Effects of dietary supplementation with natural carotenoid sources on growth performance and skin coloration of fancy carp, *Cyprinus carpio* L.

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Abstract

We evaluated the effects of natural carotenoid sources on the growth performance and skin pigmentation of *Cyprinus carpio*. Samples with mean initial weight of 51.23 ± 5.04 g were fed with four experimental diets in quadruplicate for 8 weeks: A control diet without carotenoid supplementation and three other diets with three different kinds of natural carotenoid sources, *Phafia rhodozyma*, *Paracoccus* sp., and *Haematococcus pluviallis*, at a level of 45 mg kg^{-1} total carotenoids. The growth and feed utilization efficiency did not significantly differ between the dietary treatments ($p>0.05$). The lightness (L*) in the red zone of the fish on the control diet tend to increase significantly from the other treatments ($p<0.05$). The *P. rhodozyma* diet provided the highest redness (a*) and chroma (C*), and a strongly reddish hue (H°), with a significant difference to the other diets ($p<0.05$). The yellowness (b*) in the red and the white zones had no significant difference between the dietary treatments ($p>0.05$). In addition, the fish fed with a *P. rhodozyma* diet had high accumulated carotenoid levels in the red and the white scales, as well as in the serum. Furthermore, the carotenoid deposition capacity was higher in the fish skin than in the scales and the serum, and the carotenoids were deposited more in the red than in the white tissues. The results indicate that diet supplementation with *P. rhodozyma* enhances the red skin color of fancy carp.

Keywords: *Cyprinus carpio*, Carotenoid, Pigment, Growth, Skin color

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Introduction

Fancy carp (*Cyprinus carpio* L.) is one of the globally most popular ornamental fish species, and a wide variety of colors and patterns have been classified for this species (Tamadachi, 1990; Blasiola, 1995; Gomelsky *et al.*, 1996). The fancy carp with a white body and a red marking is one of the most popular among the color types (Kuroki, 1981; Tamadachi, 1990). The skin pigmentation of fancy carp is the most important quality parameter determining the market value and consumer acceptability. A frequent problem found in fancy carp culture is that, the fish tend to lose their color as if fading when maintained in captivity, and this decreases their market value (Yuangsoi *et al.*, 2010). Therefore, several studies have been focused on improving the fish skin coloration. Various factors were contributed to the color intensity of aquatic animals, such as the source and type of pigments, water temperature, brightness, feeding rate, diet composition, species, size and physiological conditions (Leng and Li, 2006). However, carotenoids that are lipid-soluble pigments are the most effective and consistent means to enhance the skin coloration in ornamental fishes (García-Chavarría and Lara-Flores, 2013). Since fishes are incapable of syntesizing carotenoids *de novo* in their body, so these pigments must be supplied in the diet (Johnson and An, 1991; Gouveia *et al.*, 2003; Gupta *et al.*, 2007). A variety of carotenoids from both natural and synthetic sources, such as astaxanthin, cantaxanthin, β-carotene, lutein and xanthophylls, have been incorporated into fish diets for color enhancement (Gouveia *et al.*, 2003; Kalinowski *et al.*, 2005; Kurnia *et al.*, 2007; Yeşilayer *et al.*, 2011; Yuangsoi *et al.*, 2011; Liu *et al.*, 2014; Yi *et al.*, 2014; Sornsupharp *et al.*, 2015; Boonyapakdee *et al.*, 2015). However, the use of syntetic carotenoids may have negative impacts, such as environmental deterioration and carcinogenic effects, and they tend to have a high cost (El-Baky *et al.*, 2007; Gupta *et al.*, 2007; Sun *et al.*, 2012; Teimouri *et al.*, 2013), so recent efforts have emphasized the potential use of coloring agents from alternative natural sources, such as fairy shrimps (Seidgar, 2015; Seidgar *et al.*, 2016), beetroots (Devi *et al.*, 2016) to replace the syntetic chemicals. In prior studies the natural compounds derived from red yeast, marine bacteria, and green algae, were efficient as synthetic carotenoids for improving the skin pigmentation in several ornamental fish species, including goldfish (*Carassius auratus*) (Gouveia *et al.*, 2003; Xu *et al.*, 2006), Kenyi cichlids (*Maylandia lombardoi*) (Karadal *et al.*, 2017), and tomato clownfish (*Amphiprion frenatus*) (Hekimoglu *et al.,* 2017). Therefore, the objective of the present study was to evaluate the effects of *Phafia rhodozyma*, *Paracoccus* sp., and *Haematococcus pluviallis,* as natural sources of carotenoids, on the growth, feeding efficientcy and skin pigmentation of *C. carpio*. In addition, the total carotenoid concentrations deposited in the skin, scales and serum

