การสร้างแคโรทีนอยด์ในรูปนาโนพาร์ติเคิลจากน้ำมันปาล์มดิบสำหรับเร่งสีผิวปลาทอง (*Carassius auratus*)

Creation of Nanoparticle Carotenoid from Crude Palm Oil to Enhancing the Skin Color of Goldfish (*Carassius auratus*)

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บทคัดย่อ

การเพาะเลี้ยงปลาสวยงามจำหน่ายเป็นอาชีพที่สามารถเพิ่มมูลค่าให้กับสินค้าได้เป็นอย่างดี โดยเฉพาะอย่างยิ่งปลาที่มีสีสันตามที่ตลาดต้องการ สารสีจากธรรมชาติถูกนำมาใช้ในการเพิ่มสีสันของปลาสวยงาม เนื่องจากปลาสวยงามไม่สามารถสร้างแคโรทีนอยด์ได้เอง จำเป็นต้องได้รับจากอาหารเท่านั้น แต่พบปัญหาเรื่องการเสื่อมสภาพของแคโรทีนอยด์และความสามารถในการดูดซึม การศึกษานี้มุ่งเน้นในการพัฒนาการสร้างอนุภาคขนาดเล็กเพื่อเก็บกักแคโรทีนอยด์จากปาล์มน้ำมันสำหรับเพิ่มสีผิวปลาทอง แคโรทีนอยด์ในขนาดนาโนพาร์ติเคิลถูกสร้างขึ้นโดยใช้กระบวนการก่อเกิดเจลแบบไอออนโนโทรปิก (ionotropic gelation) แบ่งสารผสมที่ได้ไปผ่านกระบวนการทำแห้งแบบแช่เยือกแข็ง จากนั้นถ่ายภาพด้วย Scanning electron microscopes (SEM) และใช้โปรแกรม Image J วัดขนาดอนุภาค พบว่ามีค่าเฉลี่ยของ nanoparticle carotenoid (NPC) มีค่าเท่ากับ 131.56 ± 32.35 nm และแบ่งไปวิเคราะห์ขนาดและการกระจายตัวของอนุภาคด้วย Zetasizer พบว่ามีขนาดเฉลี่ย 177.567 ± 14.00 nm และมีค่าศักย์ไฟฟ้าที่ผิว (zeta potential) เท่ากับ -30.233 ± 0.702 mV เมื่อนำแคโรทีนอยด์ขนาดนาโนใช้เป็นแหล่งแคโรทีนอยด์ในอาหารปลาทอง 6 ระดับที่ความเข้มข้น 0, 100, 200, 300, 400 และ 500 µg kg^{-1} เมื่อเลี้ยงเป็นเวลา 8 สัปดาห์ วัดสีผิวด้วยระบบ CIE (L*, a*, b*) และ ระบบ (L*, c*, h*) ผลการทดลองพบว่าสารสกัดที่ระดับความเข้มข้น 500 µg kg^{-1} ให้สีผิวของปลาเข้มที่สุดอย่างมีนัยสำคัญ ($p<0.05$) โดยไม่มีผลกระทบต่ออัตราการรอดตาย อัตราการเจริญเติบโต รวมถึงผลกระทบข้างเคียงอื่นๆ ที่มีต่อสุขภาพปลา

คำสำคัญ : แคโรทีนอยด์ ; ปลาทอง ; นาโนพาร์ติเคิล ; การเร่งสี ; สารสี

Abstract

Production and trade of ornamental fish are profitable because of their aesthetic value. Fish that have particular colors and shapes are supplied to fulfill customer orders. Natural pigment is added to the skin color of ornamental fish by feed but reduction in ability of pigment absorption can cause color deterioration. Development of nanoparticle carotenoid from crude palm oil was studied to enhance goldfish skin color. Carotenoid nanoparticles were created by an ionotropic gelation process. A prepared solution containing carotenoids was freeze-dried and captured by a Scanning Electron Microscope (SEM). Mean particle diameter was determined as 131.56 ± 32.35 nm with Image J program and particle size and electrical potential of the surfaces were assessed using a Zetasizer, mean diameter was 177.567±14.00 nm with electrical potential of -30.233 ± 0.702 mV. NPC was incorporated into the feed at 0, 100, 200, 300, 400 and 500 μg kg^{-1} feed and fed to goldfish for 8 weeks. Measurement of goldfish color was performed by the CIE (L*, a*, b*) and (L*, c*, h*) system. The goldfish skin color intensity measured by CIE had shown that the fish fed with 500 μg kg^{-1} containing significantly highest skin color ($p<0.05$) without interfering on growth rate, survival rate and other side effect was not observed.

