, consumers were willing to pay significantly more for salmon fillets with normal or above-normal redness, compared with paler salmon fillets in an experimental market of Atlantic salmon (Alfnes et al., 2006). In the catfish industry, ivory to lightly pink fillet are considered high quality and preferred by customers. Yellow coloration in catfish flesh is generally considered a color defect. The yellow color is perceived as different and consequently undesirable. Some markets even refuse catfish fillets with visible yellow color resulting in unwanted product that must be disposed of. Due to the unacceptability to customers, the yellow coloration has become the center of quality concern for the catfish industry.

The prominent role of color in consumer decisions has resulted in the food industry putting considerable effort into controlling and standardizing the color of their products. Color standard for food grading has been developed in many industries. The salmon industry is a mature industry with well-developed grades for both flesh color and skin color. The Roche SalmoFan^{TM} chart is a widely used color standard for salmon industry. However, the catfish industry, which is the leading aquaculture fish industry in the U.S., has not yet to adopt a color standard. To help address the yellow fillet problem and normalize the catfish industry in terms of product color, a color standard needs to be developed. To facilitate automated sorting or grading

it would be best if the system could utilize digital color codes. Towards this end, a photo-based color standard using CIE (1976) LAB color system to evaluate catfish fillet was developed by Cline (2011) and has been proposed as a tool to automate color sorting of fillets.

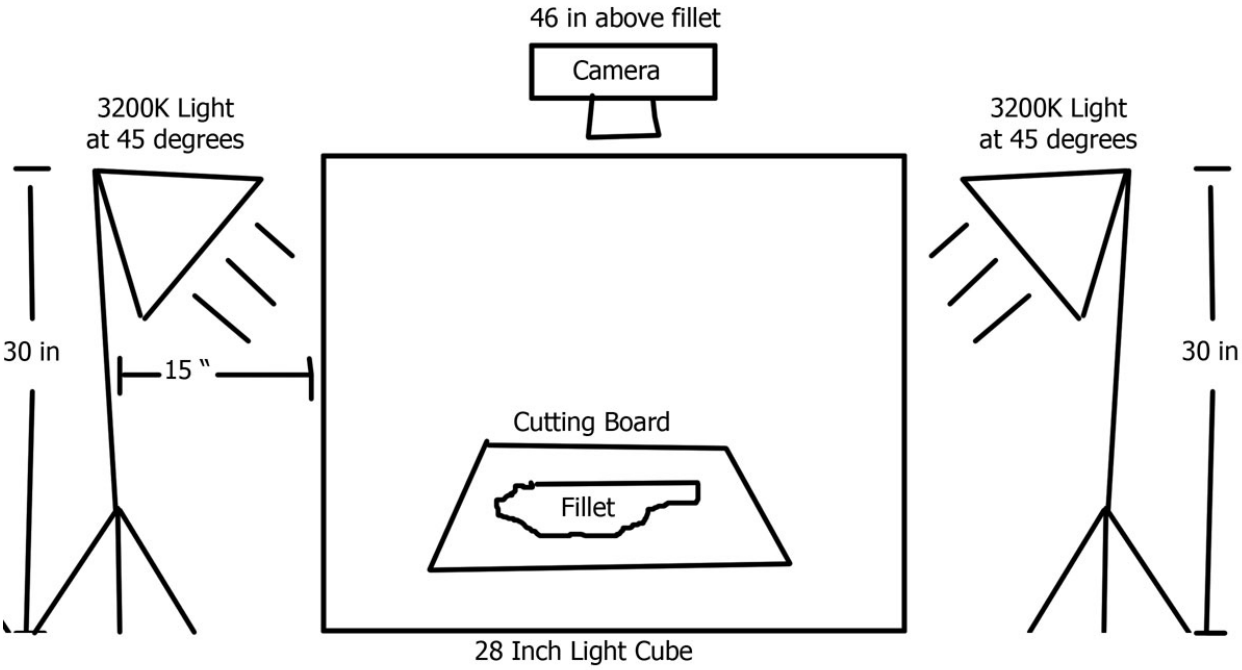
The principle pigments responsible for the quality-related color in seafood include carotenoids, heme proteins and melanins (Bremner, 2002). Carotenoids are the pigments responsible for the yellow color in catfish fillet, and xanthophyll is the main class of carotenoids found in catfish fillet (Lee, 1987; Tsushima, 2002; Li et al., 2007). To better understand the yellow coloration in catfish fillet, a correlation between yellowness in color standard and xanthophyll contents needs to be developed.

2. Materials and Methods

Measurement of color

Sixty catfish fillets were taken directly from the processing line. The measurement of catfish fillet color employed a method developed by Cline (2011) with CIE LAB color space (International Commission on Illumination, 1976). Catfish fillets were placed on a uniform white board in a light box. The light box was made of white translucent material and lit with 3200-K video spotlights on two sides (Fig. 1). White foam core boards were placed against the other two sides and underneath of the light box. Photographs of the fillets were taken by a Canon 40D digital camera in the raw image format. In Adobe Photoshop, the white balance of all the photographs was calibrated with X-Rite ColorChecker Passport software (X-Rite Inc., 2010).

Figure 1. Light cube setting for photograph of catfish fillets.



The fillet was isolated from the background and blurred to get an average color of the whole fillet. The CIE LAB values of the fillets in Adobe Photoshop were recorded, where L represented lightness, A redness and B yellowness (Fig. 2). The B value was used to define the yellowness of fillets.

Xanthophyll analysis

The extraction and analysis of xanthophyll was modified from a method developed for channel catfish by Liu et al. (2012) as well as the general procedure described by Rodriguez-Amaya (2001). Individual catfish fillets were ground in a Black & Decker Quick-N-Easy Food Processor to produce a homogenous sample. A 5-g sample of the ground fillet was mixed with 25 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole) in a test tube (16×150 mm). The mixture was homogenized by Brinkmann Polytron Homogenizer PT10/35 (Brinkmann Instruments, Westbury, N.Y.) and then filtered under light vacuum through 1.0 µm filter paper into another test tube. The test tube with remaining tissue and extract on the filter paper were washed three times with 5 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole) each. To get a preliminary purified carotenoid sample, 10 ml hexane/ethyl ether mixture (1:1, v/v) was gently added into the test tube with filtered samples, followed by 20 ml distilled H_2O. The mixture was allowed to stand for 15 minutes to allow separation. The upper layer containing pigments was then removed using a Pasteur pipette and transferred to a separate test tube. Finally, the pigments were concentrated by evaporating the solvents under nitrogen flow. The collecting test tube containing extracted pigment samples were sealed and stored in a freezer for subsequent HPLC analysis. A gradient elution program was used for analysis of xanthophyll in Shimadzu LC-20 HPLC system (Moros et al., 2002; Liu et al., 2012). The identification of carotenoids was

confirmed by comparing their mass measurement and HPLC retention time to those of standard compounds (Liu et al., 2012). The total xanthophyll level in catfish fillet was calculated as the sum of lutein, zeaxanthin and alloxanthin.

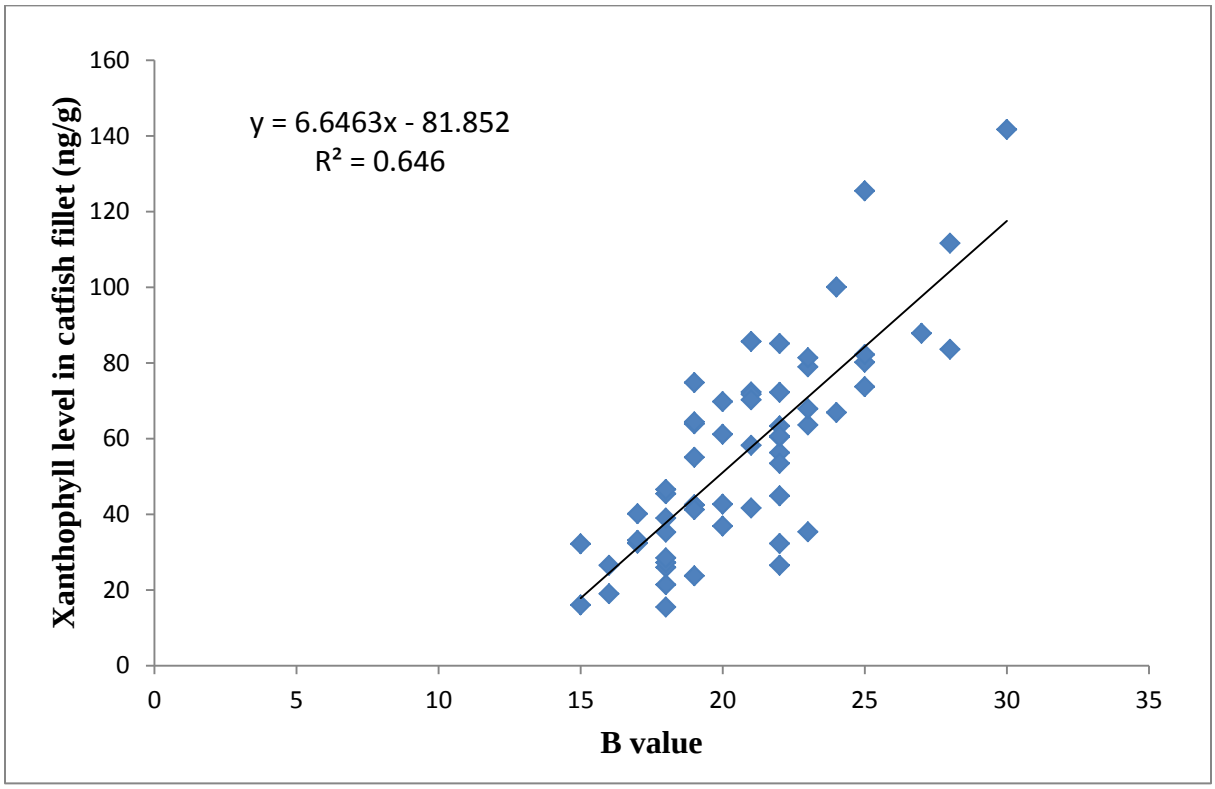
Statistics

A correlation between the CIE LAB B value and actual xanthophyll level was established. Correlation and regression analysis between B values and xanthophyll levels in catfish fillets was conducted using regression module (Zar, 1999) in SAS 9.1(Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2008).

3. Results

Lutein was found as the major xanthophyll in catfish fillet, followed by zeaxanthin and alloxanthin. The composition of the three xanthophylls varied in catfish. Lutein contributed 35-82% of the total xanthophyll, zeaxanthin 16-60% and alloxanthin 0-31%. No other carotenoid was identified in catfish fillet. Total xanthophyll concentration in catfish fillet varied from 16 to 142 ng/g. The CIE LAB B values of catfish fillets ranged from 15 to 30. The correlation between B values and xanthophyll levels in the sixty catfish fillets was significant (Fig. 3). The linear regression equation between B values and xanthophyll levels in catfish fillets was $y = 6.65x – 81.85$ with an R-square value of 0.646, where x standed for B values and y standed for xanthophyll concentrations in catfish fillets (ng/g).

Figure 2. Relationship between CIE LAB B values and xanthophyll levels of sixty catfish fillets taken directly from processing line.



4. Discussion

Variation in catfish fillet color resulting in consumer dissatisfaction warrants the development of ways to produce a more uniform product. The development of color standards would provide a tool for sorting by color resulting in a more uniform color. Towards this goal, CIE (1976) LAB color space was chosen to give a measurement of the yellow coloration in catfish fillet. CIE LAB is the most complete color space to describe all the perceivable colors by human eyes, whose gamut is wider than RGB or CMYK color spaces. In addition, positive B value in CIE LAB indicates yellowness which directly describes the yellow coloration of catfish fillet. This should be convenient for the development of automated sorting technologies as CIE LAB B value can be used directly without any conversion.