were also investigated. In case such natural carotenoid supplemented diets were effective on improving the skin pigmentation of fancy carp, the costs and concerns from using synthetic carotenoids could be reduced without loss of the market value, which depends on the pigmentation.

Materials and methods

Experimental diets

Four different experimental diets were used during the experimental period for 8 weeks. A commercial herbivorous fish diet (Starfeed 5931[TM], Charoen Pokphand Foods Public Company Limited, Thailand) without additional pigment was used as the baseline or control diet. The three other diets were formulated to contain 45 mg kg^{-1} total carotenoids from three different natural pigment source, namely, *P. rhodozyma* (Brineshrimpdirect Co., Ltd., USA), *Paracoccus* sp. (Brineshrimpdirect Co., Ltd., USA), and *H. pluvialis* (Naturose[TM], Cyanotech corporation, USA). The supplement pigments were in powder form, and were coated onto the surface of the basal diet by first completely mixing with egg white (100 g kg^{-1}) and spreading evenly, while the control diet was only coated with egg white. All the experimental feeds were air dried, stored in re-sealable bags at -20°C, and kept away from light and moisture throughout the experiment. The proximate analysis of the control diet, amount of supplemented pigments and total carotenoid concentrations of the experimental diets are given in Tables 1 and 2.

The fish and their rearing conditions

Mixed sex fancy carp were obtained from a commercial fish farm in Nakornpatom province, Thailand. Before these fish were transported to conduct the feeding experiments at Prince of Songkla University, Surat Thani campus, Thailand, they were first raised in an earthen pond and fed with a commercial fancy carp diet. Before starting the experiment on campus, the fish were fed with a control diet for 2 weeks to acclimatize them to the rearing system, and to equalize carotenoid contents in their bodies. A total of 256 individual fish with an initial mean weight of 51.23±5.04 g were randomly divided into 16 indoor tanks (300 L each) at a stocking density of 16 fish per tank. Each group of fish were stocked in a 75×75×60 cm^3 indoor tank, and the freshwater flush rate was 0.5 L min^{-1}. Each dietary treatment was randomly assigned to four of the tanks, for four replicate treatment groups. The fish were fed their assigned experimental diets by hand three times a day (8:00, 12:00, and 16:00) at the feeding rate of 3% of their body weight for 8 weeks. The amount of feed was adjusted every 2 weeks after the fish had been weighed. The mortality and the amount of feed used were recorded daily. During the feeding trial, the fish were reared under natural photoperiod. The water temperature ranged from 26 to 28.5°C, the dissolved oxygen was in the range from 7 to 10 mg L^{-1}, and the pH was 6.5-7.0; these parameters were monitored daily. At the end of the experiment, the fish were individually

weighed. The observations were used to calculate the growth, the survival, and the feeding efficiency of fancy carp during the 8-week trial.

Skin color measurement

At the beginning and every 2 weeks after feeding experiment, a total of 3 individual fish from each tank were randomly selected and anesthetized using clove oil solution, to measure the coloration on red and white zones of the fish skin using a tristimulus colorimeter (SC80B, Sino Age Development Technology Ltd., Beijing, China). The color was assessed in terms of L*, a*, and b*, where L* represents the lightness with range from 0 for black to 100 for white. The +a* expresses red while -a* indicates green, and +b* represents yellow while -b* indicates blue, in accordance with the recommendations of the International Commission on Illumination (The International Commission on Illumination (CIE), 1977). The hue (H°) and the chroma (C*) values were calculated from a* and b*. The H° is an angular measure of the visible colors in the electromagnetic spectrum, with 0°, 30°, and 60° assigned to red, orange and yellow hue, respectively. The C* represents the intensity of a given color. The transforms used were H°= arctan (b*/a*) and $C*=\sqrt{a*^2+b*^2}$ (Hunt, 1977).