Keywords : carotenoid, goldfish, nanoparticle, enhancing color, pigmentation

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Introduction

Carotenoids are naturally yellow-red pigment synthesized by photosynthetic organism and non-photosynthetic like eukaryotes and prokaryote. However, it was usually found in plants and subsequently acquired by animals (Ho *et al.*, 2013), have an important role on coloration, reproduction, immunity and antioxidant capacity in all vertebrates (Christian *et al.*, 2018; Villar-Martínez *et al.*, 2013; Yahia, 2018). However, animals cannot fully synthesize carotenoids and also have limited supply from their natural diet (Gouveia *et al.*, 2003; Yanong, 1999). Under intensive breeding and feeding with inadequate color additives, fish tend to lose their coloration. The value of fancy carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) increases with intensity of skin color is one of the most important quality criteria setting the market value of ornamental fish (Wallat *et al.*, 2005; Yanar *et al.*, 2008). As in the goldfish farm use carotenoids to enhance fish coloring such as astaxanthin or zeaxanthin that are both natural and synthetic substances in ratio of 100 mg kg^{-1} of food.

However, these imported food additives are high-cost (Li *et al.*, 2018). Palm seed oil is a lipid extract from fresh mesocarp of the fruits from the oil palm tree (*Elaeis guineensis*),containly the highest carotenoid content.(Chiu *et al.*, 2009; Ping & Gwendoline, 2006), with high productivity as the most consumed vegetable oil. Furthermore, the composition of the carotenoids in crude palm oil shows that α- carotenes (35.16%) and β-carotenes (56.02%) are the major components. (Zou *et al.*, 2012). Production of palm oil for human consumption involves a refining process to remove carotenoids (Ng, 2002; Ribeiro *et al.*, 2018). This is done because consumers prefer to buy oil that has a clear color. The dark orange colored carotenoids are commonly removed by absorbents such as activated carbon, acid-activated clay or neutral earth (Almeida *et al.*, 2019; Ribeiro *et al.*, 2018; Silva *et al.*, 2013, 2014), generating industrial waste. However, these pigments are sensitive to quick degradation, especially by heat and oxygen; therefore, the processing and extraction process may alter the amount and bioavailability of the obtained pigment. However, a group of cyprinid fish, especially carp and goldfish, have the highest ability to transfer carotenoids to the skin. Bjerkeng (2008) describe carp type, which can use and convert lutein into astaxanthin. Also, several carotenoids in various forms and these sources vary in their digestibility and complicated to interpret. Carotenoid in nanosized can be increased the selectivity of drug action, stability rate and adsorption and bioaccessibility as specific interaction with certain tissue (Sergeev & Klabunde, 2013). Accordingly, we hypothesized that carotenoids, extracted from crude palm oil (CPO) were encapsulated into alginate nanoparticles would enhance their stability, for help carotenoid absorption and storage in skin of goldfish for used as a color enhancing substance combined in fish feed after 16 weeks of treatment on body weight and making their pigmentation efficiency, resulting in their vivid color.

Methods

Preparation of carotenoid concentrate of crude palm oil via saponification.

CPO from palm seed pulp was obtained from Udomchai Palm Oil Co., Ltd., Tha Sae district, Chumphon Province, Thailand. The methodology followed Ping and Gwendoline (2006); Rodriguez-Amaya and Kimura (2004). CPO (100 g) was dissolved in 600 ml absolute ethanol and then 100 ml of aqueous KOH (50% wt v^{-1}) was added and homogenized. The mixture was added 0.01% Butylated hydroxytoluene (BHT) and refluxed in a water bath at 100°C for 1 hour. Then, 1,000 ml diethyl ether/hexane (50:50 by volume) was added to extract the carotenoids. The extraction was repeated until all the non-saponified materials were recovered. The extract was washed with water until neutral, dehydrated with anhydrous Na_2SO_4 and evaporated at 40°C to dryness in a stream of nitrogen. Total carotenoid concentration was determined using an Ultraviolet- visible Spectrophotometer (Agilent Cary 60 UV-Vis Spectrophotometer, USA) at 450 nm. Carotenoid concentration was calculated according to the equation (1) of Rodriguez-Amaya and Kimura (2004)

$$\text{Total carotenoid } (\mu g\ g^{-1}) = \frac{A \times B \times 10^4}{A\ (1\%)\ /\ (1cm)} \quad (1)$$

Where, A = absorbance; B = total volume of extract; A 1% $1cm^{-1}$ = Absorbance at 450 nm which is recommended for mixtures.