The salmon industry has well-established color standards, which can be used as reference for the color standard development in the catfish industry. Roche SalmoFan^{TM} color standard is widely used in the salmon industry to evaluate color of salmon fillet by physically comparing the color standard with salmon fillets. This color evaluation method is simple and low-cost, but it needs human senses to select the best-match color grades. It yields various results because of different sensitivities of people, and can't provide an unbiased uniform color standard to the industry. In addition to the Roche color standard, colorimeters are also used as a traditional method to measure color (Sarkar, 1991). For example, CR-200 Chroma Meter (Minolta, Japan) is a common used colorimeter in food industry. Colorimeter measurement is quick and accurate, but it does not work well when the surface of food is not uniform. This is because colorimeters use a small probe window to catch the sample color, which is much smaller than catfish fillet. To get the fillet color, several locations need to be chosen to get the colorimeter readings, and the average reading will be determined as the fillet color. However, the yellow coloration is not

evenly distributed within the catfish fillet, but concentrated along the front and dorsal part of the fillet (Li et al., 2011). The result would vary with different choices of locations. Thus, the use of colorimeters cannot provide a uniform color standard to the catfish industry, either. Additionally, one must consider increased labor costs and possible product contamination due to additional handling making these methods problematic. Instead of human sorting and colorimeters, computer vision method with digital camera has been developed to evaluate the color of food product. In salmon industry, a computer vision system with digital camera was evaluated as a powerful tool to sort salmon fillets by color in a fast and nondestructive manner (Misimi et al., 2007). In a study on color of sturgeon fillets, it was concluded that computer vision could outperform other colorimeters when recording and estimating color changes in foods (Oliveira and Balaban, 2006). Though computer vision has many advantages, human vision is the final arbiter of color in the market. It has been reported that there were no significant difference between computer vision and human visual evaluation of Atlantic salmon fillet color (Misimi et al., 2007) indicating automated systems are viable.

In the present study, a computer vision-based color standard was developed to evaluate the color of catfish fillet. A digital camera using a specific configuration and standardized color palette was used to photograph catfish fillets. This method removed the subjectivity of human vision and provided an objective standardized method of color analysis. The color of objects is affected by light source whose color is determined by its temperature. In the present study, standard color calibration by X-Rite ColorChecker passport provided consistent color rendition under various light conditions and surroundings. Foam cores placed around the light box could also help prevent alteration of fillet colors due to the reflection of surroundings. The reading of color was given after “blurring” the whole fillets in Adobe Photoshop, which ensured the reading

a uniform average value of the whole fillet. Overall, the setting of this method made it consistent and repeatable.

Because standardized configuration is difficult to achieve, special concerns need to be addressed to help maintain the color standard: (1) Sheets of white foam core need to be placed under and around the light cube to avoid color contamination from nearby objectives. (2) The photos of ColorChecker standardized color target and all the fillets need to be taken under the same lighting conditions. (3) Fillet photos should be taken in the raw image format because this format maintains the most color information in the scene.

The yellow coloration in catfish flesh is due to a group of pigments called xanthophylls. Previous studies have recognized lutein and zeaxanthin as the two major xanthophylls in channel catfish fillets (Lee, 1987; Li et al., 2007). Li et al. (2007) demonstrated the visual yellow color intensity was highest for fish fed lutein, followed by zeaxanthin in all the 5 xanthophylls tested. Maoka and Akimoto (2011) identified alloxanthin in skin, fins, and gonads of the Japanese common catfish (*Silurus asotus*), which belongs to a different family from the channel catfish. Liu et al. (2012) identified alloxanthin as a primary xanthophyll in channel catfish fillet for the first time. In the present study, alloxanthin was also identified in catfish fillet, together with lutein and zeaxanthin. Alloxanthin contributed up to 31.5% of the total xanthophyll in the 60 catfish fillets and could be recognized as one principal xanthophyll in the fillets of channel catfish. The correlation between B values and xanthophyll as a sum of lutein and zeaxanthin (R-square = 0.60) was a little weaker than that between B values and xanthophyll as a sum of all the three pigments (R-square = 0.65). This revealed that using a sum of lutein, zeaxanthin and alloxanthin as the total xanthophyll provided a better indication of the yellowness of catfish fillet. There was a positive linear relationship between xanthophyll levels and CIE LAB B values

of the catfish fillets. This result is associated with the fact that all three main xanthophylls in catfish fillet are yellow in color. This linear equation (Fig. 2) could be used to quickly estimate the xanthophyll levels based on the B values.

The development of this color standard is a small step in the quality standardization for the catfish industry. It provides a means to sort fillets into different categories for different markets. Furthermore, this color standard can be used for the establishment of catfish fillet grades based on consumer preference, and helps with the development of color management practice which can ensure the product quality.

In summary, a consistent and unbiased color standard was developed in this study. A specific photograph configuration and color calibration software were used to warrant the accuracy of this color standard. A correlation was built up between the B value and the xanthophyll content in catfish flesh which could be used for quick estimation of the xanthophyll content.

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CHAPTER IV

DEPOSITION AND DISSIPATION OF XANTHOPHYLL IN CHANNEL CATFISH FILLET

Abstract

Variations in the color of catfish fillets have always been a problem. However, more recently it has become the center of attention as consumers are requesting a more consistently colored product. Finding a way to address, prevent or solve the yellow fillet problem has become an important issue for the industry. To help us better understand the yellow color problem in catfish, an indoor aquarium trial was conducted to evaluate the deposition and dissipation rate of xanthophyll in channel catfish fillet under various dietary treatments. Channel catfish with initial weight of 22.3g were stocked into 21 aquariums and fed twice daily based on a set percentage of body weight over a 16 week experiment. Four diets with increasing xanthophyll content were used in this study to produce 7 treatments. Treatments 1 to 4 were designed to evaluate the deposition of xanthophyll at different dietary levels so each diet was offered through the 16 weeks culture period. The concentration of xanthophyll in fish flesh increased rapidly at first and then trended to level off depending on the dietary xanthophyll concentration. Xanthophyll levels in fish fillets and in diets were linearly correlated after 16 weeks of culture. Treatment 5, 6 and 7 were fed the highest xanthophyll (diet 4) for the first few weeks and then reassigned low-

xanthophyll diets to observe the dissipation rate. Treatment 5 and 6 were switched to diet 1 and diet 2 after 8 weeks, respectively. Treatment 7 was switched to diet 2 after 12 weeks. Xanthophyll levels decreased linearly over the 8 weeks. No significant difference was found in the dilution rate between treatment 5 and 6. Fish with higher initial xanthophyll level appeared to have a faster reduction in xanthophyll levels. Based on these results it appears that deposition of xanthophyll is fast and dependent on the level of intake, while dissipation was quick with those started at a high xanthophyll level having the fastest clearance. From a practical standpoint, yellow coloration can be cleared if the fish are placed on a low xanthophyll feed prior to harvest.

1. Introduction

Channel catfish (*Ictalurus punctatus*), is a white-fillet fish which is one of the top seafood consumed in the U.S. (Hanson and Sites, 2012). Variations in catfish fillet color have always been a problem but typically did not result in consumer disapproval. More recently, yellow coloration has become a concern as it has led to price-reductions or even refusal by some markets. Quite often consumers associate the shift in color with poor quality or perceived problems and do not want to purchases off-color fillets. Thus, yellow catfish fillet has become the center of attention as consumers are requesting a more consistently colored product. Finding a way to address, prevent or solve the yellow fillet problem has become an important issue for catfish industry.

Carotenoids are the pigments that are responsible for the yellow coloration in catfish fillet (Lovell, 1998). Carotenoids can be divided into two classes: xanthophyll and carotene. Xanthophyll contains oxygen while carotene does not. β-carotene, is the most widely distributed carotene. It is not deposited in catfish flesh or skin but slight depositions can be found in the liver and abdominal fat (Lee, 1987). Xanthophyll is the carotenoid class that makes up the primary deposits in catfish flesh. Traditionally, lutein and zeaxanthin are considered as two major xanthophylls found in catfish flesh and responsible for the yellow coloration (Lee, 1987; Li et al., 2007). Liu et al. (2012) identified alloxanthin as another principal xanthophyll in catfish fillet.

Carotenoids are found to have various functions. Carotenoids, especially xanthophylls, can serve as antioxidants. Carotenoids can also enhance general performance as well as specific functions in reproduction and metabolism of fish (Torrissen and Christiansen, 1994). The color of carotenoids can assist in communication among species. Lutein and zeaxanthin are the only

two carotenoids accumulated in the tissue of the eye and serve a variety of roles as the macular pigments of the retina (Stringham and Hammond, 2005). Carotenoids are potential precursors of vitamin A, but the ability of converting carotenoids to vitamin A varies in different species. Rainbow trout (*Oncorhynchus mykiss*) can convert zeaxanthin into vitamin A_1 and A_2 (Schiedt et al., 1985). Several freshwater species can convert lutein to vitamin A, such as *Saccobranchus fossilis* (Barua et al., 1973), *Clarias batrachys* (Goswami and Bhattacharjee, 1982), and goldfish, *Carassius auratus* (Benjamin and Tito, 1983). Nile tilapia (*Oreochromis niloticus*) can convert both lutein and zeaxanthin into vitamin A_2 alcohol (Katsuyama and Matsuno, 1988). However, channel catfish do not convert either to vitamin A (Lee, 1987). Conversions among carotenoids have also been observed in some species. It was reported that zeaxanthin and possibly lutein were the metabolites of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (Schiedt et al., 1985). The conversion of zeaxanthin to astaxanthin has been reported for goldfish and common carp (Hata and Hata, 1972). Although there are some discussions about the function and conversion of carotenoids, the mechanism of absorption and metabolism of carotenoids, such as lutein, zeaxanthin and alloxanthin, has not yet been clarified.

Carotenoid must come from food source as they are not synthesized by catfish. Catfish feed and natural prey items are the two contributing factors for the yellow fillet. Commercial feed with more than 7-11 mg/kg carotenoid will impart yellow fillet (Lee, 1987; Li et al., 2009; Lovell, 1998). Various feed ingredients containing high levels of carotenoid can contribute to yellow catfish fillet. Carotenoids contents in whole corn, corn gluten feed, corn gluten meal and distillers dried grains solubles are about 20 mg/kg (Moros et al., 2002), 17-30 mg/kg (Lovell, 1989; Robinson et al., 2001b), 145-350 mg/kg (Lovell, 1989; Moros et al., 2002) and 4-34 mg/kg (NRC 2011), respectively. The combination of high-carotenoid feed ingredients needs to be

controlled to keep carotenoid concentration under the threshold (7-11 mg/kg) to avoid incidence of yellow catfish fillet.

Presently, there is limited information regarding how fast the color develops or dissipates from tissues. To better understand this process, an indoor aquarium trial was conducted to quantify the deposition and dissipation of xanthophyll in channel catfish fillet under various dietary treatments.

2. Materials and Methods

This experiment consisted of 7 treatments, with 3 replicates. Treatments 1 to 4 were designed to look at the deposition of xanthophyll in catfish fillet, and treatments 5 to 7 were designed to examine the dissipation of xanthophyll. Four experimental diets (Table 1) were formulated to contain different levels of naturally occurring xanthophyll (lutein plus zeaxanthin) by controlling the percent of yellow corn and corn gluten meal (high xanthophyll ingredients) with white corn and wheat gluten (low xanthophyll ingredients). Diets were manufactured by extruding at Auburn University, Auburn, AL, USA. The analyzed xanthophyll contents in diet 1, 2, 3 and 4 were 4.1, 9.0, 15.5 and 34.2 ppm, respectively. For catfish under treatment 1, 2, 3 and 4, each of the treatment was fed one of the four diets for 16 weeks to get the corresponding deposition rates. Treatment 5, 6 and 7 were fed highest xanthophyll diet 4 for the first few weeks and then reassigned low-xanthophyll diets to observe the dissipation rate. Treatment 5 and 6 were switched to diet 1 and diet 2 after 8 weeks, respectively. Treatment 7 was switched to diet 2 after 12 weeks (Table 2).