Sampling procedure and total carotenoid analysis

At the end of the feeding trial, three individual fish from each replicate tank were first used for color measurement and then sacrificed with an overdose of clove oil solution. A 300 µl blood sample was collected from each individual, from the caudal vein with a 1 ml non-heparinized disposable syringe fitted with a 0.7×25 mm disposable needle. The blood samples for each replicate tank were pooled into a single tube, and allowed to clot on ice before centrifugation at 300 g for 10 min to separate out the serum. The serum (200 µl per replication) was transferred to an empty tube and vortexed with 400 µl of ethanol for 30s, before 800 µl of petroleum ether was added to the mixture and vortexed for 1 min. The petroleum ether was then separated by centrifugation at 300 g for 10 min (White *et al.*, 2002). The absorbance of the supernatant was measured at 450 nm using a UV-visible spectrophotometer (U2900; Toshiba, Tokyo, Japan). The total carotenoid concentration was estimated using the specific extinction coefficient $E_{1\%,\ 1cm}=$ 2,500 with Beer Lambert's law (Britton *et al.*, 1995).

Skin and scales from the red and the white zones were collected from three individuals per replication for evaluating the total carotenoid concentrations. Samples representing each replicate tank were pooled separately for the white and the red skin, and the scales, into re-sealable bags covered with aluminium foil that were stored at -20°C until further analysis. The total carotenoid analysis of the fish skin and the scales followed the method of Britton *et al.* (1995), with minor modifications. Briefly, the

sample was accurately weighed to 1.00 g fresh weight, and homogenized in acetone using a small mortar and a pestle until the sample appeared colorless. After homogenizing, the solid debris have been removed out by filtering through a small pad of cotton wool. The filtered mixture was transferred to a separating funnel and an equal volume of diethyl ether was added. After that, the mixture was mixed gently, then two volumes of water were added to the mixture, gently mixed and set aside for a while until the mixture was separated into two phases. The upper phase was collected, then the maximum absorbance was determined over the wavelength range 350-550 nm. The maximum absorbance value (λ max) was used to estimate the total carotenoid concentration using the specific extinction coefficient $E_{1\%, 1cm}=2,500$ (Britton *et al.*, 1995).

Statistical analysis

All results are presented as mean±SE. All the data were subjected to a one-way analysis of variance (ANOVA) using SPSS 15.0 for Windows, and Tukey's test was used to determine significant differences between individual means used in assessing the total carotenoids in experimental diets and the effects of the dietary treatments. The differences were considered statistically significant when $p<0.05$. Correlation and regression analyses were used to determine the relationship of a* and H° values on the red zone with the carotenoid deposition in the red scales.

Results

Treatment effects on growth and feed utilization efficiency

The effects of the experimental diets on the growth performance and feed utilization efficiency were shown in Table 3. There were no significant differences in WG, SGR, FCR or PER between the treatments ($p>0.05$). The average WG values, relative to initial weight, across the experimental diets were in the range from 0.69 to 0.84. The SGR values varied from 0.95 to 1.31. The FCR ranging from 2.4 to 3.11 was related to the PER, from 1.69 to 2.15. The survival rates by treatment were between 92% and 96%.

Treatment effects on the body color intensity

The effects of experimental diets on the lightness (L*), redness (a*), yellowness (b*), hue (H°), and chroma (C*) values of fancy carp were shown in Table 4 and in Figs. 1 and 2. There were no significant differences in the L*, a*, and b* values for the red zone between the dietary treatment groups at the beginning of the experiment ($p>0.05$) (Fig. 1). The L* for the red zone of fish fed with the control diet tended to increase during the feeding trial, and by the end of the experiment it was significantly higher than the other treatments ($p<0.05$) (Fig. 1A).

