

Improving skin coloration of koi carp (*Cyprinus carpio*) fed with red dragon fruit peel meal

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Abstract. Generally, synthetic astaxanthin is used to maintain and enhance the skin color of ornamental fish. However, it is expensive and cause increasing cost production of aquaculture because of feed cost. Therefore, it should be looked for alternative ingredient for skin coloration to replace high price of synthetic astaxanthin with cheaper and locally available product. The objective of this study was to evaluate the influence of different dosage of red dragon fruit peel meal as an alternative pigment source on the skin coloration of ornamental koi carp fish (*Cyprinus carpio*). Three experimental diets were formulated with three different dosages of red dragon fruit by product meal (RDFM), namely 5% (Diet A), 10% (Diet B) and 15% (Diet C) of RDFM in the diet and one of commercial diet (Diet D) as a control diet. A total of 60 koi carp (initial weight: 1.43 ± 0.14 g) were distributed in 12 glass tanks (5 fish/tank) size: 20×20×35 cm. The fish were fed diet in two times a day (08.00 a.m. and 04.00 p.m.) and till satisfied for 42 days of feeding trial. Variables observed were weight gain, skin color brightness and survival rate. The skin color brightness was determined by using Toca Colour Finder methods. After six weeks of feeding, there were significant differences ($p < 0.05$) in skin coloration in orange. The fish fed with the diet containing 15% of RDFM has a more intense orange color (3.5 of 4 value) and black color (4.3 of 5 value), respectively. This study concluded that supplementation of 10% RDFM in the diet could be added into the diet to enhance skin coloration of koi carp.

Key Words: synthetic astaxanthin, alternative pigment source, toca colour finder, weight gain, survival rate.

Introduction. The koi fish (*Cyprinus carpio*) is one of the most precious ornamental fish in the world and In Indonesia. It is an imported fish from Japan for this reason; a large number of fish are annually propagated and farmed. The price of ornamental fish including koi carp depend on the skin color brightness of fish. Fish coloration and pigmentation of koi is due to absorption and deposition of carotenoid in the body. Skin coloration of fish reared in aquarium will be pale if their feed will contain no carotenoid, because fish couldn't synthesize *de novo* carotenoid in their body and therefore, it should be supplemented in their diet (Kurnia et al 2007, 2008).

To obtain the good performance of skin coloration of ornamental fish, generally their diet it is supplemented with synthetic carotenoids such as astaxanthin and cantaxanthin. However, synthetic carotenoids are costly and have deteriorating effects on the environment. Hence, there is a great demand for inclusion of natural carotenoids in aqua feed to ensure bright coloration in fish. Utilization of low cost pigment ingredients should be supplied in the ornamental fish diet. Gupta et al (2007) reported the possibility of using naturally available carotenoid rich ingredients such as micro algal *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina*, *Phaffia rhodozyma*, *Xanthophyllomyces dendrorhous* that have received much attention. Plant sources like *Spirulina* have been used as a source of carotenoid pigments for trout and koi (Choubert 1979; Boonyaratpalin & Phromkunthong 1986; Alagappan et al 2004) while marigold petal was used for ornamental fishes like sword tail and barb (Boonyaratpalin & Lovell 1977).

One of potentially carotenoid ingredient from plant is dragon fruit peel (*Hylocereus polyrhizus*). Dragon fruit peel could be utilized as natural pigment in the food production, industry and pharmacology industries (Cahyono 2009). The peel of dragon fruit is about 30-35% of total fruit and it is always treated as a residue. Dragon fruit peel contain anthocyanin as natural pigment which is used for alternative red color in food products as substitution of synthetic pigment and it has high antioxidant effect (Li et al 2006; Kristanto 2009). Anthocyanin is a group of red and blue pigment compounds found in many plants. Anthocyanin is classified as pigment known as flavonoid. Extract of red dragon fruit peel contain 26.5 ppm of anthocyanin (Handayani & Rahmawati 2012). The red dragon fruit mostly contain β -caroten (Farikha et al 2013). According to Mahattanatawee et al (2006) the red dragon fruit contains 0.005-0.12 mg/100 g of carotenoids. Use of low cost and easily available natural additives to enhance coloration of fishes, minimize the expenditures and help ornamental fish entrepreneurs to maximize their earnings. Red dragon peel meal, as waste of fruit, is easily available in local market and can be smoothly incorporated as feed ingredient. Red dragon fruit peel meal has been chosen for this reason as a color enhancer and increasing fish growth.