Carotenoid Nano encapsulated preparation

Nanoparticle carotenoid (NPC) were produced in two-steps. The first consisted of emulsion and internal gelation. An emulsion was prepared using the water in oil (W/O) emulsion technique according to Lertsutthiwong *et al.* (2008) with ionic crosslinking in a sodium alginate solution. A solution of alginate (0.6 mg ml^{-1}) was spread at high-frequency waves and then mixed with 5% (wt v^{-1}) ethanolic carotenoids using polyoxyethylene sorbitan monooleate (Tween 80) solution 1% as a binder. The particles were added with $CaCl_2$ solution 0.67 mg ml^{-1} at 0.2 psi using a 0.5 mm nozzle spray adapted from Arpagaus *et al.* (2018), This divided the solution into two parts to compare particle size. 1) The solution was freeze-dried and captured by a Scanning Electron Microscope (SEM: SU 8020: Hitachi High-Technologies Corporation, Japan) and measured by with the Image J program. 2) Particle size was analyzed by dynamic light scattering (dls; z-average), with diffraction of dynamic light scattering in the colloid stage for electrical potential surfaces using a Zetasizer® (Nano-ZSP, Malvern Instruments, UK). Samples were previously diluted in a prefiltered (0.45 µm) aqueous solution at 1% (v v^{-1}). Carotenoid concentrations were calculated for diet formulae in the next experiment.

Experimental diets

Six experimental basal diets were supplemented with NPC at concentrations of 0, 100, 200, 300, 400 and 500 µg kg^{-1} and analyzed according to Proximate Analysis; AOAC (2000) (following crude protein with Kjeldahl method, crude fat with solvent extraction methods, ash with direct method, moisture with air oven method and crude fiber analysis with filter bag technique), Proximate composition of the diet was composed of 33.16% crude protein, 10.75% crude fat, 9.12% ash, 6.80% moisture and 10.4% crude fiber. NPC were mixed with the basal diet and the experimental diets were made into extrudates with 2 mm diameter. All diets were stored at -20°C to avoid oxidation of pigmentations throughout the experiment.

Experiment fish

An Oranda strain of goldfish were purchased from a commercial farm in Ratchaburi Province, Thailand. The goldfish were first acclimatized in the experimental laboratory culturing system for 120 days, Fish were satiated with the basal diet 2 times a day at 9 am and 3 pm and then randomly distributed into 18 groups of 8 fish each (6 treatments, with 3 replicate). 144 individual fish were selected base on the skin color. The fish were cultured in an aquarium measuring 65 x 30 x 30 cm height and supplied with continuous aeration. Illumination was provided by LED lamp (Philips 13 W, 68x0.2 W) with L: D (light: dark) cycles of 12:12 h. The necessary ethical clearance was obtained from the relevant authorities at Kasetsart University for use of fish (Registration Id: ACKU 61-FIS-016). Color and weight measurement of goldfish were done at the same time with a speed to reduce the damage of the fish.

Feed and rearing conditions

Observations on glass tank were made over 8 weeks with goldfish average initial weight of 16.11 g., that market size of goldfish, Fish were feed with the experiment diet 2 times a day at 9 am and 3 pm. Before morning feeding, Feed residuals and water wastes were removed by siphoning and 1/3 of water in each tank was exchanged with fresh water. The water was completely changed and clean fish tanks every 2 days. Experiment were assigned into the 1st step, color conditioning for fish to adjust its color according to changing environment, was dealt with basal diet within 8 weeks (weeks 0-8) to ensure the skin color was not different. The 2nd step was then continuously about 8 weeks carried out using the experimental diet about 8 weeks (weeks 9- 16) for testing the efficacy of NPC on fish skin color. In this step divided into 6 concentrations of 0 (control), 100, 200, 300, 400 and 500 µg kg^{-1} of diet. Approximately 6% of net weight were fed per day.