Table 1. Composition of experimental diets (%, as-fed). All diets were formulated to contain 32% protein and 6% lipid, and designed to meet or exceed nutrient and energy requirement of channel catfish.

	Diet 1	Diet 2	Diet 3	Diet 4
Menhaden fish meal[a]	4.00	4.00	4.00	4.00
Soybean meal[b]	28.00	28.00	28.00	28.00
Fish oil[a]	3.20	3.20	3.20	3.20
Corn gluten meal[c]	2.00	4.00	8.00	16.00
Wheat gluten[d]	13.25	11.62	8.35	1.81
Corn starch[d]	4.45	4.08	3.35	1.89
Wheat middlings[e]	10.00	10.00	10.00	10.00
Yellow corn[f]	4.00	8.00	16.00	32.00
White corn[g]	28.00	24.00	16.00	0.00
Catfish mineral premix[h]	0.50	0.50	0.50	0.50
Catfish Vitamin premix[i]	1.00	1.00	1.00	1.00
Stay C[j]	0.06	0.06	0.06	0.06
Dicalcium phosphate[d]	1.50	1.50	1.50	1.50
Choline[d]	0.04	0.04	0.04	0.04
Proximate composition				
Xanthophyll[k] (mg/kg)	4.08	8.99	15.47	34.22

[a]Omega Protein Inc., Reedville, Virginia, USA.
[b]De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.
[c]Grain Processing Corporation, Muscatine, IA, USA.
[d]MP Biomedicals Inc., Solon, Ohio, USA
[e]Alabama Catfish Feed Mill Llc., Uniontown, AL, USA
[f]Faithway Feed Co. Llc., Guntersville, AL, USA.
[g]Hybrid seed corn, DKC63-45, Dekalb brand, Monsanto Company, St. Louis, MO, USA.
[h]Catfish mineral premix (g/kg premix): cobalt chloride 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate 40.00; magnesium sulfate anhydrous, 138.62; manganese sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; alpha-cellulose, 679.64.
[i]Catfish vitamin premix (g/kg premix): thiamin.HCL, 0.44; riboflavin, 0.63; pyridoxine hydrochloride, 0.91; D-pantothenic acid, 1.72; nicotinic acid, 4.58; biotin, 0.21; folic acid, 0.55;

inositol, 21.05; menadione sodium bisulfite, 0.89; vitamin A acetate, 0.68; vitamin D3, 0.12; dL-alpha-tocoperol acetate, 12.63; alpha-cellulose, 955.59.

[j]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[k]Xanthophyll content was analyzed by High-Performance Liquid Chromatography following the procedure described in the present study.

Table 2. Feeding strategy of the indoor aquarium experiments for channel catfish.

Week\treatment	Trt1	Trt2	Trt3	Trt4	Trt5	Trt6	Trt7
week1-4	Diet1	Diet 2	Diet 3	Diet 4	Diet4	Diet 4	Diet 4
week5-8	Diet1	Diet 2	Diet 3	Diet 4	Diet 4	Diet 4	Diet 4
week9-12	Diet1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 4
week13-16	Diet1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 2

This experiment consisted of 7 treatments, with 3 replicates. Treatments 1 to 4 were designed to evaluate the deposition of xanthophyll in catfish fillet, and treatments 5 to 7 were designed to examine the dissipation of xanthophyll. Four experimental diets (Table 1) were formulated to contain different levels of naturally occurring xanthophyll (lutein plus zeaxanthin) by controlling the percent of yellow corn and corn gluten meal (high xanthophyll ingredients) with white corn and wheat gluten (low xanthophyll ingredients). Diets were manufactured by extruding with boiling water at Auburn University, Auburn, AL, USA. The analyzed xanthophyll contents in diet 1, 2, 3 and 4 were 4.1, 9.0, 15.5 and 34.2 ppm, respectively. For catfish under treatment 1, 2, 3 and 4, each of the treatment was fed one of the four diets for 16 weeks to get the corresponding deposition rates. Treatment 5, 6 and 7 were fed highest xanthophyll diet 4 for the first few weeks and then reassigned low-xanthophyll diets to observe the dissipation rate. Treatment 5 and 6 were switched to diet 1 and diet 2 after 8 weeks, respectively. Treatment 7 was switched to diet 2 after 12 weeks (Table 2).

This aquarium trial was conducted in an indoor recirculating system over a 16 week period. Twenty juvenile channel catfish were randomly stocked into 21 75-L flow-through aquariums, with mean initial weight of 22.3g. Water temperature was maintained between 26°C to 30°C. All other water quality parameters were maintained in acceptable ranges for juvenile catfish (Table 3). A diel light:dark cycle was set at 14:10h. Fish were maintained by feeding commercial catfish diets (Cargill Aquafeed WW4010 1/8", Cargill Inc., Minneapous, MN, USA) before the experiment. During the 16-week experimental period, fish were fed twice daily based on a set percentage of body weight. Fish were weighed every two weeks and the feed amount was adjusted accordingly. Three catfish were taken out of each aquarium every 4 weeks as sample and frozen at -50˚C before xanthophyll analysis. All the four experimental diets and

sampled fish were analyzed by HPLC (High-performance Liquid Chromatography) to determine xanthophyll content.

Carotenoid analysis

The extraction of carotenoid was modified from a method on channel catfish developed by Liu et al. (2012) as well as the general procedure described by Rodriguez-Amaya (2001). For each fish sample, whole fillets of three fish from each aquarium were pooled together by blended in a Black & Decker Quick-N-Easy food processor to produce a homogenous sample. A 5-g sample of the ground fillet was mixed with 25 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole) in a test tube (16×150mm). The mixture was homogenized by a Brinkmann Instruments homogenizer and then filtered under light vacuum through 1.0 μm filter paper into another test tube. The test tube with remaining tissue and extract on the filter paper were washed three times with 5 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole). Samples were then mixed with a secondary organic solvent for preliminary purification and concentration. For this, 10 ml hexane/ethyl ether mixture (1:1, v/v) was gently added into the test tube, followed by 20 ml distilled H_2O. The mixture was allowed to stand for 15 minutes to allow separation. The upper layer (containing pigments) was then removed using a Pasteur pipette and transferred to a separate test tube. The pigments were then concentrated by evaporating the solvents under nitrogen flow. The collecting test tube containing extracted pigment samples were sealed and stored in a freezer for subsequent HPLC analysis. A gradient elution program was developed for analysis of xanthophyll in Shimadzu LC-20 HPLC system (Liu et al., 2012; Moros et al., 2002). Xanthophyll concentration was calculated as the sum of lutein and zeaxanthin. For diet sample, the diets were ground and 1-g sample was taken for the following extraction. Appropriate

dilution was applied before HPLC analysis to ensure the concentration of xanthophylls within the linear range determined in Liu's study (Liu et al., 2012)

Statistics

The collected data was analyzed using SAS 9.1(Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2008). Growth data was subjected to analysis of variance (ANOVA), followed by the Student–Neuman–Keuls multiple comparison test to determine if significant difference ($P < 0.05$) existed between means (Montgomery, 1997). To provide a better description of the response, correlation and regression analysis between xanthophyll level in diets and in catfish fillet were conducted using SAS regression module (Zar, 1999). The initial data on week 0 of treatment 1 was excluded for the linear regression analysis because of the high initial xanthophyll level resulted from the commercial feed used before the experiment.

3. Results

Catfish under all the seven treatments grew normally and equally. There was no significant difference in final weight ($P = 0.11$) or percent weight gain ($P = 0.14$) due to the dietary treatments (Table 3). Lutein and zeaxanthin were detected as primary xanthophylls in the catfish fillet. Trace alloxanthin was also observed in very few samples, which could not cause significant shift of total xanthophyll amount.

Treatments 1 to 4 were designed to look at the deposition of xanthophyll in catfish flesh (Fig. 1). Poor linear relationship was found between xanthophyll concentration and culture time in treatment 1 (R-square=0.22) and treatment 2 (R-square=0.01). Xanthophyll level in fish under treatment 1 fluctuated between 15-80 ng/g, while treatment 2 between 30-100 ng/g during the 16

weeks. For treatment 3, xanthophyll levels increased linearly (R-square = 0.61) during the 16 weeks, with a linear regression equation of Y = 8.4X + 74.5, where X stands for culture time (week), Y stands for xanthophyll concentration in catfish fillet (ng/g). Compared to linear regression model (R-square=0.70), quadratic regression model fit data of treatment 4 better (R-square=0.77, Fig. 1). Across the four treatments, deposition of lutein and zeaxanthin followed similar trend as total xanthophyll (Fig. 2). The final xanthophyll, lutein and zeaxanthin levels in treatment 1 to 4 were all linearly correlated with the xanthophyll, lutein and zeaxanthin levels in diets (Fig. 3). The correlation equations for xanthophyll in catfish fillet and in diets was Y = 1.5X - 3.7, with an R-square of 0.938, where y stands for the xanthophyll concentration in catfish flesh (ng/g) and x stands for the xanthophyll concentration in diets (mg/g).

Treatments 5 to 7 were designed to look at the dissipation effect. In treatment 5 and 6, xanthophyll level decreased linearly during the 8 weeks after switched to low-xanthophyll diet 2 (Fig. 4). After 8-week dilution, xanthophyll concentration in treatment 5 and 6 dropped to similar levels. The final xanthophyll contents at week 16 in treatment 5 and 6 were 195.3 ng/g and 194.5 ng/g, respectively. After diluted by diet 2, treatment 6 and treatment 7 lowered their xanthophyll levels by 80.8 ng/g within 8 weeks and 256.7 ng/g within 4 weeks, respectively. Xanthophyll concentration in treatment 7 decreased up to 50.6% within 4 weeks.

Table 3. Water quality of the indoor aquarium experiments for channel catfish.

Parameters	Unit	Mean±SD[a]
Morning temperature	˚C	27.7 ±1.5
Afternoon temperature	˚C	27.7±1.6
Morning dissolved oxygen	mg/L	5.7±0.7
Afternoon dissolved oxygen	mg/L	5.5±0.7
Total ammonia nitrate	mg/L	0.12±0.10
Nitrite	mg/L	0.07±0.07
pH		7.7±0.2

[a]SD= Standard deviation

Table 4. Growth performance of channel catfish under seven treatments after 16 weeks of culture in the indoor aquarium experiment.

Treatment	1	2	3	4	5	6	7	P-value
Initial weight (g)	22.1	22.5	21.6	22.8	22.7	22.5	22.2	0.16
Final weight (g)	121.7	117.9	82.0	138.0	129.1	130.5	132.9	0.11
Weight gain (g)	99.6	95.4	60.5	115.3	106.4	108.1	110.7	0.11
Survival (%)	90	82.5	87.5	75	77.5	83.3	91.7	0.57

Figure 1. Xanthophyll concentrations in channel catfish under treatments 1 to 4, fed with diet 1 to diet 4 during 16 weeks. Xanthophyll concentrations in diet 1 to diet 4 were 4.1, 9.0, 15.5 and 34.2 ppm, respectively. Xanthophyll= lutein + zeaxanthin. The linear regressions of treatment 2 and 3 were calculated with all the data, while treatment 1was calculated excluding the initial data on week 0 because of the high initial xanthophyll level resulted from the previous feeding with commercial feed.