In contrast, the a* for the red zone of fish fed with the control group tended to decrease during the trial, and showed the lowest a* (6.50±0.74) at the end of experiment ($p<0.05$), while the *P. rhodozyma* diet provided the highest a* (26.80±1.26) followed by *Paracoccus*

6

sp. (22.32±1.50), and *H. pluvialis* (21.73±1.34), respectively (Fig. 1B). Moreover, the b* values of the red zone showed no significant differences between the dietary treatments throughout the trial ($p>0.05$) (Fig. 1C). In terms of the white zone, no significant differences in the L*, a*, and b* values were observed between the dietary treatment groups at the beginning of the experiment ($p>0.05$) (Fig. 2). During the feeding trial, the L* for the white zone of fish fed with the control diet tended to decrease and by the end of the feeding trial, it was the lowest for the control group, and was higher in the other groups with carotenoid supplementation (Fig. 2A). Over the first 6 weeks of trial, there were no significant differences in the a* values on the white zone, but at the end of the trial, the highest a* (3.10±0.47) on the white zone was found with *P. rhodozyma* in the feed ($p<0.05$) (Fig. 2B). The b* values of the white zone showed no significant differences between the dietary treatments throughout the trial ($p>0.05$) (Fig. 2C). Nevertheless, the b* value on the white skin zone of fish on the control diet tended to increase significantly ($p<0.05$) during the trial. Furthermore, the highest C* and lowest Hº were found in the fish fed with the *P. rhodozyma* diet (Table 4). Overall, at the end of the trial, the fish fed with natural carotenoid supplements had significantly higher skin redness than the control group ($p<0.05$), and the *P. rhodozyma* diet had the strongest effects on skin coloration in terms of L*, a*, C* and Hº.

Total carotenoid contents of integuments and serum
Effects of the experimental diets on the total carotenoid deposition in the fish skin, the scales and the serum were presented in Table 5. Samples of both white skin and scales showed no significant effects, while the carotenoid concentrations in the red skin and scales had significant differences between the experimental diets. The diet with *H. pluvialis* gave the highest carotenoid levels in both red skin (53.05±1.77) and scales (35.60±0.69), while the control group had the lowest levels ($p<0.05$). The differences between diets with *P. rhodozyma, Paracoccus* sp., and *H. pluvialis,* were not significant. The highest serum carotenoid contents were found with *P. rhodozyma* (1.50±0.13) and *Paracoccus* sp. (1.51±0.14) containing diets, though without statistical significance. When the total contents were compared between the fish skin, the scales and the serum, the carotenoid deposition capacity was the highest in the fish skin, and more carotenoids were deposited in the red than in the white tissues. Expected for, the fish fed with carotenoid supplemented diets had more carotenoids in their skin, scales and serum, than the fish in the control group.

Regression analysis
The relationships between a* and Hº to the carotenoid levels in the red scales were summarized in Table 6. Regression analysis revealed that an increase in a* values matched an increase in the carotenoid deposition in

red scales with $R^2=0.90$, while such change in deposition matched a decrease in H° of the red scales with $R^2=0.92$ ($p<0.05$).

Table 1: The proximate composition (% dry weight) of the control diet

Composition	Contents (%)
Crude protein	19.56±0.00
Crude fat	3.93±0.03
Crude fiber	17.64±0.08
Moisture	14.80±0.03
Ash	11.24±0.00

Values for the proximate composition are expressed as mean±SE.

Table 2: Supplemented pigments (g kg^{-1}) and total carotenoid contents (mg kg^{-1}) in the experimental diets

Item	Control	*P. rhodozyma*	*Paracoccus* sp.	*H. pluvialis*
Pigment (g kg^{-1})	0.00	9.77	5.19	9.58
Total carotenoids (mg kg^{-1})	22.93±1.25[b]	47.40±0.46[a]	48.20±1.27[a]	46.00±1.75[a]

Values of total carotenoids in the experimental diets are expressed as mean ±SE. Different superscripts in the same row of total carotenoids in the experimental diets indicate significant differences at $p<0.05$.