Color analysis

All fish was become completely anaesthetized using solution 200 mg of clove oil per lite (pure clove oil (90-95% eugenol) was dissolved in ethyl alcohol (92.8%) in 1:9 ratio (clove oil: ethyl alcohol). While fish were loss of equilibrium, which were determined for skin color throughout the experimental period and weight

and color changes were measured on fish body (Figure 1) by a Chroma Meter (CR-400, Konica Minolta, Japan) with a calibration plate (CR-A43, Konica Minolta, Japan). Skin color was assessed by reflectance spectroscopy with transformation into color parameters based on the tristimulus values L*a*b* and L*C*H* every 2 weeks to calculate ΔE and ΔH according to the method of Fairchild (2013).

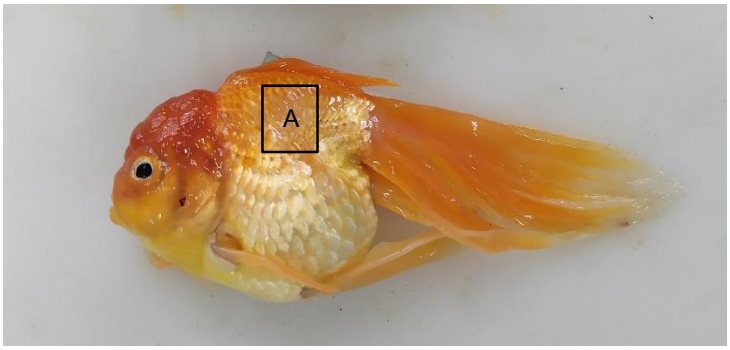

Figure 1 (A) Sample areas for color measurement, were taken on both side for all fish used in the experiment.

Growth performance and physical indicators

The fish were weighed and measured for color every 2 weeks; feed intake was measured daily. Average daily gain (ADG), specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR) was determined in equation (2), (3), (4), (5) and (6), following the parameters used to evaluate growth performance by Suyom (2020)

a. Weight gain (WG ; g) = final weight– initial weight (2)

b. Average daily gain (ADG; g day^{-1}) = (final weight – initial weight) / age (days) (3)

c. Feed conversion ratio (FCR) = (feed consume) / (weight gain) (4)

d. Specific growth rate (SGR; % day^{-1}) = [Ln (final weight) - Ln (initial weight)] /days × 100 (5)

e. Survival (%) = 100 × (final count of the fishes) / (initial count of the fishes)) (6)

Statistical analysis

All data statistically analyzed using one-way analysis of variance (ANOVA) and using Kruskal-Wallis H test for a rank-based nonparametric test, followed by Tukey's HSD post hoc test. $P < 0.05$ was regarded as the statistically significant level, with results presented as mean ± standard deviation.

Results

Size and stability of nano capsules

The measured diameter technique according to Mazzoli and Favoni (2012), by dragging the cross section to the size of the particles were then averaged. After the freeze-drying process and compared with Zetasizer measurements, stability was analyzed at a concentration of 1%. At alginate 0.6 mg ml^{-1} and $CaCl_2$ 0.67 mg ml^{-1} average particle diameter size was 131.56 ± 32.35 nm (Figure 2) and particle size diameter measured by Zetasizer was 177.567±14.00 nm

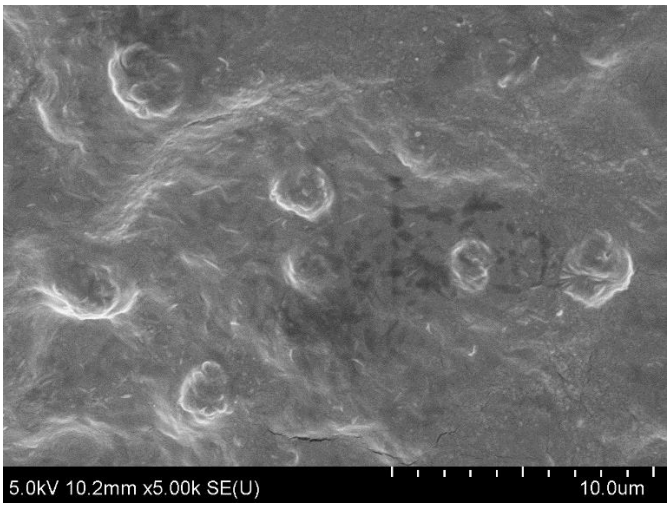

Figure 2 Representative SEM images of nanoparticle carotenoid (NPC) with scale bar