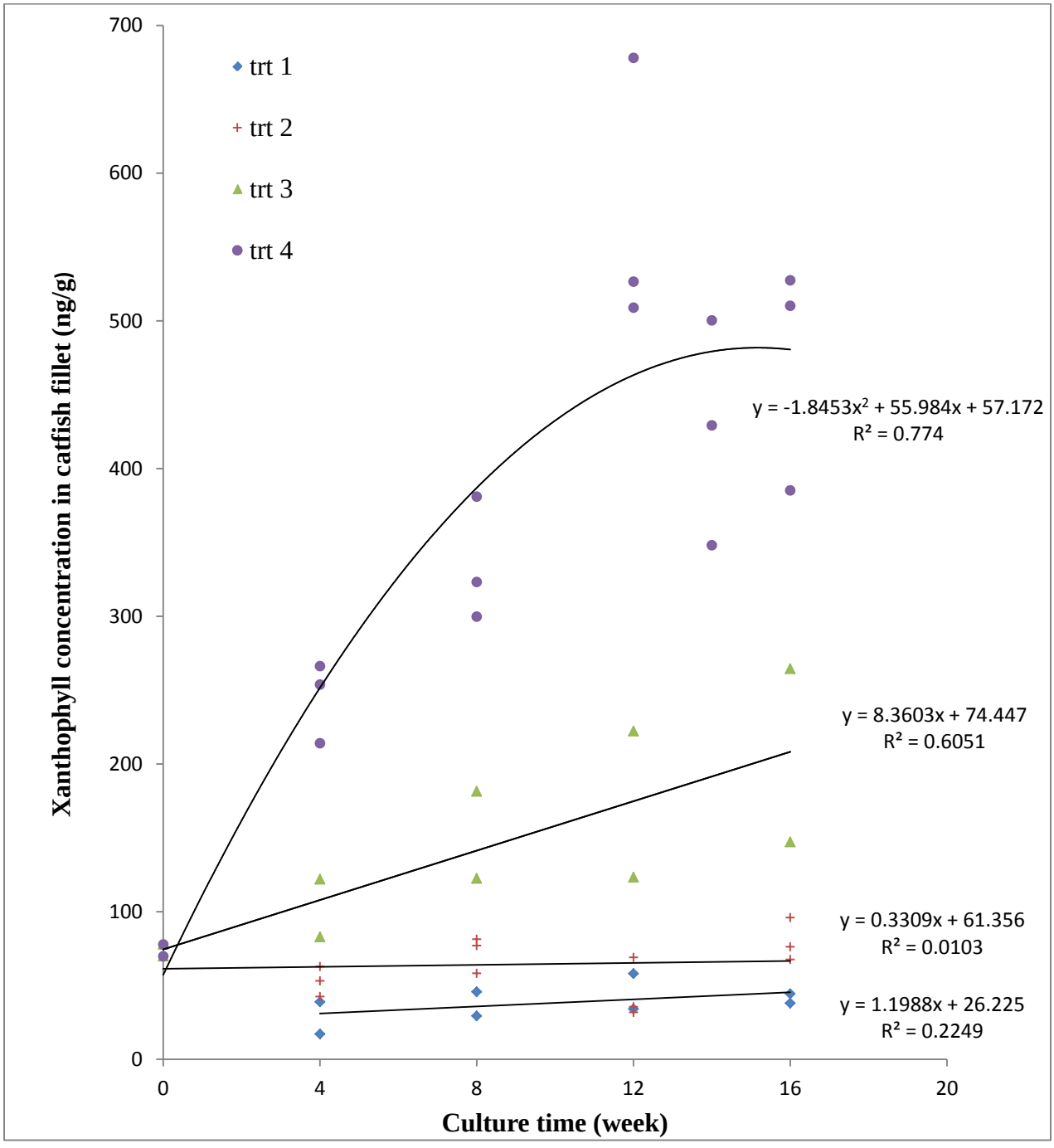


Figure 2. Lutein, zeaxanthin and total xanthophyll concentrations in channel catfish fillets under treatments 1 to 4, fed with diet 1 to diet 4 during 16 weeks. Xanthophyll concentrations in diet 1 to diet 4 were 4.1, 9.0, 15.5 and 34.2 ppm, respectively. Xanthophyll= lutein + zeaxanthin.

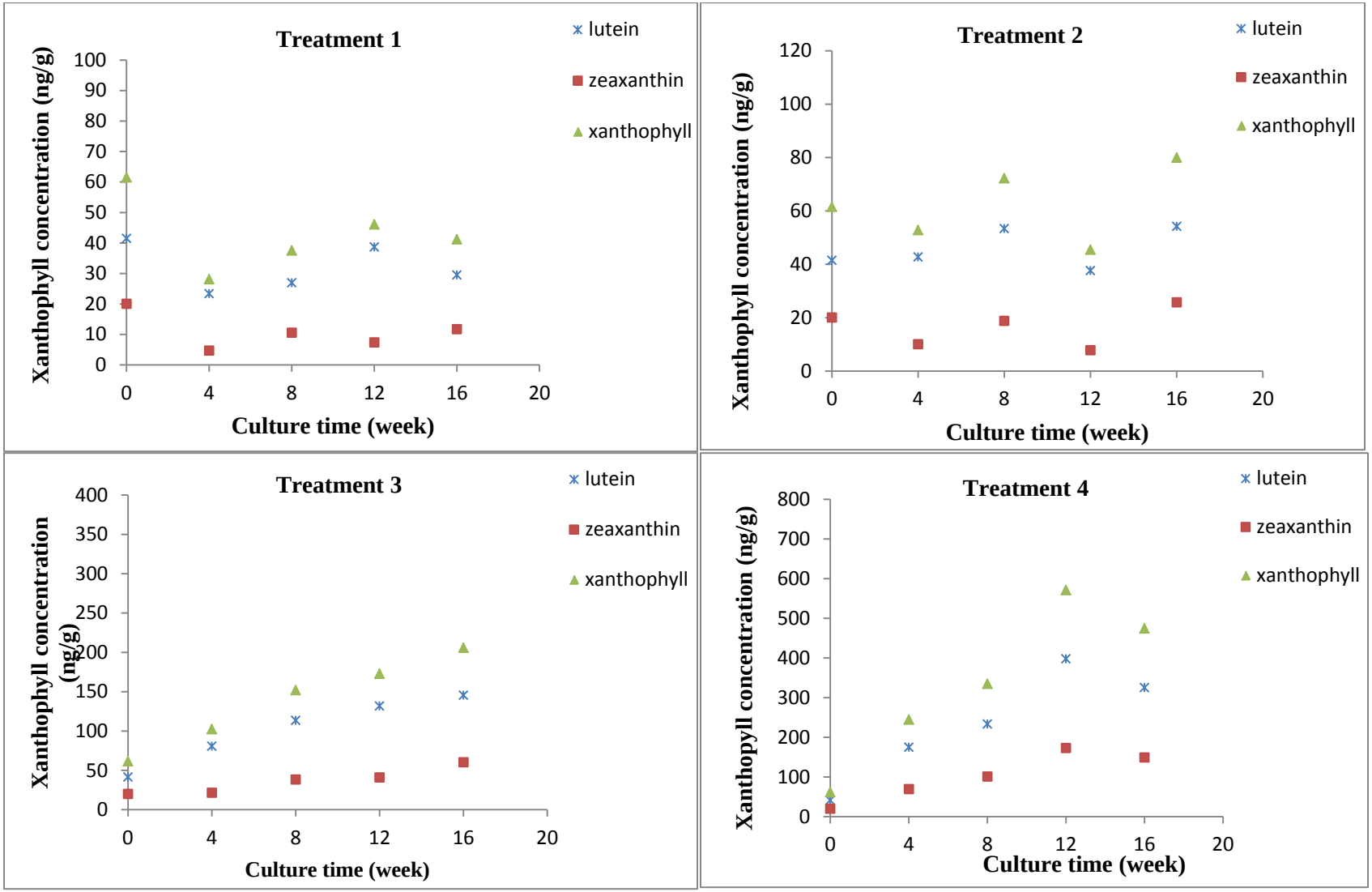


Figure 3. Correlation between lutein, zeaxanthin and xanthophyll in diets and in catfish fed with diet 1 to 4 at week 16. Xanthophyll concentrations in diet 1 to diet 4 were 4.1, 9.0, 15.5 and 34.2 ppm, respectively. Xanthophyll= lutein + zeaxanthin.