Table 3: The growth performance and feed utilization efficiency of fancy carp when fed with different experimental diets for 8 weeks

Parameter	Experimental diets			
	Control	*P. rhodozyma*	*Paracoccus* sp.	*H. pluvialis*
WG	0.69±0.10[a]	0.84±0.09[a]	0.74±0.18[a]	0.77±0.05[a]
SGR (% day^{-1})	1.18±0.03[a]	0.99±0.04[a]	0.95±0.07[a]	1.31±0.26[a]
FCR	2.40±0.11[a]	2.88±0.12[a]	3.11±0.28[a]	2.49±0.27[a]
PER	2.15±0.10[a]	1.79±0.07[a]	1.69±0.14[a]	2.15±0.29[a]
Survival rate (%)	98.44±1.56[a]	92.25±2.96[a]	96.88±1.80[a]	96.11±1.12[a]

Values are expressed as mean ±SE. Values with the same superscript letter in the same row indicate no significant differences at $p>0.05$.
WG (Weight Gain)=Final body weight (g)-Initial body weight (g)
SGR (Specific Growth Rate)=[ln (final body weight)-ln (initial body weight)]/days of feeding trial×100
FCR (Feed Conversion Ratio)=Total feed intake (g)/Weight gain (g)
PER (Protein Efficiency Ratio)=Weight gain of fish (g)/Protein intake (g)

Table 4: Effects of the different diets on the skin color of fancy carp after 8 weeks of treatment

Color parameter	Dietary supplement			
	Control	***P. rhodozyma***	***Paracoccus* sp.**	***H. pluvialis***
White zone				
L*	77.57±1.72[b]	81.02±0.99[ab]	82.14±0.42[a]	79.28±0.60[ab]
a*	1.45±0.28[b]	3.10±0.47[a]	1.86±0.36[ab]	2.62±0.45[ab]
b*	3.83±0.55[a]	2.94±0.42[a]	3.00±0.50[a]	2.67±0.46[a]
Red zone				
L*	51.33±1.30[a]	41.35±1.55[b]	43.84±1.34[b]	44.20±2.50[b]
a*	6.50±0.74[c]	26.80±1.26[a]	22.32±1.50[ab]	21.73±1.34[b]
b*	26.29±1.70[a]	26.55±1.46[a]	28.53±1.23[a]	27.35±1.73[a]
H°	1.31±0.04[a]	0.78±0.04[b]	0.91±0.03[b]	0.90±0.05[b]
C*	27.26±1.59[b]	38.15±0.86[a]	36.43±1.53[a]	35.36±1.43[a]

Data represent as mean±SE. Different superscripts in the same row indicate significant differences at $p<0.05$.

Table 5: Effects of the different diets on the total carotenoid concentration in the skin, the scales, and the serum of fancy carp, after 8 weeks of treatment

| Total carotenoids (mg kg^{-1})	Dietary supplement			
	Control	*P. rhodozyma*	*Paracoccus* **sp.**	*H. pluvialis*
White skin	10.75±1.58^a	12.88±0.85^a	11.68±1.68^a	13.56±1.62^a
Red skin	42.15±0.13^b	49.45±2.24^{ab}	52.73±4.95^a	53.05±1.77^a
White scales	5.21±0.51^a	5.63±1.21^a	5.24±0.73^a	4.79±0.60^a
Red scales	26.27±2.17^b	35.07±0.90^a	33.20±2.32^{ab}	35.60±0.69^a
Serum (µg ml^{-1})	1.07±0.03^a	1.50±0.13^a	1.51±0.14^a	1.30±0.16^a

Data represent as mean±SE. Different superscripts in the same row indicated significant differences at $p<0.05$.