Effect of experimental diet on the body color

Results for average L* during the 16-weeks trial are shown in Figure 3. At the end of the 16th week, fish fed 0 µg kg^{-1} diet had the highest L* value ($P < 0.05$). The control group maintained the highest lightness from 10th weeks until the end of the trial ($P < 0.05$). The lightness of all fish groups tended to higher levels showing increased skin brightness (light shade). In general, a* and b* values are known on the CIE-L*a*b* scale as redness and yellowness, respectively. In this research, the trend of average red (a*) value of the control group decreased, while groups 300, 400 and 500 µg kg^{-1} maintained significantly higher levels than the control group from 10th week to the end of the trial ($P < 0.05$) (Figure 4), the trend of average yellowness (b*) values in all experimental groups reduced at the start of the experiment (week 9th), while the control group was significantly lower than the others at 10th week until the end of the experiment ($P < 0.05$) (Figure 5).

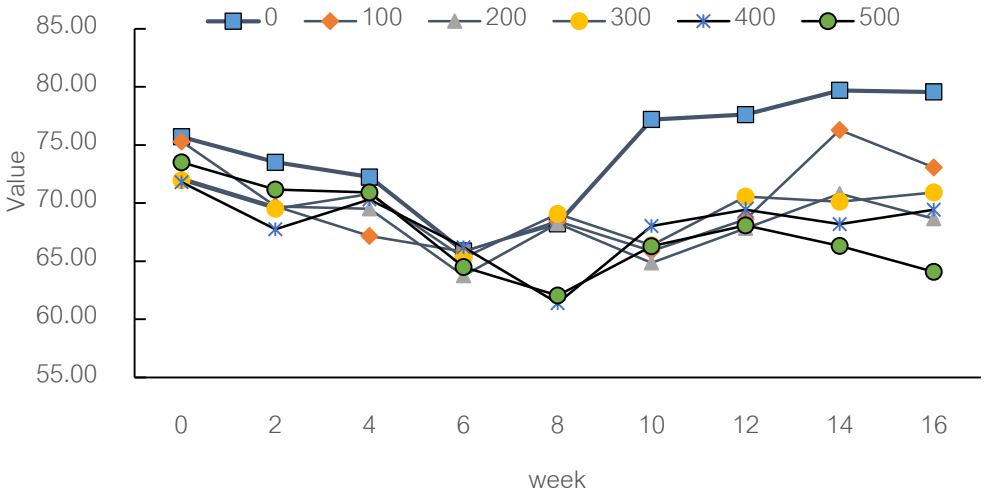

Figure 3 The trend of lightness (L*) of goldfish skin during 16 weeks trial

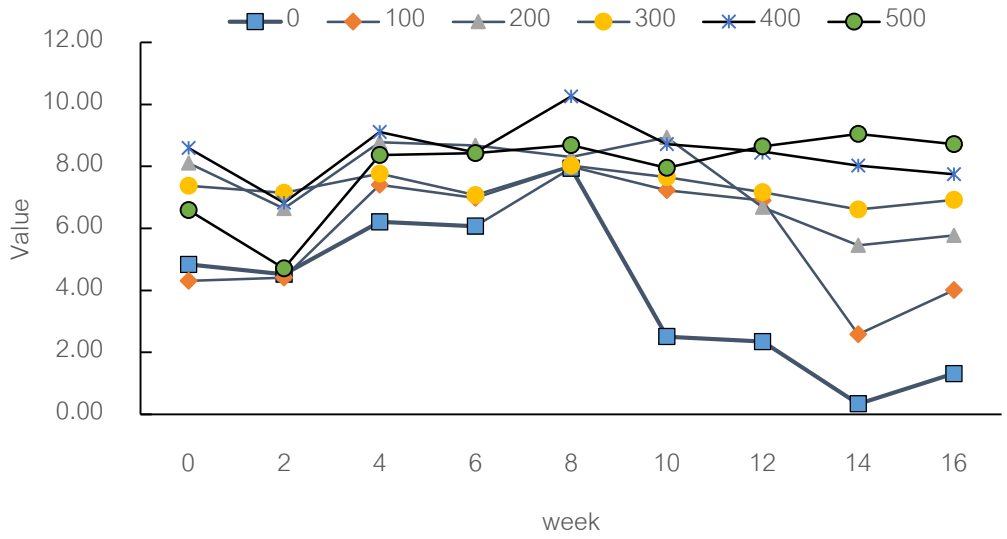

Figure 4 The trend of redness (a*) of goldfish skin during 16 weeks trial

Average skin color of goldfish showed more brightness (L*) in all experiments. Conversely, the 500 μg kg^{-1} diet concentration group gave lower mean intensity of skin color ($P < 0.05$), while brightness (L*) and redness (a*) declined in every experimental level. The diet of 500 μg kg^{-1} of carotenoid gave (a*) greater redness ($P < 0.05$), as shown in Figure 4. While yellow color (b*) in all groups was dropped during the experiment (Figure 5).