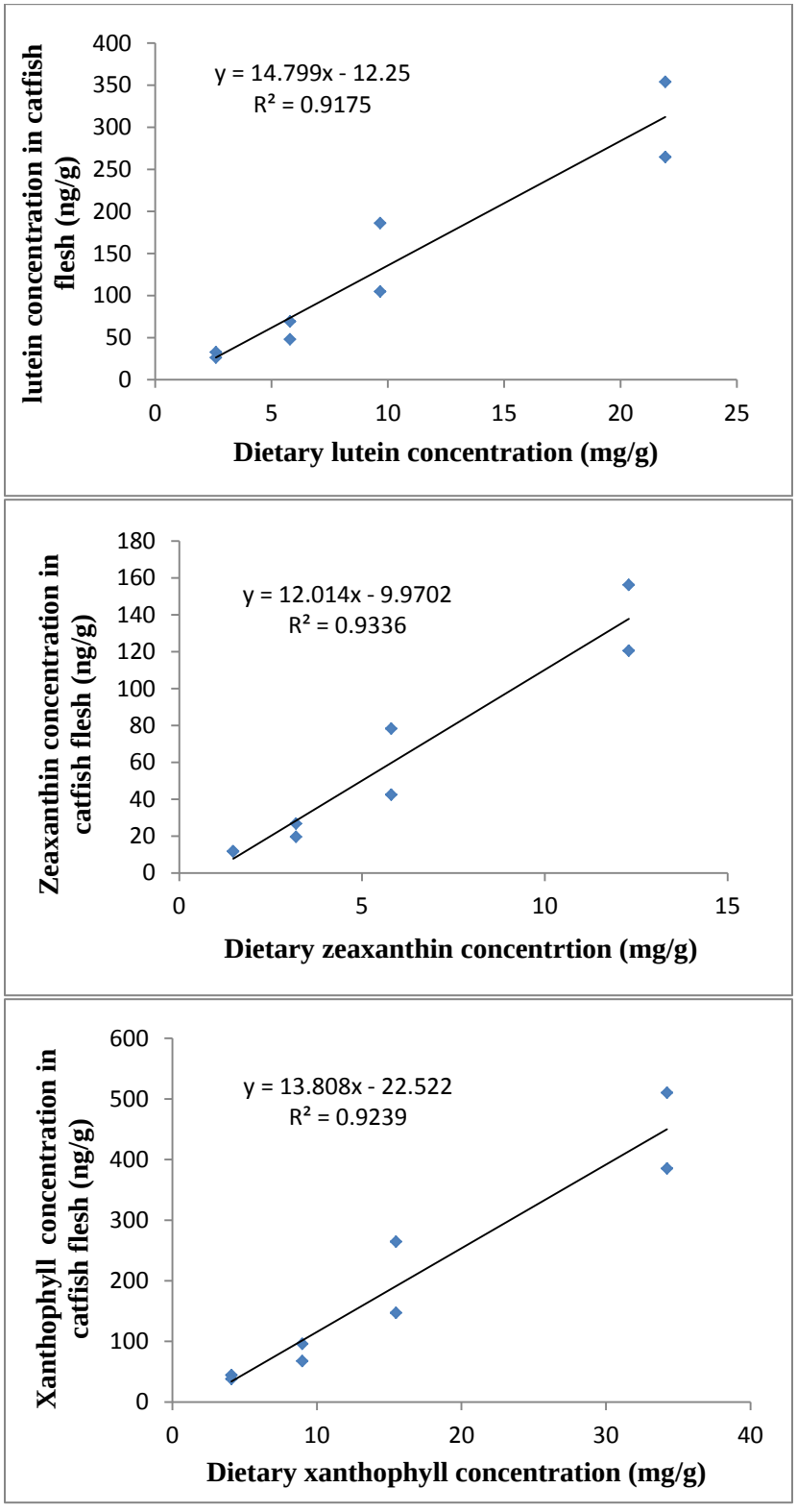
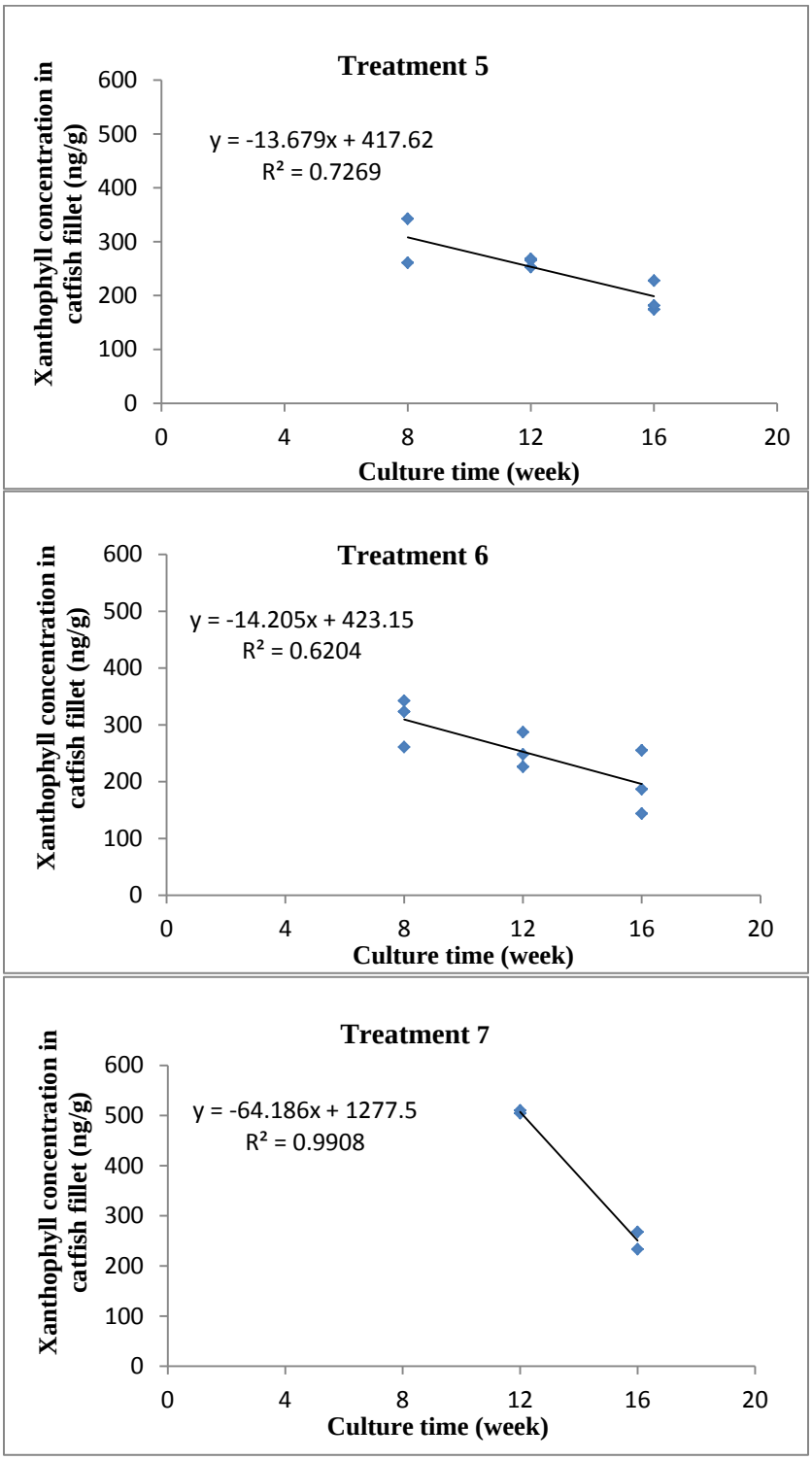


Figure 4. Dissipation of xanthophyll concentrations in catfish fillets under treatments designed for depletion experiment. Treatment 5 and 6 were fed with diet 4 for the first 8 weeks and switched to diet 1 and diet 2 during week 9-16, respectively. Treatment 7 were fed with diet 4 for 12 weeks and switched to diet 1 during week 13-16. Xanthophyll concentrations in diets 1, 2 and 4 were 4.1, 9.0 and 34.2 ppm, respectively. Xanthophyll= lutein + zeaxanthin.



4. Discussion

Xanthophyll is selectively deposited in animal tissues with wide variations among species. Channel catfish can accumulate lutein and zeaxanthin in flesh much better than canthanxanthin and astaxanthin, but poorly for astaxantin and β-carotene (Li et al., 2007). In contrast, the absorption of lutein and zeaxanthin is 10 to 20 times less efficient than astaxanthin and canthaxanthin in salmon and rainbow trout, (Schiedt et al., 1985). In chickens, the absorption of zeaxanthin was three times of astaxanthin (Schiedt et al., 1985). Additionally, some species e.g. rat, pig and sheep, do not accumulate carotenoids (Goodwin, 1980). Besides species selection of carotenoids, interactions between carotenoids can also affect the absorption. It was reported that dietary zeaxanthin could enhance lutein absorption in quail while lutein did not influence zeaxanthin absorption (Toyoda et al., 2002). In contrast, zeaxanthin supplementation did not influence plasma lutein in spider monkeys (Snodderly et al., 1997). When lutein was given simultaneously with β-carotene to human, an inhibitory effect of lutein on absorption of β-carotene was found (van den Berg, 1998). In channel catfish, the absorption and retention of xanthophyll have not been clearly studied. A better understanding of deposition and dissipation of xanthophyll in catfish is needed to help catfish industry better manage the yellow coloration in catfish fillet.

Data from this deposition experiment generally indicated that by feeding xanthophyll-containing diets, xanthophyll concentration in catfish fillet would increase (or decrease) until it reached a plateau or steady state which was related to the dietary xanthophyll concentration. As noted, before the start of this experiment, catfish were fed with xanthophyll-containing commercial feed, resulting in initial xanthophyll content in fillet. Consequently, fish maintained on diet 1 initially responded reducing the xanthophyll level in the muscle tissue and then the

xanthophyll level in the catfish fillet stayed constant. This was presumably because the initial body burden of xanthophyll was slightly higher due to levels in the commercial feed. The xanthophyll level in treatment 2 stayed constant around the initial xanthophyll level, which indicated that the body capacity of xanthophyll associated with diet 2 was close to the initial xanthophyll concentration. In treatment 3, the xanthophyll level in fillet linearly increased over time but did not appear to reach a clear plateau by the end of the 16 weeks. While in treatment 4, xanthophyll level increased rapidly and appeared to level off after 12 weeks. This was probably because the xanthophyll level reached the capacity of the tissue.

At week 16, a linear relationship between xanthophyll concentration in fish flesh and in diets was found in the present study (Fig. 2), which agreed with Lee's study (1987) and a study conducted in pond by Hu et al. (2012). This is also supported by a study conducted in chick's in which increasing levels of dietary xanthophyll resulted in a linear increase in the skin and serum xanthophylls (Dua et al., 1967). However, the dose response was not always linear. Lee (1987) found that dietary lutein levels of 100-120 mg/kg gave maximal lutein concentration in the catfish flesh. Another study conducted in broilers reported that 55-88 mg/kg lutein gave maximal color to the shanks (Fritz et al., 1957).

Under commercial conditions catfish are reared for an extended period of time on commercial feeds which would allow xanthophyll concentration to reach the capacity plateau. Thus, the xanthophyll level in catfish flesh could be roughly estimated based on the linear relationship between dietary xanthophyll level and flesh xanthophyll level obtained in this and other studies. Albeit, this will provide a good estimate of final levels, other factors could also affect the xanthophyll level in catfish, such as the presence of natural foods rich in carotenoids and most likely genetic variations in xanthophyll metabolism of the fish.

The mechanism of xanthophyll metabolism in channel catfish is not clear. Lee (1987) demonstrated that in channel catfish, lutein and zeaxanthin could not be converted to vitamin A and they were deposited in flesh, skin, liver and abdominal fat. The deposition of lutein and zeaxanthin in muscle tissue was in free forms without modification. Li et al. (2007) reported that channel catfish had limited ability to convert lutein to echinenone in the muscle tissues. In the present study, lutein and zeaxanthin were only found as free form in the fillet.

Once xanthophylls are accumulated in catfish flesh, they can be depleted if switched to low-xanthophyll feed (Li et al., 2011). In catfish farms with yellow fillet incidence, application of low-xanthophyll feed before harvest could correct the flesh color resulting in satisfying product. Thus, it is important to understand dissipation of xanthophyll with diets containing different xanthophyll contents. In treatment 5 and 6, xanthophyll concentration decreased linearly after the fish were switched to reduced xanthophyll diets at week 8. The linear reduction of xanthophyll levels generally agreed with a study conducted by Li et al. (2011). They reported that lutein plus zeaxanthin concentration in catfish fillet decreased linearly during the 12 weeks after switched to control diet at 20˚C and 30˚C. In the present study, treatment 5 and 6 ended with very close xanthophyll levels after the fish were offered diet 1 and 2 for 8 weeks, respectively. This indicated that diet 1 and diet 2 produced similar levels of reduction. The xanthophyll concentration of diet 1 and 2 were both below 11ppm, which was a threshold reported by Lovell (1998) that would not impart yellow color in catfish fillet. Li et al. (2009) reported a lower threshold level under pond conditions that lutein plus zeaxanthin level needed to be maintained below 7 ppm to avoid yellow fillet. Because natural productivity may also contribute to the xanthophyll concentration in catfish, the influence of natural food under different pond conditions needs to be considered for the threshold determination. Under pond

conditions, the xanthophyll concentration in diet which could cause yellow fillet should be lower than that in aquarium system without natural productivity.

Treatment 6 and 7 were designed to look at the clearance process of xanthophyll using the same diluting diet but starting with different initial xanthophyll levels. The linear regression slope of treatment 7 (slope=64.2) was significantly higher than that of treatment 6 (slope=14.2), while the starting xanthophyll concentration of treatment 7 (352 ng/g) was higher than that of treatment 6 (233 ng/g). This indicated that catfish with higher initial xanthophyll concentration had a quicker dilution rate of xanthophyll, if diluted by the same low-xanthophyll diet. The depletion rate was not only related to initial xanthophyll level, but it may also be associated with water temperature. Catfish in warm water (20˚C and 30˚C) had a quicker clearance rate of lutein and zeaxanthin than in cold water (10˚C). It needed 8 weeks for catfish to washout most of yellow pigment at 20˚C and 30˚C, but more than 12 weeks at 10˚C (Li et al., 2011). Hence, consideration about the depletion rate of xanthophyll in catfish should be given to both water temperature and initial xanthophyll levels when the catfish industry applying low-xanthophyll diet to dilute xanthophyll level in flesh. Under pond conditions, natural foods could also contribute to the xanthophyll level in catfish flesh resulting in a hidden “additional dietary xanthophyll”. Thus, feed applied to dilute xanthophyll level before harvest in ponds was suggested to be lower than that applied in aquariums.