The aim of the present study was to observe the effect of supplementation of red dragon peel meal in the diet on coloration and growth performance of koi fish.

Material and Method

Experimental protocol. The feeding trial was conducted at the Laboratory of Breeding and Fish Production, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia for a three months of rearing period between January and March 2018. The koi fish were obtained from ornamental fish division, Fisheries and Marine Affair Department of Southeast Sulawesi Province. All the fish were prophylactically treated with a sodium chloride salt bath (1%) for several hours; acclimatized for one weeks until the experiments began, in one square fibertank (sized 200×50×40 cm) with 60 koi fish stocked. After acclimation, five fish of showa koi carp (initial weight: 1.43 ± 0.14 g) were randomly distributed into 12 aquarium glass tanks (25 L, 25x20x25 cm). About one-third of the water was exchanged every day, and replaced with filtered freshwater from a well, kept in a reservoir tank at room temperature. Water quality conditions in each tank were monitored and maintained at 24-26°C, a dissolved oxygen level of 5-7 mg L⁻¹, pH of 7.0-7.1, and the fraction of unionized ammonia was never detected above 0.01 mg L⁻¹. The room where the experiment was conducted, was illuminated with a fluorescent light set to a 12 h light (1,200 lux) and 12 h darkness cycle. The feeding trial had a duration of 45 days.

Experimental diets. Three experimental diets were formulated to contain three doses of red dragon fruit meal (RDFM) and one commercial diet as a control diet. The ingredient compositions of the diets are presented in Table 1. Diet 1 was formulated to contain 50 g kg⁻¹ RDFM; diet 2 contained 100 g kg⁻¹ RDFM; diet 3 contained 150 g kg⁻¹ RDFM; and diet 4 was commercial diet as a control diet. The ingredients and proximate composition of four experimental diets are shown in Table 2. All ingredients were finely ground in a hammer mill and passed through a 250- μ m mesh sieve and weighed according to the composition of diet. The ingredients of each diet were mixed in a 3D dynamic mixer. The ingredients of each diet were blended to a homogeneous mixture. Water (approximately 50% of the diet weight) was added, and the dough was extruded to form strands and then pelleted to suitable size through a 2.5 mm die using a multifunctional spiral extrusion machinery. The pellets were dried outside (open air) for two days and then placed at room temperature and sealed in plastic bags. The fish were fed to apparent satiation twice a day (08.00 a.m. and 04.00 p.m.).

Table 1
Formulation and proximate analysis of experimental diet

<i>Ingredients</i>	<i>Diets (g/kg diet)</i>			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D (commercial diet)</i>
Fish meal	210	210	210	-
Shrimp head meal	200	200	200	-
Soybean meal	250	200	150	-
Dragon fruit peel meal	50	100	150	-
Corn meal	55	55	55	-
Sago meal	50	50	50	-
Wheat flour meal	40	40	40	-
Brain by product meal	80	80	80	-
Fish oil	5	5	5	-
Squid meal	5	5	5	-
Corn oil	5	5	5	-
Mineral and Vitamin mix.	50	50	50	-
Total	1000	1000	1000	

Table 2
Results of proximate analysis of experimental diets

<i>Nutrient (%)</i>	<i>Diet (% dry weight)</i>			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Crude protein	26.23	24.34	26.23	34.09
Crude lipid	7.21	8.27	6.75	3.41
Fiber	11.41	11.41	11.44	4.00
Crude ash	13.73	15.34	14.85	12.0
NFE ¹	41.42	40.64	40.73	46.50
GE ²	384.48	380.66	377.24	413.61

¹NFE: nitrogen free extract; ²GE = gross energy (Watanabe 1988); 1 g protein = 5.6 kcal, 1 g NFE = 4.1 kcal, 1 g lipid = 9.4 kcal.