Table 6: The relationships of a* and H^o on the red zone to the carotenoid content of the red scales

	r	**Regression equation**	R^2	p-**value**
a*	0.95	y=0.46x+23.58	0.90	< 0.05
H^o	0.96	y=-17.90x+49.99	0.92	< 0.05
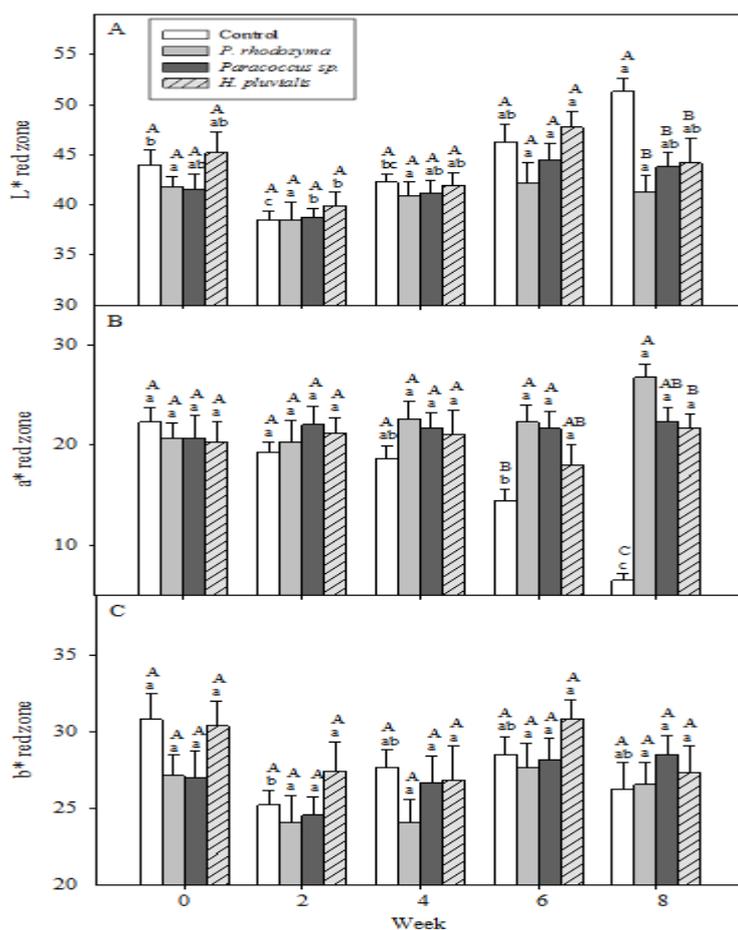
Figure 1: Mean values of L*, a*, and b* on the red skin zone with the four experimental diets observed at 0, 2, 4, 6, and 8 weeks. Different capital letters indicate significance with $p<0.05$ when the experimental diets are compared at a given treatment time. Different small letters indicate significance on comparing different treatment times within one dietary treatment.
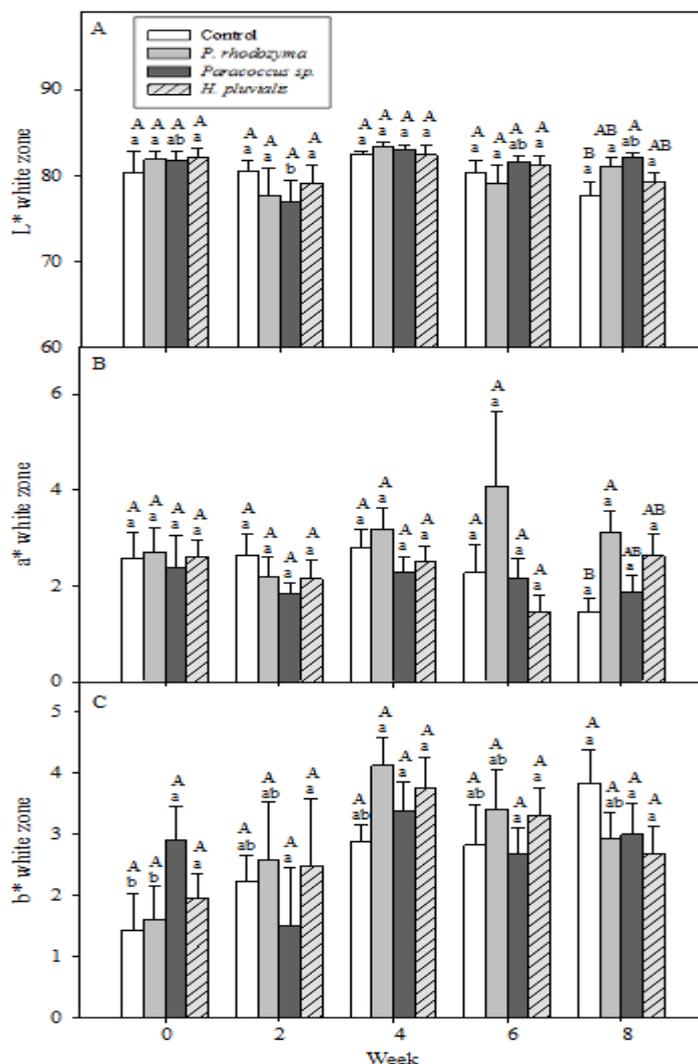
Figure 2: The mean values of L*, a*, and b* on the white skin zone for the four experimental diets observed at 0, 2, 4, 6, and 8 weeks. Different capital letters indicate significance with *p*<0.05 when the experimental diets are compared at a given treatment time. Different small letters indicate significance on comparing different treatment times within one dietary treatment.