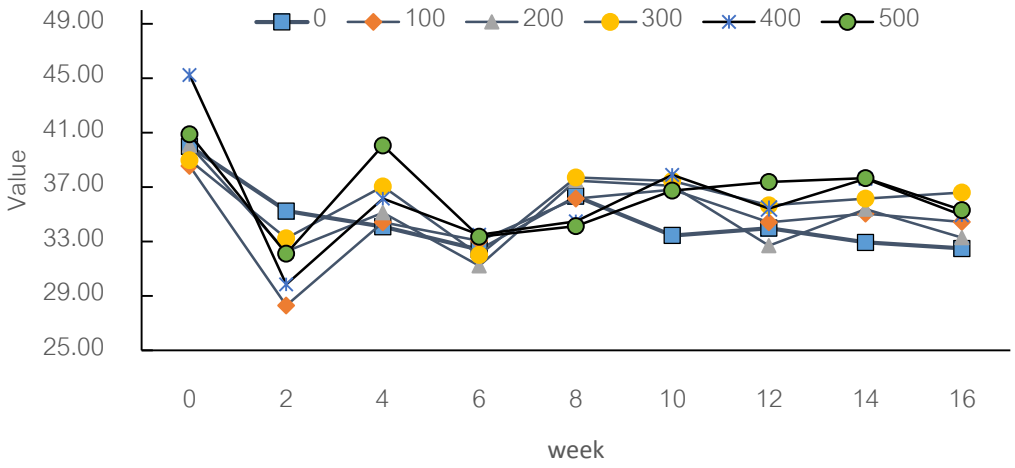

Figure 5 The trend of yellowness (b*) of goldfish skin during 16 weeks trial

At 100 μg kg^{-1} the mean yellowness was greatest decreased, while the experimental set with NPC diet group of 500 μg kg^{-1} had the accumulated yellowness increased when considering the total color difference (ΔE). A little change of average skin color was observed at the level of 300 μg kg^{-1}.

Table 1 Changes in skin color of goldfish fed different carotenoid concentration in diet throughout the experiment period in CIE L* C *H* (L*; Lightness, C*; Chroma and H*; Hue)

Concentration (μg kg^{-1})	C*(Initial)	C*(Final)	H*(Initial)	H*(Final)	ΔL*	ΔC*	ΔH*
0	40.487±7.023^{a}	32.702±4.289^{a}	83.501±5.493^{bc}	88.061±6.006^{d}	3.871	-7.785	4.56
100	38.917±7.610^{a}	34.876±5.496^{abc}	83.747±5.207^{c}	83.280±6.249^{c}	-2.233	-4.041	-0.467
200	41.162±8.466^{ab}	34.040±4.553^{ab}	78.677±7.765^{s}	80.110±7.081^{bc}	-3.484	-7.122	1.433
300	39.952±7.034^{a}	37.426±5.376^{c}	79.848±7.003^{sb}	79.305±5.867^{ab}	-1.063	-2.526	-0.543
400	46.379±14.588^{b}	35.948±5.952^{bc}	79.009±6.959^{s}	77.461±5.678^{ab}	-2.367	-10.431	-1.548
500	41.658±7.085^{ab}	36.638±6.104^{bc}	81.325±6.205^{sbc}	76.260±6.801^{a}	-9.410	-5.02	-5.065
P-value	0.002	0.000	0.000	0.000	-	-	-

Values are a means ± s.d., Means with the same row with different superscript letters are significantly different (P<0.05).