Summary

The deposition of xanthophyll in fish flesh was directly correlated with dietary level and finally tended to level off around a certain level related to the dietary xanthophyll level. The lutein, zeaxanthin and total xanthophyll levels in fish fillets and in diets were linearly correlated.

This provided important information for the prediction of xanthophyll level in catfish flesh. The depletion of xanthophyll was linear within 8 weeks. Fish with higher initial xanthophyll level had quicker depletion rate. The dilution abilities of diets with 4.1 ppm and 9.0 ppm xanthophyll were similar to each other in aquarium system within 8 weeks. This indicated that feed with a xanthophyll level of up to 9 ppm could be applied to dilute the xanthophyll in catfish flesh when feed was the exclusive food source. Under pond conditions, contribution of natural foods should be considered.

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CHAPTER V

EVALUATION OF XANTHOPHYLL IN CATFISH FILLET, PLANKTON, SHAD AND SNAILS IN CATFISH PRODUCTION PONDS

Abstract:

Yellow coloration of catfish fillets has always been a problem. Recently increased consumer scrutiny has brought this to the forefront as a major industry concern. As catfish cannot synthesize xanthophyll, commercial feeds and natural prey items are the initial sources of the yellow color. To better understand the contribution of natural foods, a field study was conducted to identify if there were correlations of yellow fillet and natural productivity under commercial pond conditions. Upon notification from a west Alabama fish processor, yellow catfish fillets and natural organism samples (plankton, shad and snails) were collected from different farm ponds in west Alabama. “Clean” catfish fillets (no visible yellow coloration) and natural organisms were also collected at multiple farms where problematic yellow fillets were not present. In addition to large food items, microorganism samples from the ponds were collected by pumping pond water through two filters (sizes 20 micron and 75 micron) for a period of 10 minutes. The fillet and field samples were analyzed quantitatively for xanthophyll levels through High-Pressure Liquid Chromatography (HPLC). Results revealed a correlation between xanthophyll level in catfish fillets and in microorganisms (>75 microns). Results indicate that natural productivity consumed by gizzard shad and other filter feeders appear to contribute to the xanthophyll levels in catfish fillets.

1. Introduction

Channel catfish, *Ictalurus punctatus,* is a primary species of farm-raised fish in the United States. Even though the catfish industry is a mature industry with a long history of success, it faces numerous challenges while it evolves and adapts to shifting markets. The occurrence of yellow fillet is one of many challenges facing farmers. Because catfish is considered a white-fillet fish, catfish fillets with a discernible yellow color are considered of low quality, particularly by consumers that purchase the product fresh, not frozen. Due to customer preference of white catfish fillets, yellow catfish fillets are often price-reduced in the market or may be refused. More recently, the yellow coloration in catfish fillets has become a major industry concern because of increased consumer scrutiny. A considerable amount of resources have been invested by the catfish industry to address the yellow fillet problem.

Xanthophylls are the pigments responsible for the yellow coloration of catfish fillets (Lee, 1987; Tsushima et al., 2002; Li et al., 2007). Xanthophylls are yellow to red pigments with an oxidized tetraterpenoid structure. Most fish species examined to date are xanthophyll selectors, with xanthophyll as the primary carotenoid present in their natural food (Tacon, 1981; Matsuno, 2001). Xanthophylls exist widely in many aquatic species in various forms, such as free forms, esters, glycosides, sulfates and carotenoproteins (Matsuno, 2001). Different species of fish accumulate different kinds of xanthophylls. Salmonids deposit astaxanthin as the primary xanthophyll (Matsuno and Hirao, 1989). Zeaxanthin is the dominant xanthophyll in anchovies and sharks, while lutein and alloxanthin is more pronounced in freshwater fish (Matsuno and Hirao, 1989). In catfish flesh, lutein and zeaxanthin have been considered the two major xanthophylls in catfish fillets (Lee, 1987; Li et al., 2009). Alloxanthin was recently identified as another principal xanthophyll in catfish fillets (Liu et al., 2012).

Xanthophylls exist widely in bacteria, fungi, plants and animals. They serve a wide array of functions. As pigments, xanthophylls produce a variety of colors which assist in behavioral communication. Xanthophylls serve many functions related to light absorption. Zeaxanthin and antheraxanthin, as the major xanthophyll cycle carotenoids, can mediate the key energy dissipation progress in plants (Demmig-Adams and Adams, 1996). Xanthophyll can protect cells from oxidative damage by deactivating singlet oxygen and free radicals. Xanthophyll cannot be converted to vitamin A in mammals, but can serve as vitamin A precursor in lower animals, such as astaxanthin in crustaceans (Goodwin, 1984). Xanthophyll can generally enhance the growth of fish and crustaceans (Torrissen and Christiansen, 1994).

Xanthophylls are synthesized by microorganisms and plants, whereas, animals are generally considered not capable of synthesizing xanthophyll. Consequently, catfish must obtain xanthophyll from food sources. Under commercial production conditions, commercial feed and natural foods are the two sources of xanthophyll available to catfish. To minimize the incidence of yellow fillets, maximum allowable levels of xanthophyll in the feed have been proposed. Lovell (1998) recommended feed with less than 11mg/kg xanthophyll, while Li et al. (2009) suggested no more than 7 mg/kg under pond conditions. Catfish are opportunistic feeders and will feed on a wide array of organisms. Consequently, natural foods present in the pond can contribute to the xanthophyll concentration in catfish flesh following consumption. At commercial catfish farms, fish (e.g. gizzard shad *Dorosoma cepedianum*, threadfin shad *Dorosoma petenense* and fathead minnow *Pimephales promelas*), microorganisms (mainly large plankton and zooplankton) and snails (e.g. marsh ramshorn snail *Planorbella trivolvis*) are representative natural feed items available to catfish and are also potential sources of xanthophylls.

Presently there is limited information regarding natural productivity as an initial source of xanthophylls in commercially produced catfish. To better understand the contribution of natural foods, the present study was conducted to identify if there were correlations of yellow fillet and natural productivity under commercial pond production conditions.

2. Materials and Methods

Sample collection

Channel catfish fillets and gizzard shad were obtained from a commercial catfish processor, and then marsh ramshorn snails and plankton samples were obtained from commercial catfish ponds from which the samples originated. Samples were collected from ponds with and without an incidence of yellow fillet based on quality control indices at SouthFresh processing plant (Eutaw, Alabama, USA) during the spring months of 2011 (March to May). The decision of the presence of yellow fillet was made by a judgment call by the technicians and the Quality Assurance/Quality Control (QA/QC) manager working at the catfish processing plant based on their experience. Wholesale buyers have criteria for yellowness of fillet and each fish plant is responsible that these criteria are met for each customer. Twenty ponds from nine commercial catfish farms were sampled over a three month period. Farms were located in Greene, Hale, Perry and Dallas Counties, Alabama (Table 1). Three catfish fillets and two shad from each pond were collected at the processing plant. Plankton and snail samples were collected from catfish ponds within 24-48 hours after harvest. The sampling protocol and system were designed as a modification of the sampling system utilized by Lindley and Phelps (2009). To collect phytoplankton samples, a bilge pump was used to pump pond water through two filter sizes (20

microns and 75 microns) for a period of 10 minutes. The bilge pump was a submersible pump by Rule (Rule 3700) with a flow rate of 61 gallons per minute. Two nylon monofilament bags (Aquatic Eco-systems Inc., Apopka, FL, USA) were used as filters, which are 32" long with 7" diameter mouths and polypropylene rings. Pond water was first pumped through the 75 micron filter and then the 20 micron filter. Water was pumped from a depth of 1-2 m. Contents of the two filters were emptied into 200 ml of distilled water and frozen at -20˚C until analysis.

Xanthophyll analysis

The extraction of carotenoid was modified from the method proposed by Liu et al. (2012) as well as the general procedure described by Rodriguez-Amaya (2001). Three catfish fillets and two whole shad from each pond were separately blended in a Black & Decker Quick-N-Easy Food processor to produce homogenous samples. A 5-g subsample of the ground fillet or shad was mixed with 25 ml acetone (with 6 mg/ml 3-tert-butyl-4-hydroxyanisole) in a test tube (16 × 150mm). The mixture was homogenized using a Brinkmann Instruments homogenizer (Polytron PT 10/35, Westbury, N.Y.) and then filtered under light vacuum through 1.0 µm filter paper and captured in a test tube. The test tube with remaining tissue and extract on the filter paper were washed three times with 5 ml acetone. Samples were then mixed with a secondary organic solvent for preliminary purification and concentration. For this, 10 ml hexane/ethyl ether mixture (1:1, v/v) was gently added into the test tube, followed by 20 ml distilled H_2O. The mixture was allowed to stand for 15 min to allow separation. The upper layer (containing pigments) was then removed using a Pasteur pipet and transferred to a separate test tube. Finally, the pigments were concentrated by evaporating the solvents under nitrogen flow. The collecting test tube containing extracted pigment samples were sealed and stored in a freezer at -20˚C for subsequent HPLC

Table 1. List of dates, regions and ponds where the catfish fillets, gizzard shad, plankton and snails were collected.

Collect date	County	Farm	Pond	Yellow fillet
4/4/2011	Dallas	1	8	w
4/4/2011	Perry	2	19	w
4/11/2011	Greene	8	23	w/o
3/17/2011	Dallas	1	2	w
3/31/2011	Dallas	1	12	w
3/22/2011	Marengo	3	C12	w
3/17/2011	Dallas	1	5	w
3/25/2011	Perry	4	16	w
3/25/2011	Greene	5	3	w
4/11/2011	Marengo	3	53	w/o
3/14/2011	Marshall	2	5	w
3/31/2011	Hale	6	12	w
4/1/2011	Greene	7	3	w
3/28/2011	Marengo	3	C13	w
4/12/2011	Marshall	2	13	w/o
4/5/2011	Marshall	2	6	w
4/19/2011	Greene	8	27	w/o
4/8/2011	Greene	9	8	w
3/18/2011	Greene	5	6	w
4/19/2011	Greene	9	2	w/o

(High-performance Liquid Chromatography) analysis. A gradient elution program was developed for analysis of xanthophylls using a Shimadzu LC-20 HPLC system (Liu et al., 2012; Moros et al., 2002).

Whole snails were ground by mortar and pestle, and then divided into two subsamples. The two subsamples were weighed and then processed for xanthophyll analysis as previously described. The frozen phytoplankton samples were thawed, homogenized and two 20 ml sub samples removed for freeze-drying. The dry sample was then extracted and analyzed by HPLC as previously described. Appropriate dilution was applied to ensure the concentration of xanthophylls were within the linear range determined in Liu's study (Liu et al., 2012). Xanthophyll concentration was calculated as a sum of lutein, zeaxanthin and alloxanthin.