Skin color observation. Observation of color brightness of fish skin was conducted by eight panelists with no color blindness and their objective in observation. The observation of fish skin was performed two times (at the beginning and at the end of experiment) by using Toca Colour Finder (TCF) modified method. Color was judged by test panels of eight persons randomly recruited from lecturers and students of the Department of Aquaculture, Faculty of Fisheries and Marine Science Halu Oleo University. The treatments were not revealed to the individuals who were asked to rank the fish according to intensity of color. Two kinds of color determined were orange and black. Color ranking was by a score of 1-7 (one being the lowest) for fish with four treatment groups (Figure 1). Scores were subjected to ANOVA and was continued with Duncan test if there was one or more treatment which showed significant difference.

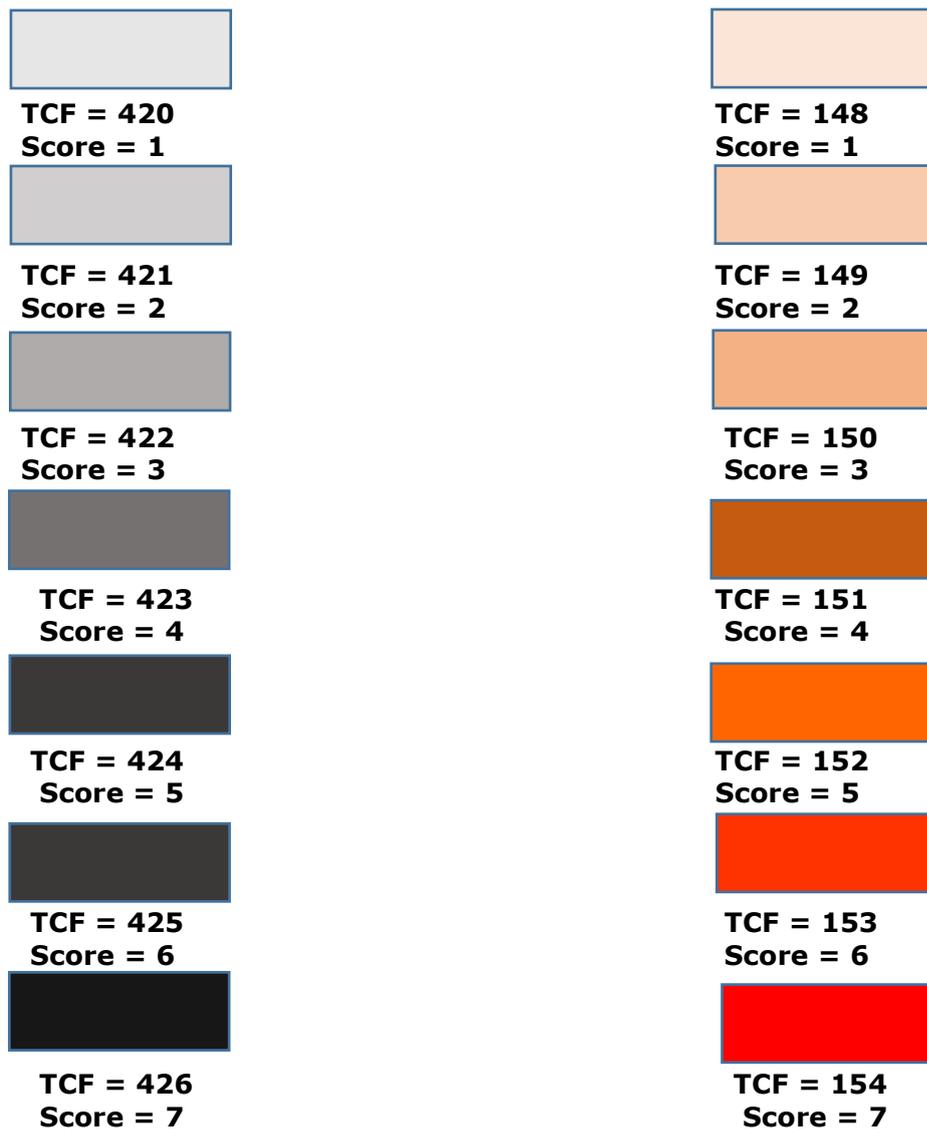


Figure 1. Direction for colour determination of koi fish by using Toca Colour Finder.

Results. The water quality such as water temperature, pH, and dissolve oxygen (DO) was measured and recorded every day during feeding trials. During the feeding trial, the temperature, pH and DO were varied between 28.0 and 29.0°C, 6.5 and 7.0 and 2.5 and 4.9 mg L⁻¹, respectively. At the end of experimental time (45 days of rearing period), weight gains, specific growth