Original article DOI: 10.19027/jai.18.1.54-69

Enhancement of colour quality, growth, and health status of rainbow Kurumoi fish *Melanotaenia parva* through dietary synthetic carotenoids supplementation

Peningkatan kualitas warna, pertumbuhan, dan status kesehatan ikan rainbow Kurumoi *Melanotaenia parva* dengan suplementasi karotenoid sintetis dalam pakan

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(Received February 28, 2018; Accepted November 30, 2018)

ABSTRACT

Carotenoids were known as pigment sources, vitamin A precursor, antioxidant, and can improve the health status of fish. Furthermore, several studies revealead carotenoids function in bone formation and metabolism. This study was conducted to determine the effect of different carotenoids at two different doses in the feed on growth, pigmentation, vitamin A conversion, blood profile, antioxidant activity, and calcium bone of the rainbow fish. Twenty-one aquariums with a volume of 20 L each stocked with 20 fish (1.08 ± 0.03 g of body weight and 4.56 ± 0.07 cm of body length). The experiment applied seven experimental diets (in triplicates) consisted of three types of carotenoids were astaxanthin (AS), canthaxanthin (CS), and lutein (LS) and two doses (130 and 260 mg/kg), i.e. AS-130, AS-260, CS-130, CS-260, LS-130, LS-260 and basal (without carotenoids) feed. The fish were fed for 56 days of experimental period. The results showed that carotenoid diets were able to increase growth, total carotenoids, percentages of chromatophores, vitamin A conversion, erythrocyte, leukocytes, packed cell volume (PCV), neutrophils, and hemoglobin (Hb) compared to the control. Fish fed dietary astaxanthin at a level of 260 mg/kg was superior compared to other diets. Dietary carotenoids were also capable of decreasing the endogenous antioxidant activity of superoxide dismutase (SOD) and malonyl dialdehyde (MDA) and increased the calcium level in fish bone than basal diet.

Keywords: carotenoids, growth, health status, *Melanotaenia parva*, pigmentation

ABSTRAK

Karotenoid diketahui sebagai sumber pigmen, prekursor vitamin A, antioksidan, dan dapat meningkatkan status kesehatan ikan. Selain itu, karotenoid juga memiliki peran dalam formasi dan metabolism tulang. Penelitian ini dilakukan untuk mengevaluasi pengaruh jenis dan dosis karotenoid yang berbeda terhadap pertumbuhan, pigmentasi, konversi vitamin A, gambaran darah, aktifitas antioksidan dan kalsium tulang ikan rainbow Kurumoi. Sebanyak 20 ekor ikan (bobot tubuh rata-rata 1,08 ± 0,03 g dan panjang total rata-rata 4,56 ± 0,07 cm) dan diberi makan pakan yang mengandung karotenoid. Pakan uji terdiri atas tiga jenis karotenoid dengan tiga ulangan yaitu astaksantin (AS), cantaksantin (CS), dan lutein (LS) dan dua dosis (130 dan 260 mg/kg) dikodekan dengan AS-130, AS-260, CS-130, CS-260, LS-130, LS-260 dan basal (tanpa karotenoid) sebagai kontrol. Ikan diberi makan selama 56 hari pemeliharaan. Hasil penelitian menunjukkan bahwa karotenoid dapat meningkatkan pertumbuhan, total karotenoid, persentase kromatofora, konversi vitamin A, eritrosit, leukosit, hematokrit, neutrofil, dan hemoglobin dibandingkan dengan kontrol. Ikan yang diberi pakan mengandung astaksantin 200 mg/kg lebih baik dibandingkan denga pakan uji lainnya. Pakan yang mengandung karotenoid juga mampu menurunkan antioksidan endogenus superoxide dismutase (SOD) dan malonyl dialdehyde (MDA), serta meningkatkan kalsium tulang ikan dibandingkan pakan kontrol.

Keywords: *Melanotaenia parva*, karotenoid, pertumbuhan, pigmentasi, status kesehatan.

INTRODUCTION

Kurumoi rainbowfish *Melanotaenia parva* is an indigenous fish species from Lake Kurumoi, Bintuni, Papua, Indonesia. This species is on the red list of IUCN with *vulnerable* status because its only habitat has been threatened (Allen, 1996). Since 2008, the Research Institute for Ornamental Fish Culture, Depok, West Java has cultured this species from its natural habitat. The expansion of *Melanotaenia* sp. is widely promising followed by various enthusiasts, high economic value, and high demand on both local and export market, i.e. Europe and the United States of America.

As a potential candidate of ornamental fish, the Kurumoi rainbowfish has to fulfill several benchmarks, such as colour, shape, fin shape, size, and colour pattern (Yuangsoi *et al.,* 2010). Compared to the other species in its natural habitat, the cultured species is constrained by colour degradation. Another issue is about the downgrade of the growth performance and survival rate caused by poor quality of the feed and it does not meet the feed requirement.