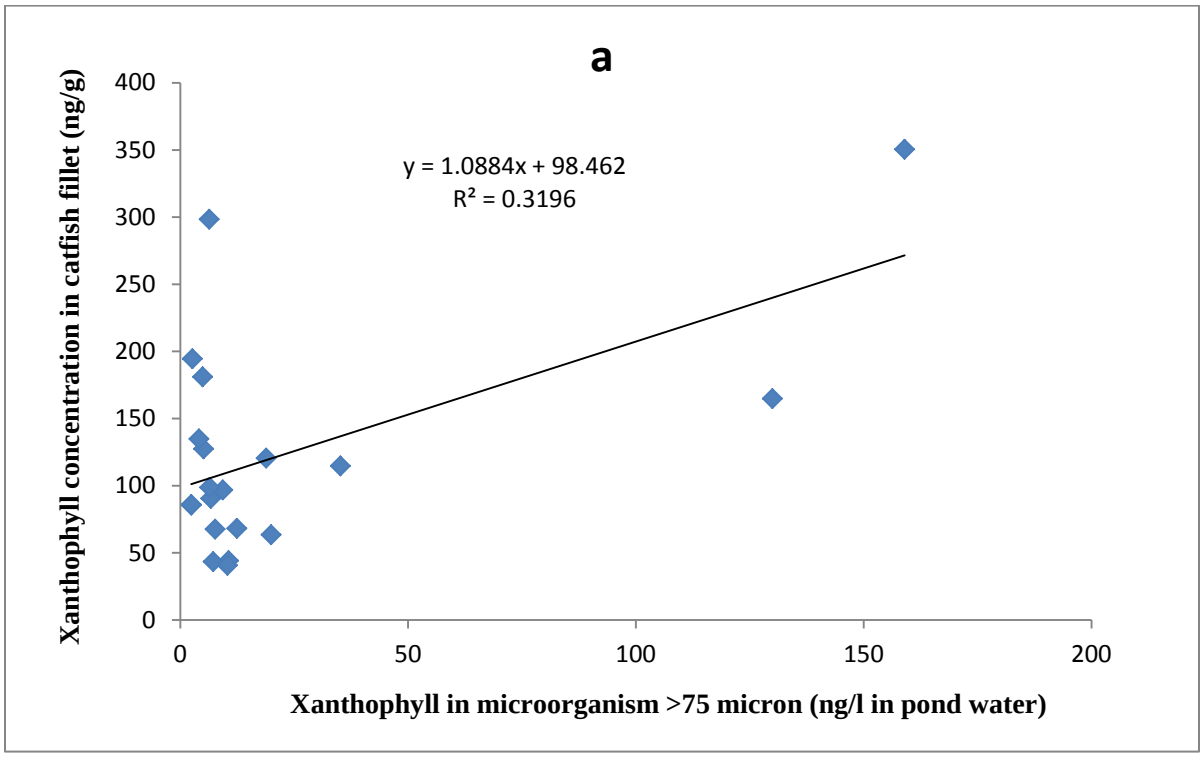
Statistics

Data was analyzed by SAS 9.1 (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2008). A mean of the xanthophyll concentration of the duplicate subsamples was used as the xanthophyll concentration of the sample. Correlation and regression analysis between xanthophyll levels in fillets and in pond samples were conducted in SAS (Zar, 1999).

3. Results

Xanthophyll levels in catfish fillets ranged from 40 ng/g to 350 ng/g. Shad whole body levels of xanthophyll ranged from 24 to 439 ng/g. In plankton samples with sizes of 20-75 microns and above 75 microns, the xanthophyll levels were 9-127 ng/L and 2-159 ng/L in pond water, respectively. Sample snails accumulated a high level of xanthophyll with whole body levels ranging from 9949 ng/g to 18215 ng/g.

Figure 1. Relationship between xanthophyll concentrations in catfish fillets and plankton collected by filtration with a size >75microns. Fig. a represents the data from 21 sample ponds; Fig. b represents data from ponds with xanthophyll level above 7ng/l in pond water.



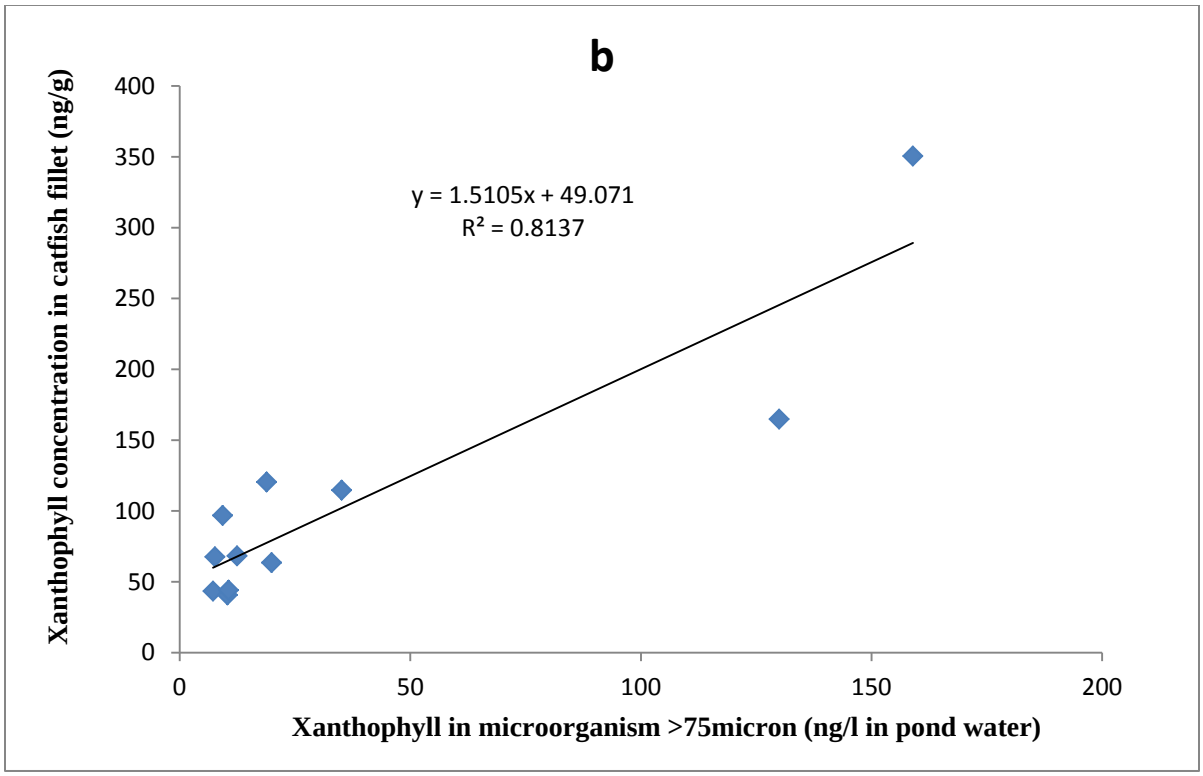


Figure 2. Scatter plot of xanthophyll concentrations in catfish fillets and in plankton captured by filtration (20-75 microns) from 21 commercial catfish ponds in west Alabama.

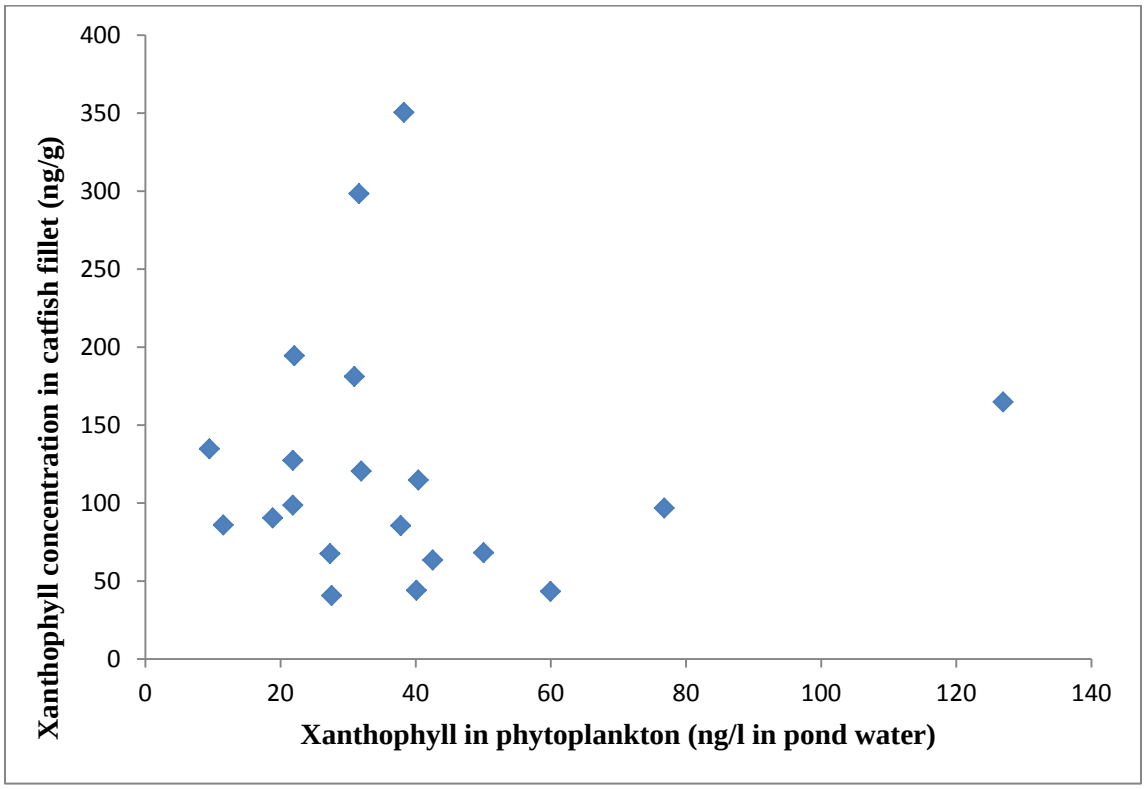


Figure 3. Relationship between xanthophyll concentrations in catfish fillets and in gizzard shad from 21 commercial catfish ponds in west Alabama.