Discussion

Effects of natural carotenoid supplemented diets on growth and feed utilization efficiency

Carotenoids are primary class of compounds that can affect the skin coloration of fish. Besides their beneficial effects on pigmentation, carotenoids also play a significant role in enhancing nutrient utilization that may contribute to survival and growth performance (Shahidi and Brown, 1998; Amar *et al.*, 2001). In the present study, fish fed with carotenoid supplemented diets did not significantly differ from the control group in the growth, feed utilization efficiency or survival rate. These results are in accordance with previous studies carried out with gilthead seabream

(*Sparus aurata*) (Gomes *et al.*, 2002), red porgy (*Pagrus pagrus*) (Chatzifotis *et al.*, 2005; Kalinowski *et al.*, 2005), goldfish (Yeşilayer *et al.*, 2011) and large yellow croaker (*Larimichthys croceus*) (Yi *et al.*, 2014). However, in some studies a dietary carotenoid supplement improved the growth and feed utilization efficiency in fish (Liang *et al.*, 2012; Sun *et al.*, 2012).

Effects of carotenoid supplemented diets on color parameters

The color parameters have been widely used to quantify fish skin coloration. In this study the inclusion of natural pigment in the diets significantly improved skin pigmentation. The fish fed with *P. rhodozyma* containing diet had the highest a* and C*, and the lowest L* and Hº in the red zone, while the control group had the lowest a* and C* and the highest L* and Hº. Interestingly, the red zone a* values of fish fed with the control diet tended to decrease during the trial, while the carotenoid supplementation maintained or improved skin redness. The continuous decrease in the skin redness of the fish fed with the control diet may be explained by the effects of carotenoid suspension in a control group. Since the fish had been raised in an earthen pond, which contained a variety of phytoplanktons, and fed with a commercial fancy carp diet for a long period of time before they were used in the experiment. Thus, fish had accumulated carotenoids from phytoplanktons and mainly from the commercial fancy carp diet, prior to the dietary treatments of the trial. The loss

of pigment supplementation in the feed caused a slight decrease in redness on the skin over the first 4 weeks, followed by a significant drop over the rest of the trial. During carotenoid suspension, the fish cannot synthesize carotenoids in their body. Therefore, the initial carotenoid contents continuously decreased, due to utilization in intermediary metabolism and other functions, and this affected the skin redness. A similar result to this study has been reported by Yuangsoi *et al.* (2010), who also observed that carotenoid suspension decreased the redness of fish skin. We note that, in our case, it took at least ten weeks for a significant drop in the skin pigmentation, appearing as faded color.