rate, and survival were not significantly different among fish fed different diets (Table 2). Weight gains of fish for 45 days of rearing were ranged between 1.28±0.5 and 1.38±0.1 g. There were no significant differences in fish growth at different level of supplemented RDFM in the diet.

Results of color observation by panelists for black color of fish skin showed that increasing dosage of dragon fruit peel meal supplemented in the diet was followed by increasing of skin brightness. Statistically, analysis of variance showed that the fish fed with experimental diets were significantly different in black skin color (P<0.05). Total score of panelist for skin black color is showed in Figure 2. The results showed that total highest score of fish fed with 15% of RDFM in the end of experiment had enhancements in black color brightness of their skin (Total score: 108.0) and the lowest score was observed in the fish fed with 5% of RDFM in the diet (Total score: 95.4 value) as showed in Figure 3. In addition, the highest average score of black skin color was also observed in fish fed with 15% RDFM (average score: 4.5) and the lowest one was observed in fish fed with 5% RDFM in the diet (average score: 3.97).

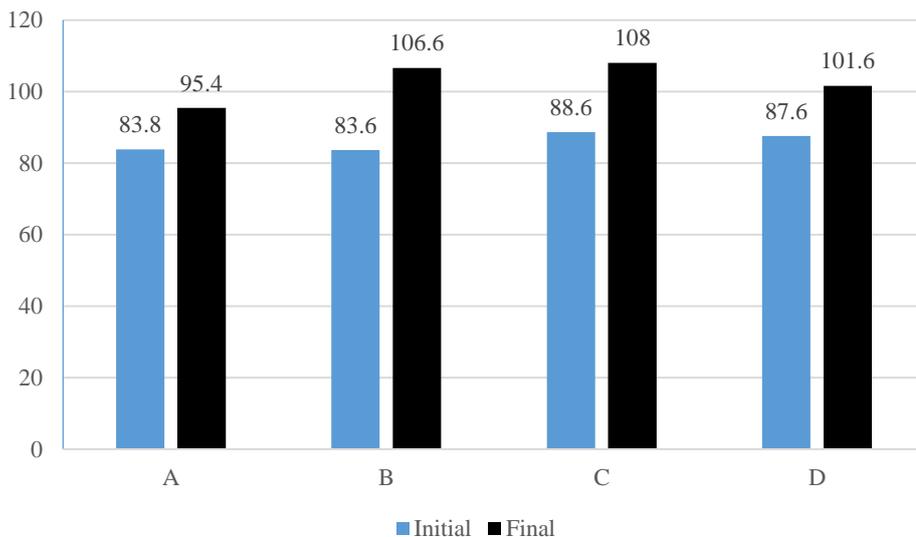


Figure 2. Total score of black color brightness of koi carp skin. Diet A: 5% RDFM, Diet B: 10% RDFM, Diet C: 15% RDFM and Diet D: Ornamental fish commercial diet.