The ornamental fish feed usually has a lot of specific criteria than common fish. The specific requirement is especially carotenoid pigments to boost or defend the colour quality (Das & Biswas, 2016; Li *et al*., 2016). Carotenoid functions as an antioxidant (Gramza-Michałowska & Stachowiak, 2016) and induces immune system (Chow *et al*., 2016). Carotenoid also acts as a vitamin A precursor (Schiedt *et al*., 1985) and induce bone density (Bovier & Hammond, 2017).

The colour of ornamental fish usually comes from carotenoid precipitation in the tissues and chromatophores which contains carotenoids (Chatzifotis *et al.,* 2011). Carotenoids cannot be produced by the fish itself (de novo) Ahilan *et al.* 2008; Sujath *et al.* 2011; Jintasataporn & Yuangsoi, 2012), so that nutrient supply is a necessity in order to obtain optimum carotenoid supply. Several pigments which obtained by carotenoid are lutein (yellow-green), carotene (orange), doradexanthin (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), echinenone (red), and taraxanthin (yellow) (García-Chavarría & Lara-Flores, 2013). Various study about pigmentation on ornamental fish has been widely observed using extracted carotenoid and astaxanthin is the most commonly used and it showed effective

Table 1. The composition of test feed

Ingredients (g/kg)	Feed						
	B	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
Fish meal	637	635.7	634.4	635.7	634.4	635.7	634.4
Gelatine	120	120	120	120	120	120	120
Dextrin	110	110	110	110	110	110	110
Fish oil	63	63	63	63	63	63	63
Vitamin mix	20	20	20	20	20	20	20
Mineral mix	30	30	30	30	30	30	30
CMC[1]	20	20	20	20	20	20	20
Astaxanthin (10%)[2]	0	1.3	2.6	0	0	0	0
Canthaxanthin (10%)[2]	0	0	0	1.3	2.6	0	0
Lutein (10%)[2]	0	0	0	0	0	1.3	2.6
Nutrient composition (% dry matters):							
Crude protein[NS]	50.98	51.00	50.95	52.33	52.00	52.09	51.99
Crude fat[NS]	16.00	17.24	17.54	16.79	16.69	15.80	15.82
Crude fiber[NS]	2.82	2.47	2.64	2.91	3.05	2.90	2.69
Ash[NS]	19.96	21.50	21.20	19.62	21.78	20.65	21.09
Nitrogen free extract (NFE)[NS*]	9.94	7.79	7.68	8.35	6.48	8.56	8.41
Total carotenoids (mg/kg)	15.74	103.50	137.07	106.00	151.09	104.07	142.63

[1]Carboxy methylcellulose

[2]Formulated based on the coefficient of carotenoids digestibility (Meilisza *et al.,* 2017)

*NFE = 100−(crude protein+fat+fiber+ash)

NS = no significant amongst treatments

to result in colour enhancing on different ornamental fish species (Niu *et al*., 2014; Das & Biswas, 2016). The other application of different source of carotenoids, such as canthaxanthin and lutein, is also proved in enhancing the colour of ornamental fish and affected the health status of the fish positively (Talebi *et al*., 2013; Rahman *et al*., 2016; Chow *et al*., 2016; Meilisza *et al*., 2018).

The supplementation of synthesized carotenoid, such as astaxanthin, canthaxanthin, and lutein, followed by suitable dosage was expected to enhance the colour of ornamental fish and increase its economic value. This study was also predicted to maintain health status and increase growth performance. It is also important to review the dopisition of carotenoid in several tissues and evaluate the other possible roles.

MATERIALS AND METHODS

Tested fish

This study used Kurumoi rainbowfish fry with an average body length of 4.56 ± 0.073 cm and an average body weight of 1.08 ± 0.03 g, which was obtained from natural spawning. The test fish was reared in an aquarium with water volume of 20 L. The aquarium was set in an indoor aerated stagnant system using a photoperiod of 10 h light and 14h dark.

Tested feed

Basal feed, as the control, and the test feed contained semi-purified ingredients (Table 1). Several carotenoids used in this experiment were synthesized astaxanthin (AS-carophyll pink 10% cold water soluble), canthaxanthin (CS-carophyll red 10%), and lutein (LS-carophyll yellow 10%) (DSM Nutritional Products Ltd, Basel, Switzerland). Each of carotenoids was split into two different concentrations (130 mg/kg and 260 mg/kg). Therefore, the tested diets were encoded B (basal feed), AS-130, AS-260, CS-130, CS-260, LS-130, and LS-260.

All of the ingredients were grounded and sieved. The feed was made using feed extruder and then oven dried at 60°C and stored in -20°C until further use. Proximate analysis was done according to AOAC (2002) (Table 1). The tested diets was delivered twice a day (08.00 and 15.00) to apparent satiation for 56 days of experimental period.