ΔL^* *(+ ; brighter than initial sample, - ; less bright than initial sample), initial sample = week 0*

ΔC^* *(+ ; more vivid than initial sample, - ; less vivid than initial sample), initial sample = week 0*

ΔH^* *(+; more saturated than initial sample, - ; less saturated than initial sample), initial sample = week 0*

Changes in goldfish skin color shade are shown in Table 1. The CIE L*C*H* system explained the color shade and vividness of the color better than the CIE L*a*b* system. At the end of the experiment, the control group (0 μg kg^{-1}) had the highest lightness of color ($P<0.05$), while the 500 μg kg^{-1} fed group had the lowest lightness of color. The purpose of experiment was to use the ΔC* value to show variable results with a tendency of maintain vividness following increase in normal carotenoid feed concentrations (50 - 100 mg kg^{-1}). The exception in this work because we used less than one thousand fold concentration compared to the dosage used in other experiments. The result show, the value of ΔC* could be able to describe less vivid skin than initial sample. According to the initial yellowness were dropped in first week and thoroughly maintained until final of experiment (Figure 5). ΔH* results showed less color saturation according to feed concentration. The control group turned more yellow (+4.56), while the other feed concentration groups turned toward redness. The result for 200 μg kg^{-1} fed group (1.433) was suspect due to infection by parasites during the trial period (ΔH*, + means more yellowness, - means more redness). NPC diet group of 500 μg kg^{-1} feed showed the lowest recommended dosage for visible color intensity increase.

Table 2 Growth parameter of goldfish fed the experimental diet, for a rearing period 8 weeks (week 8-16) growth

Treatment (μg kg^{-1})	Weight Gain (WG; g)	Average Daily Gain (ADG; g day^{-1})	Feed Conversion Ratio (FCR)	Specific Growth Rate (SGR; % day^{-1})	Survival rate (%)
0	18.57±4.55^{a}	0.33±0.08^{a}	3.67±0.89^{a}	0.23±0.06^{a}	100
100	22.30±4.63^{ab}	0.40±0.08^{ab}	3.54±0.96^{a}	0.26±0.05^{ab}	100
200	26.80±7.02^{abc}	0.48±0.13^{abc}	2.52±0.74^{a}	0.30±0.07^{ab}	100
300	34.50±6.07^{bc}	0.62±0.11^{bc}	2.26±0.48^{a}	0.35±0.04^{ab}	100
400	39.50±6.85^{c}	0.71±0.12^{c}	2.00±0.25^{a}	0.40±0.05^{b}	100
500	36.23±5.13^{bc}	0.65±0.09^{bc}	2.13±0.32^{a}	0.36±0.04^{ab}	100
P-value	0.004	0.005	0.058*	0.012	-

*Values are a means ± s.d., Means with the same row with different superscript letters are significantly different (P<0.05). *P-Value in Kruskal-Wallis H test.*

Effect of experimental diet on the growth performance

All diets were accepted equally well by the fish, and no mortality was observed with 100 % survival rate. Growth rate during the experiment is shown in Table 2. The fish received NPC at 300, 400 and 500 μg kg^{-1} were significantly different in WG and ADG ($P<0.05$) with a control group (0 μg kg^{-1}) and fish received NPC at 400 μg kg^{-1} were higher in WG and ADG in other group but were not significant differences ($P>0.05$)

with NPC 200, 300 and 500 µg kg^{-1} group. The Value of FCR were within the range 2.00±0.25 to 3.67±0.89 but were not significant differences (P>0.05). The mean of SGR of fish that fed with NPC at 0, 100, 200, 300 and 500 µg kg^{-1} were not statistically significant (P>0.05), as well as fish fed with NPC at 400 µg kg^{-1} had a higher SGR and were significantly different (P<0.05) with control group and were not significant differences (P>0.05) with NPC 100, 200, 300 and 500 µg kg^{-1} group.

Discussion

Variations of colors and patterns in fish are affected by a combination of factors including genetics, biological pigments, structural color, and diet (Hekimoglu *et al.*, 2017). Carotenoids contain oxygen in their molecules, and this influences their ability to provide pigment and offer other valuable attributes. Besides their beneficial effects on pigmentation, carotenoids also play a significant role in enhancing nutrient utilization that may contribute to survival and growth performance (Ninwichian *et al.*, 2020). Utilization of crude palm oil on coloration and growth of ornamental fish has not previously been reported. Ng (2002) mentioned that the use of palm oil in fish diets first began around the mid-1990s as a dietary lipid, not as a skin color enhancer. Here, we investigated an alternative use of carotenoid nanoparticles as a pigmentation enhancer. Results showed that particle size distribution was suitable, and the zeta potential suggested high physical stability of the system during storage condition in diet. The freeze-dried process produced smaller particle sizes than results of Arpagaus *et al.* (2018). Zeta potential values less than -30 mV and +30 mV are considered stable. J. Chen *et al.* (2017) explained that homogenization conditions and emulsification were effective for preparing oil-in-water emulsions. The zeta potential of oil droplets had good stability against aggregation due to strong electrostatic forces. On the other hand, a 1% colloid of carotenoid nanocapsules had a potential of -30.233 ± 0.702 mV. The zeta potential was greater than -30 mV, indicating good particle stability (Worranan & Tanasait, 2015).