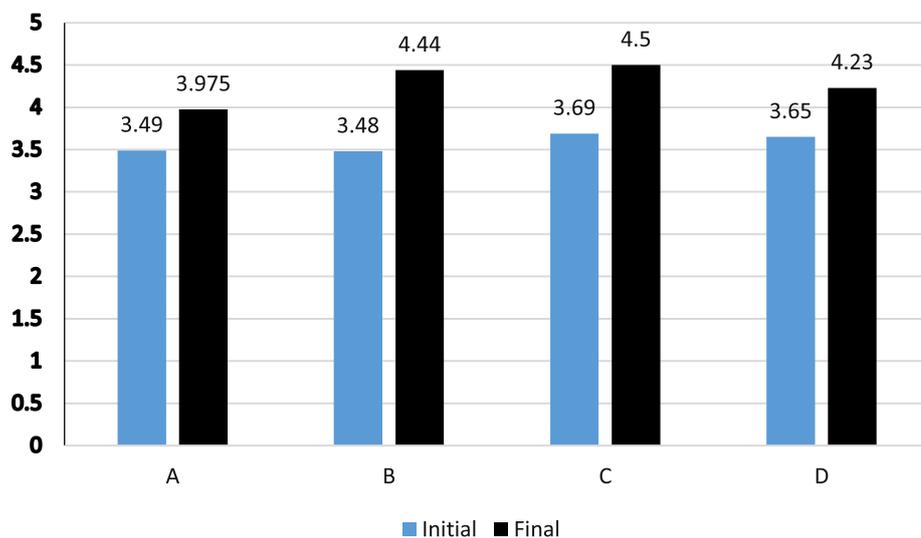


Figure 3. Average score of black color brightness of koi carp skin. Diet A: 5% RDFM, Diet B: 10% RDFM, Diet C: 15% RDFM and Diet D: Ornamental fish commercial diet.

On the other hand, the observation on orange color brightness of the fish skin was also found that the fish fed with 15% of RDFM had highest in orange color brightness (total score: 87.8) and the lowest score was observed in the fish fed 5% of RDFM (total score: 66.0) as shown in Figure 4 and Figure 5. Results of ANOVA showed that the fish fed experimental diets was significantly different in orange color brightness concerning fish skin ($P < 0.05$). Total score was recorded by eight panelists showed that the fish fed 15% of RDFM in the diet could improve skin brightness either black or orange color. Total score of black skin color was higher than total score of orange skin color as shown in Table 3.