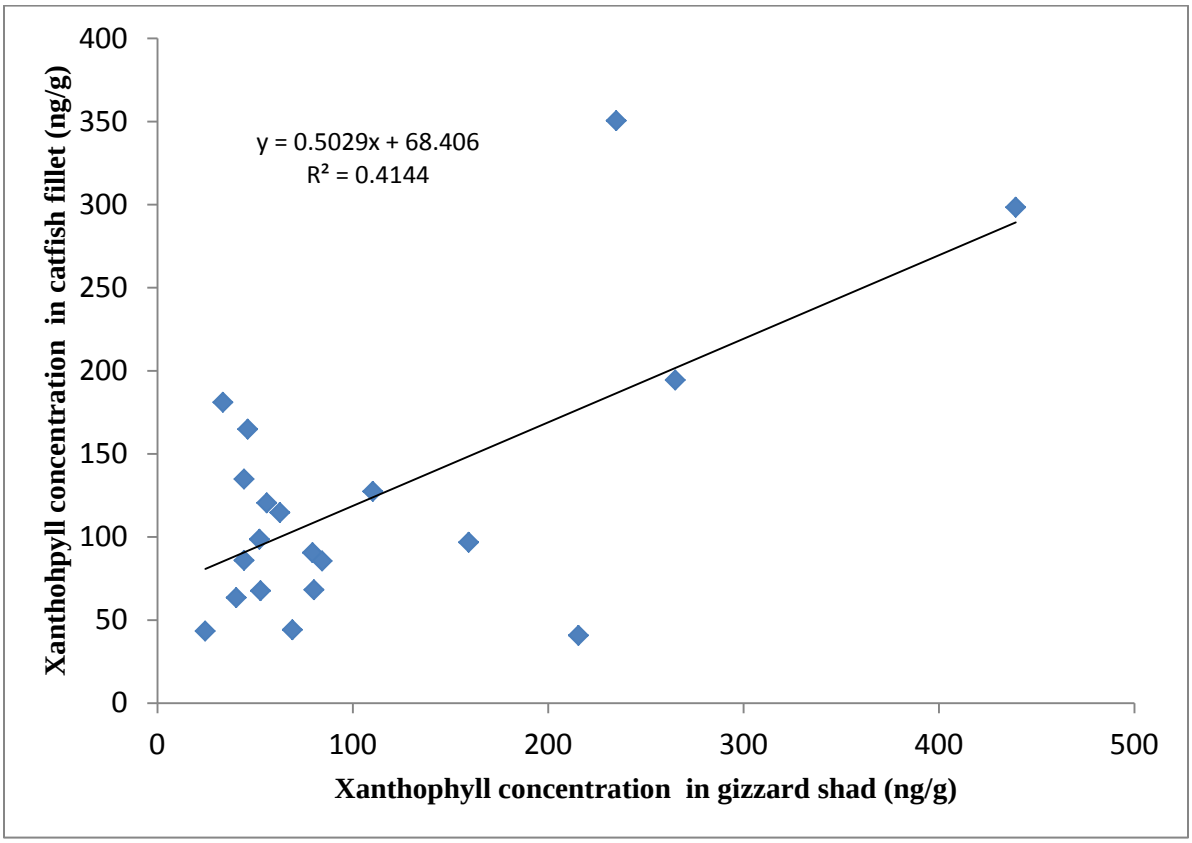
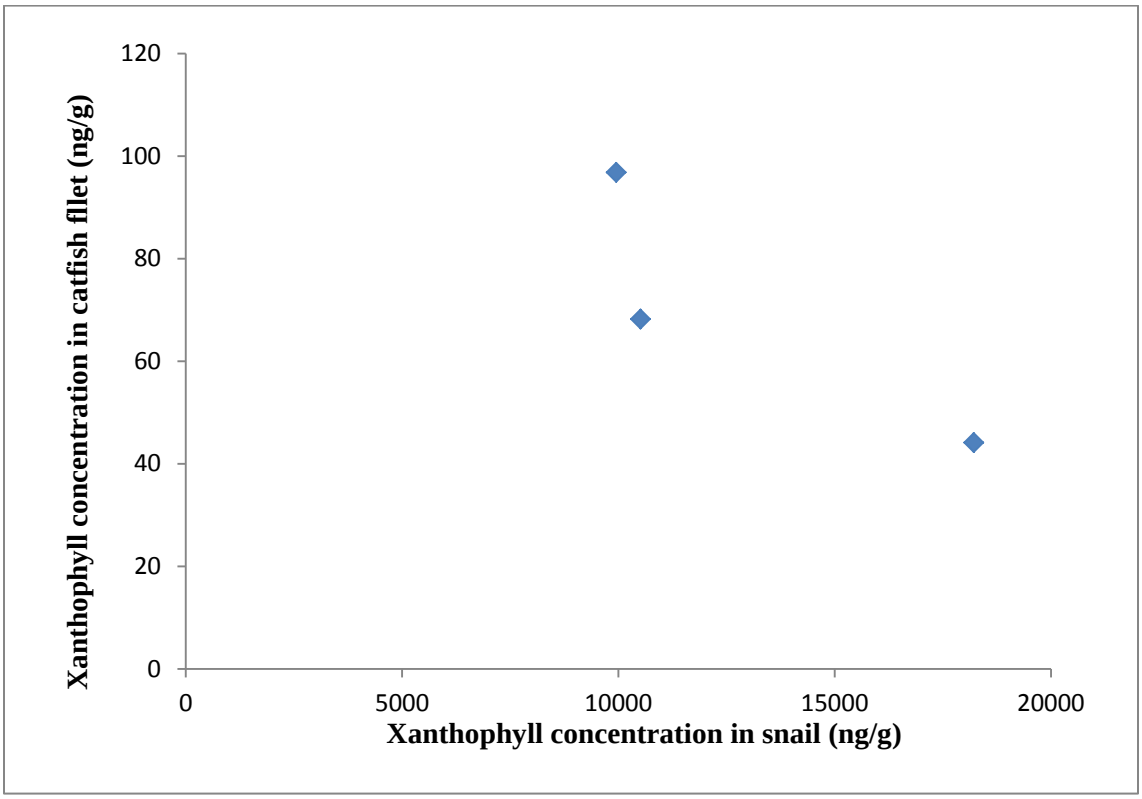


Figure 4. Relationship between xanthophyll concentrations in catfish fillets and in snails from 3 commercial catfish ponds in west Alabama.



There was a linear relationship between xanthophyll concentration in catfish fillet and in microorganisms (plankton) above 75 microns (R-square = 0.32, Fig.1). The linear relationship was much better when the xanthophyll concentration in plankton above 75 microns was higher than 7ng/l in pond water (R-square = 0.81, Fig. 2). There were no linear associations between xanthophyll concentration in catfish fillets and in plankton between 25 and 75 microns (Fig. 3). Xanthophyll concentration in catfish fillet was correlated with that in shad (Fig. 4).

Snail samples were difficult to collect in the ponds sampled in the study and were not found in most ponds. Snail samples were only collected from three ponds and no correlation was found between xanthophyll levels in catfish fillet and in snails. While the number of samples is small, it is still worth noting due to the very high amounts of xanthophylls found in the few snails that were examined.

4. Discussion

Xanthophyll is synthesized by plants and microorganisms. Animals consume xanthophyll in these food sources and deposit xanthophyll through assimilation. The retention of xanthophyll in fish depends on a wide array of factors, such as uptake, absorption, transport, metabolism and excretion (Torrissen et al., 1989). Different species selectively absorb and deposit different carotenoids. A study evaluating the carotenoids in the freshwater food chain showed a selective accumulation of carotenoid (including lutein, zeaxanthin and alloxanthin) in aquatic insects and in freshwater fish (Matsuno et al., 1999). Besides being deposited directly from food sources, xanthophylls found in animals can also be metabolically modified from other xanthophylls. Many fish species can convert one xanthophyll to another, and this ability varies among individual organisms and species (Hata and Hata, 1972; Hsu, et al., 1972; Kitahara, 1983;

Matsuno et al., 1985; Nagata and Matsuno, 1979; Schiedt et al., 1985). Li et al. (2007) reported that channel catfish had limited ability to convert lutein to echinenone in muscle tissues. To date, no study has demonstrated that channel catfish can convert other xanthophylls into lutein, zeaxanthin or alloxanthin.

Channel catfish cannot synthesize xanthophyll. The xanthophyll found in catfish tissues must originate from various food sources. Thus, feed plays an important role in the accumulation of xanthophyll in catfish fillets. Studies have demonstrated that the xanthophyll level in catfish fillet was linearly related to the dietary xanthophyll level (Lee, 1987; Hu et al., 2012). In addition to commercial feeds, xanthophyll could also be obtained through natural food sources under pond conditions (Lovell, 1984; Li et al., 2009). Given a large number of farms utilize the same feed but not all farmers or ponds have a yellow fillet problem, natural foods are likely contributors to the problem.

Primary productivity is the ultimate natural dietary source of nutrients for catfish (Riley, 1998). Aquatic weeds and phytoplankton represent primary productivity in catfish ponds. The primary productivity occupies the base of the food chain. It is consumed by small animals (eg. zooplankton, phytophagous fish) or dies decomposing into detritus. The detritus and zooplankton are consumed by aquatic insects, crustaceans, mollusks, and small fish (eg gizzard shad) which in turn can be eaten by catfish. Catfish occupy the top position in the food chain of catfish ponds. Catfish are omnivorous and can obtain all kinds of natural organisms as food, such as aquatic plants, zooplankton, aquatic insects, crustaceans, mollusks and small fish (Bailey and Harrison Jr., 1948). Thus, all these natural organisms are possible contributors to the xanthophyll level that accumulate in catfish through the food chain.

The most common plant form in catfish ponds is phytoplankton, although aquatic weeds may develop in some ponds (Tucker and Lloyd, 1984). In the present study, the samples filtered from pond water mainly consisted of phytoplankton and zooplankton. The linear correlation between xanthophyll in pond water sample above 75 microns and in catfish fillets indicated that natural organisms within this size range contributed to the xanthophyll level in catfish flesh through food chain. However, microorganisms between 25 and 75 microns were not correlated with xanthophyll levels in the catfish sampled in this study. This may be because this smaller group of microorganisms (25-75microns) was not a major part of the catfish food chain. Cyanobacteria, with a size from 0.5 to 60 microns, often dominate the phytoplankton community in catfish ponds during the summer months (Paerl and Tucker, 1995). Because cyanobacteria are generally not eaten by other aquatic organisms, they are not an important part of the food chain. However, cyanobacteria are a concentrated xanthophyll source which may be an important component in the pond water sample between 25-75microns. The existence of cyanobacteria may explain the nonlinear relationship between xanthophyll in catfish fillets and in pond water samples between 25-75 microns.

Gizzard shad are filter feeders that consume primarily phytoplankton and zooplankton. Shad are often stocked together with catfish to help control phytoplankton biomass in catfish ponds in west Alabama. Adult catfish consume shad as a primary natural food. In the present study, the xanthophyll concentration in shad was associated with the levels observed in catfish fillets, which indicated that the food sources consumed by shad may contribute to xanthophyll levels in catfish fillets. Snails feed on phytoplankton and detritus, and can be eaten by catfish. Snails analyzed in the study all accumulated high amounts of xanthophyll, which confirm reports by Li et al. (2009) that xanthophyll accumulated through the food chain. Snails could only be

collected from three ponds, and the xanthophyll levels were not correlated to those in catfish fillets. The lack of correlation could be due to sample size, variation in the snail's relative contribution as a food source or other factors. Given the high concentration of xanthophyll observed in snails, further work regarding the contribution of snails as a food source is warranted.

In the present study, the farms received feeds from two different feed mills, yet not all the ponds or all farms using these feeds exhibited yellow fillet problems. Hence it would appear that natural productivity contributed to xanthophyll content in catfish fillets in commercial ponds. Xanthophyll level in catfish flesh was related to microorganisms in pond water above 75 microns and gizzard shad. In order to prevent the yellow fillet problem, the primary productivity in catfish pond, especially the biomass of microorganisms above 75 microns needs to be controlled. The biomass threshold to prevent incidence of yellow fillets needs to be established. More studies need to be done to address other resources contributing to xanthophylls in catfish fillets.

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CHAPTER VI

SUMMARY AND CONCLUSIONS

Variations in the color of catfish filets have always been a problem. However, more recently it has become the center of attention as consumers are requesting a more consistently colored product. The main purpose of this study was designed to help the catfish industry better understand the yellow coloration on catfish fillet.

A pond study was conducted to evaluate four diets for channel-blue catfish with regards of xanthophyll level and production performance. Results from this study revealed that all the four experimental diets, with 28% and 32% protein levels, with and without 20% inclusion of corn gluten feed could be efficiently utilized by channel-blue hybrid catfish. The diets with 20% corn gluten feed produced catfish with higher levels of xanthophyll, but without causing yellow coloration problem in fillet. This indicated that all the four experimental diets could result in satisfying production with acceptable fillet color.

A color standard was developed with a digital camera and computer software to help understand the yellow color in catfish fillet. The specific configuration and following calibration guaranteed the accuracy and consistency of the yellowness reading CIE LAB B value. The development of this color standard could help sort fillets into different categories for different markets. A linear correlation was found between xanthophyll level and B value of the catfish

fillets. The relationship could be applied to quickly estimate xanthophyll level based on the B value of the catfish fillet.

An aquarium study was conducted to evaluate the deposition and dissipation of xanthophyll, which provided a better understanding of the relationship between diets and xanthophyll in fish fillet. The xanthophyll levels in fish fillets and in diets was linearly correlated if fish were fed consistent diet. Results from this study showed that the accumulation of xanthophyll in catfish flesh was fast at first and then would reach a plateau, which was related to the dietary xanthophyll level. The depletion rate of xanthophyll was associated with the initial xanthophyll concentration. Xanthophyll with higher initial concentration could get depleted quicker. The diluting ability of diets with xanthophyll level of 4 ppm and 9 ppm were similar.

To better understand the contribution of natural food to the xanthophyll level in catfish fillet, a study was conducted to evaluate various organisms in catfish pond. Result from this study showed that xanthophyll level in catfish flesh was related to microorganisms above 75 microns in pond water, as well as gizzard shad. Natural productivity contributed to xanthophyll content in catfish fillets under pond situation.

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