Brum *et al.* (2017) and L. Chen *et al.* (2020) conducted research on carotenoids with mean particle size and zeta potential similar to our results. When using the encapsulation, drug delivery due to specific tissue with easier absorption and high stability of lifetime. Their findings showed effective color enhancement in goldfish skin intensity. Carotenoids accumulation was influenced by nanocarotenoids in terms of L*a*b*. The a* values of fish fed with the control diet decreased during the experiment. Fish fed diets of 400 and 500 µg kg^{-1} NPC turned to more reddish skin hue compared with the other treatments. Our results concurred with (Ninwichian *et al.*, 2020) who noted that loss of pigment supplementation in basal feed caused a slight decrease in skin redness in fancy carp, while Das and Biswas (2016) reported that fancy carp could convert carotenoids into lutein or astaxanthin. A similar observation was made by Bundit *et al.* (2008) who found that fancy carp fed with an astaxanthin diet oxidized to lutein. And moreover, fancy carp fed with a lutein diet converted lutein

to astaxanthin too. Our results concurred with Sun *et al.* (2012) who reported that Showa koi fed the control diet showed weak red tonality redness (a*) and high difference in lightness (L*) value of the red zone. This experiment used very low levels of carotene in diets by designing a small encapsulating method and using material to promote stability and easy absorption by the ionic gelation method. In a similar, Badilli *et al.* (2018) reported that nanosizes are conventional forms with greater possibility to interact with gastrointestinal factors/enzymes, as well as gaining easier entrance to target cells to liberate their payloads. Sodium alginate acted as a mucoadhesive polymer and promoted dietary supplements to white shrimp as an immunostimulant to improve resistance against attack by bacteria (Pestovsky & Martínez-Antonio, 2019). Commercial feeds are continually modified as they are subject to least-cost formulations. In this study, we demonstrated how reducing carotenoids in feed formulations by nanosized encapsulation increased carotenoid absorption and shelf-life stability. Typically, pigments are added into the diet at varying concentrations, dietary carotenoid concentrations have varied from 60 mg kg^{-1} to 700 mg kg^{-1} of dry feed. (Chapman & Miles, 2018). However, carotenoid supplementation did not affected goldfish growth. The observations on the growth performance found that related to dietary protein intake (Bandyopadhyay *et al.*, 2005). Such as in previous study, Bell *et al.* (2019) reported the effect of lobster meal on goldfish had a SGR between 2.5 – 3.7 and an average FCR of 1.8 to 1.97 (Protein 51%). Similar to the study of Bandyopadhyay *et al.* (2005), who reported the SGR and FCR were 2.13 and 1.94 respectively (Protein 64%), as well as the experiment of da Cunha *et al.* (2020), who reported the growth rate of goldfish were kept in biofloc technology with diet with 80 mg kg^{-1} of astaxanthin, while result shown SGR 0.51% kg^{-1}, survival rate 98.33% and FCR at 3.78. Although, our results show the possibility for cost-saving in food additives at less than one thousand fold concentration compared to the dosage used in previous experiments by Sun *et al.* (2012), Wallat *et al.* (2005) and Yanar *et al.* (2008). Furthermore, the extending of deterioration rate, freeze-dry extracted carotenoid and keeping in dark and cold condition with -20 ^{o}C. This method would preserve the extraction fresh for 6 months (data not shown)

Conclusions

The conclusions drawn from the present study on growth and enhanced skin color of goldfish (*Carassius auratus*), are also plays a very important role in the outcome of a particular pigmentation or coloration. Results suggested that the inclusion of total carotenoids, primarily used as a coloring agent in goldfish, could be modified by supplementing the diet with 500 µg kg^{-1} of NPC from crude palm oil are containing significantly highest skin color ($p<0.05$) without interfering on growth rate, survival rate and other side effect was not observed. Future investigations are required to optimize carotenoid nanoparticle content and enhance the skin color of other ornamental fish. Improved skin color will add value to economic animals such as marine shrimp and deliver a cooked product that is more appetizing and appealing to consumers.

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