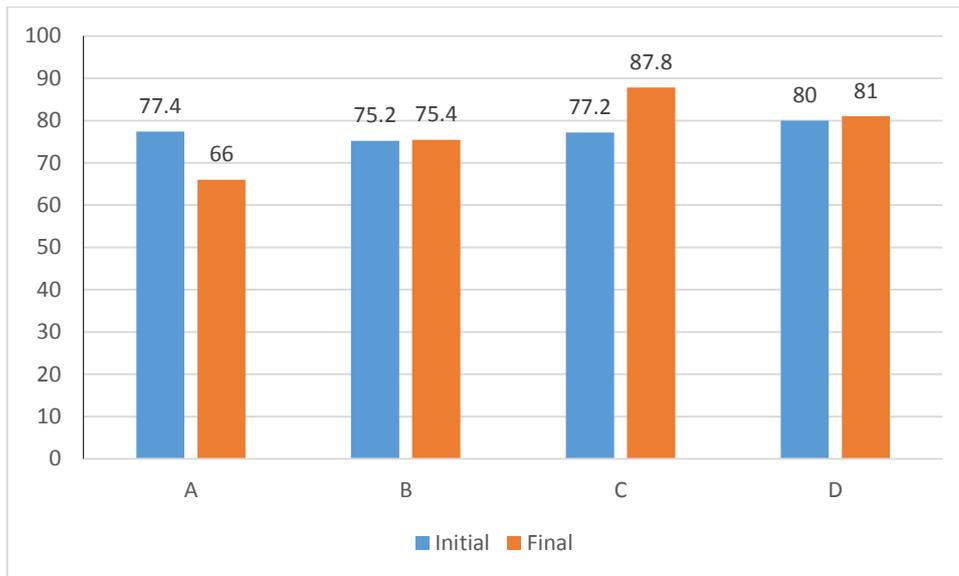


Figure 4. Total score of orange color brightness of koi carp skin. Diet A: 5% RDFM, Diet B: 10% RDFM, Diet C: 15% RDFM and Diet D: Ornamental fish commercial diet.

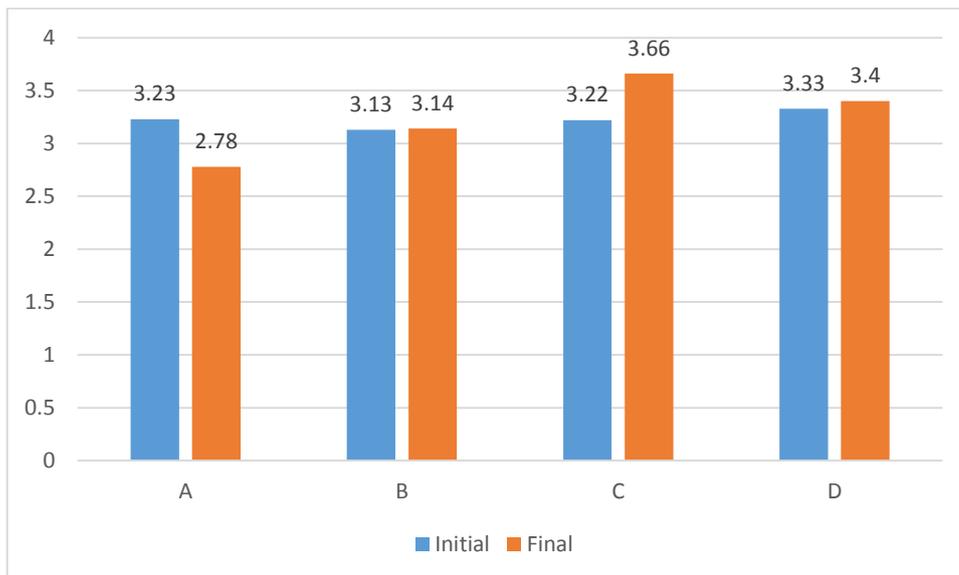


Figure 5. Average score of orange color brightness of koi carp skin. Diet A: 5% RDFM, Diet B: 10% RDFM, Diet C: 15% RDFM and Diet D: Ornamental fish commercial diet.

Table 3

The results of total score of skin color brightness at the end of experiment

<i>Treatments</i>	<i>Black color of skin</i>	<i>Orange color of skin</i>
Diet A (5% of RDFM)	95.4 ^a	66.0 ^a
Diet B (10% of RDFM)	106.6 ^c	75.4 ^b
Diet C (15% of RDFM)	108.0 ^c	87.8 ^d
Commercial diet	101.6 ^b	81.0 ^c

As a result in Table 3 is showed that total score of black skin color of fish fed the diet contained RDFM was higher than total score of orange skin color of fish. This indicated that supplementation of RDFM in the diet tend to increase black skin color brightness against orange skin color brightness. Statistically, the skin color of fish either black or orange color were significantly different in all treatments ($P < 0.05$). The fish fed with 15% of DFRM was more intense in skin coloration than in other treatments.