The b* value indicates the purity of white skin, with higher b* corresponding to more yellowness. The fish fed with *P. rhodozyma* in the diet did not significantly differ from the other treatment groups in the b* value. Meanwhile, the L* values on the red zone of fish fed with dietary pigments were significantly lower than the fish fed with the control diet, especially so with the *P. rhodozyma* diet. This is probably related to the progressive accumulation of carotenoid pigments in the red zone. Similar results with decreasing L* during carotenoid supplementation have been reported in rainbow trout (*Oncorhynchus mykiss*) (Teimouri *et al.*, 2013) and large yellow croaker (*L. croceus*) (Yi *et al.*, 2014). Furthermore, Pérez-Escalante *et al.* (2012) and Liu *et al.* (2014) have been reported that carotenoid supplementation may stimulate

chromatophore production and increase the pigment granules in chromatophores, thus ultimately increasing the fish coloration. Additionally, these chromatophore quantities on the fish tissues were dependent on the body zone (Pérez-Escalante *et al.*, 2012).

Overall, the fish fed with *P. rhodozyma* in the diet had the most redness in their skin color among all the dietary treatments. This indicates that the fish may utilize *P. rhodozyma* more efficiently than the other experimental diets to improve skin pigmentation. Such effects may be the result of cell wall disruption of *P. rhodozyma* before inclusion into the fish feed which enhance carotenoid bioavailability, and facilitate digestion and assimilation by the digestive tract. A similar explanation has been reported by Johnson *et al.* (1980) who demonstrate that trout fed with fully disrupted yeast showed more improvement in their pigmentation than those fed with only partially disrupted red yeast. Moreover, since carotenoid in *P. rhodozyma* is accumulated in the lipid droplets of cytoplasmic membranes (Johnson and An, 1991), these lipid droplets may improve the absorption of the lipid soluble pigments as reported by Guerden *et al.* (1998). The cell wall disruption and the lipid droplets of *P. rhodozyma* therefore may increase carotenoid absorption in fancy carp, leading to the most improvement in the skin redness as observed in the current study.

Carotenoid accumulation in fish skin, scales and serum

The efficiency of carotenoid deposition in some specific tissues, organs or specific positions of the fish skin is dependent on various factors, including the fish species, the carotenoid sources, quantities of chromatophores, and the distribution pattern and carotenoid deposition capacity (Barbosa *et al.*, 1999; Liang *et al.*, 2012; Pérez-Escalante *et al.*, 2012; Liu *et al.*, 2014; Yi *et al.*, 2014). In our results, there was no significant difference for carotenoid accumulations in the skin, scales, or serum among fish fed with carotenoid supplemented diets. This suggested that these dietary pigments had a similar accumulation of carotenoids. When we considered the total carotenoid concentrations across all dietary treatments, the carotenoids were more deposited in the skin than in the scales, as well as more in the red than in the white zones. These results were in agreement with the study of Liang *et al.* (2012) who state that carotenoids in fancy carp were mainly deposited in the skin more than in the scales. The possible explanation for these observations is that the deposition capacity of carotenoids and the presence of chromatophores may be higher in the skin than in the scales and also may be higher in the red than in the white zones. Besides the factors mentioned above, the number of adipose cells and the fat deposition levels in the fish may also affect the efficiency of carotenoid deposition as described by Qiufen *et al.* (2012). In addition, Mahboob *et al.* (2014)

reported that the fat content in *C. carprio* is higher in the skin than in the scales. These suggested that the fat content of the skin may facilitate the accumulation of lipid soluble pigments, leading to more carotenoid deposition in the skin than in the scales as observed in our study.

Relationship of body color and carotenoid content

In the present study, a* and Hº measured on the red body zone were linearly related to the total carotenoid content in the red scales. Our results are in agreement with previous studies by Storebakken *et al.* (2004), Teimouri *et al.* (2013) and Yi *et al.* (2014) who reported that the color parameters and the carotenoid contents in fish tissues had a linear relationship. In addition, the color parameters measured under standard conditions could be used to estimate the total carotenoid contents in the tissues (Storebakken *et al.*, 2004). This suggests that a* and Hº on the red body zone could be used to estimate the total carotenoid content in the red scales of fancy carp.

Acknowledgments

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