Discussion. The price of ornamental fish is determined by their coloration. In fish physiology, organs that are responsible for controlling the fish coloration are the endocrine and the nervous system. Coloration of fish is also determined by carotenoid sources from the diet. Generally, fish including ornamental fish couldn't synthesize de novo pigment (Chatzifotis et al 2005). Enhancement of color intensity is affected by two groups of factors, internal and external factors. Internal factors are permanent factors such as age, size, genetics, sexes and capability of fish to absorb nutrients from the diet. While, external factors come from outside of the body, for example, water quality, light and feed quality and beta carotene source (Lesmana & Satyani 2002). Supplementation of carotenoid source in the diet such as RDFM could improve the color intensity of fish. Generally, the fish could absorb carotenoids that included in the diet to perform pigment synthesis in order to increase the color of fish body (Kurnia et al 2007). The physiological change of fish color is affected by the moving of pigment granules either gathering or distributing in cell pigments are caused by temperature, light and other factors. Pigmentation in fish is controlled by nervous system and two chemical compounds produced by nervous system namely, epinephrin and acetylcholine (Evan 1993). According to Mara (2010) color formation in the fish is caused by the fact that carotenoids are being fat soluble. Then, carotenoids will be absorbed in intestine by using pancreatic lipase enzyme that will hydrolyze trygliseride to become monoglyseride and fatty acid. Carotenoids in the cytoplasm in cell mucosa of intestine will break down to retinol and absorbed by cell wall together with fatty acid absorption and combined with micelle and absorbed in limphatic tract.

This study showed that increasing dosage of RDFM in the diet could increase the color brightness of the fish skin. After 45 days of feeding the diets, the change in skin color of the koi carp could be easily distinguished within the skin color. Similarly to our results, increasing supplementation of carotenoids could improve the skin color of koi carp fish after 60 days of feeding trial was also reported by Andriani et al (2018). Enhancement of color intensity in skin coloration of koi indicates that carotenoid or color pigment that originated from RDFM supplemented in the diet could improve color intensity of koi fish. Some authors had proved that increasing pigment supplementation in the diet could increase the color brightness of some fish species (e.g., Duncan & Lovell 1993; Storebakken et al 1987; Chatzifotis et al 2005; Dharmaraj & Dhevendaran 2011; Ho et al 2014).

The diets used in this study contained different dosage of red dragon meal. After 45 days of rearing, the skin colour of the fish fed with test diet had differed from the initial colour of the fish. Studies reported differentiation of skin colour on ornamental goldfish fed with an astaxanthin-supplemented diet against fish fed control diet after 7 days (Paripatananont et al 1999). In a case of orange-red skin color oranda goldfish fed commercial diets that contained astaxanthin, lutein, or zeaxanthin it took about 28 d to observe differences from control (Wallat et al 2005). In *Salmo salar* more astaxanthin was deposited in the skin after 21 days of feeding a diet supplemented with astaxanthin compared to fish on a control diet (Storebakken et al 1987). Similarly, on an astaxanthin-rich diet, after a 21-day period of feeding, high levels of astaxanthin (40 mg kg⁻¹) were also reported in the skin of the *Sparus auratus* (Gomes et al 2002).

The fish fed with diet contained RDFM resulted higher of total score in black skin color brightness than orange skin color brightness. The results indicated that RDFM supplemented in the diet tend to increase black pigment comparing with orange pigment. RDFM meal contains anthocyanin. Similar to our study, Baron et al (2008) reported that supplementation of anthocyanin-based (Overseal *Carantho* powder) pigments in the diet did not affect orange skin color of dwarf gourami. On the other hand, this experiment found that RDFM supplemented in the diet could improve black skin color of koi fish. This founding is similar with the work of Pérez-Escalante et al (2012), which revealed that anthocyanin supplemented in the diet could increase melanophore cell as black pigment.

Conclusions. The present research concluded that supplementation of RDFM as natural pigment source in the diet didn't affect the growth of Koi carp. On the other hand supplementation of 10% and 15% of DRFM in the diet could improve the skin black color

brightness of Koi carp, however, it could not affect the skin orange color brightness. Therefore, the optimum dosage of red dragon fruit meal in the diet to increase the skin color brightness of Koi carp was 10% of red dragon fruit meal.

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