The experiment applied randomized complete design with seven treatments and three replications. The treatments were B (basal feed), AS-130, AS-260, CS-130, CS-260, LS-130, and LS-260. The applied dosage was considered as effective dosage for pigmentation, growth, and health of some fish species (Del Villar-Martinez *et al.,* 2013; Nguyen *et al*., 2014; Pham *et al*., 2014). The digestibility of 100 mg/kg dosage has been determined based on the results in the previous experiment in Kurumoi rainbowfish (Meilisza *et al*., 2017).

Sampling and observation

Sampling was done every 14 days. The measured parameters were fish body weight by digital balance, body length by milimeter block, and survival by counting the fishes. At the end of the experiment, fish growth, survival, pigmentation and deposition of astaxanthin, canthaxanthin, and lutein, and total carotenoids in muscle, skin, and fin tissues, lightness (L), colour density (C), hue (H), redness index (*a**), yellowish index (*b**), visual colour rate (TCF), and percentage of chromatophores were observed. The health status parameters were determined by measuring blood parameters including erythrocyte, leucocyte, haematocrit, lymphocyte, neutrophil, monocyte, haemoglobin (Hb), measuring superoxide dismutase (SOD) enzyme activity and malondialdehyde (MDA) in liver tissues and calcium level test in whole bone (including head).

Colour quality analysis

Visual colourcolour of the test fish was observed through the whole body surface in the beginning and at the end of the experiment. Colour appearance was analysed using Chroma meter CR-400 (Konika Minolta, Osaka, Japan). The parameters were L value for brightness (%), C value for colour density (%), H value for hue (º), *a** for red (+) or green colour (-), and *b** for yellow (+) and blue colour (-). Toca colour finder (TCF 1999 edition, Cemani Toka, Bogor, Indonesia) was also used to determined colour parameter qualitatively.

The total carotenoids in muscle, skin, and fin tissues were analysed through carotenoids extraction using Schiedt and Liaaen-Jensen (1995) method. The sample tissues were extracted using acetone so that there would not be any pigments came out. The acetone extract was required around

5 mL. The water from the extract had to be thrown so that a 5 mL of hexane was added, followed by 2 mL of water. The mixture was separated into two different phases using separating funnel. The bottom hypo phase (water) was extracted again using hexane. The upper hypo phase (hexane) which contained carotenoids was washed until three times until no extracted colour from the hypo phase. The extract was analysed using spectrophotometer (Thermo Scientific genesis 10S UV-Vis) at 350, 380, 420, 450, 180, and 663 nm of wavelength. The highest absorbance was chosen to be continued to quantification process.

Carotenoids content in the tissues were analyzed using liquid chromatography by Guillou *et al.* (1993). Sample extraction was conducted firstly by mixing the tissues with chloroform/methanol (2:1) until the volume became 20 times higher than the sample (1 g of sample in 20 mL of the solvent). The mixture was stirred for 15–20 minutes using a shaker. The homogenate was centrifuged to regain the liquid phase. The solvent was rinsed using H_2O (4 mL for 20 mL). After the mixture was stirred for a moment using a vortex, it was centrifuged in low speed (2000 rpm) to separate two phases. The upper phase was moved and the rest was rinsed two until three times using methanol/H_2O (1/1) without mixing the whole preparation. After the centrifugation and upper phase siphoning, chloroform phase which approximately 2–3 mL of lipid remained, it was stored in −30°C to be analyzed (Folch *et al.,* 1957). The extract was diluted in acetonitrile/dichloromethane (2:1) and filtered using Millex-HV (0–45 /µm) filter (Millipore) before analyzed and chromatography injection.

The standard solvent was prepared to four different concentrations, i.e. 100, 50, 10, and 1 mg/kg. The standard solvent was referred to as an Certificate of Analysis from producer. The standard solvent consisted of AdipoGen (astaxanthin) and Sigma Aldrich (canthaxanthin, lutein, and retinol). Liquid chromatography was conducted using an instrument called KNAUER. The principal method was an isocratic detector. The standard measurement showed that the wavelength and retention period for each absorption was 480 nm and 3.45 minutes for astaxanthin, 472 nm and 3.55 minutes for canthaxanthin, 450 nm and 3.60 minutes for lutein, and 326 nm and 3.50 minutes for retinol. The absorbance on each standard concentration was used to measure standard curve. Regression equation would be applied to measure the sample concentration.

We observed the chromatophores from integument of fish and calculated the number of chromatopores by percentages unit (total of numbers of chromatophores were 100% from each of observation area). The measurement of chromatophore closure was conducted using three samples each replication. The observation was done using a microscope with lower lighting in 6.3× 0.5 of magnification. The result was analyzed using Photoshop CS5 extended and classified based on the pigment, i.e. black (melanophore), yellow (xanthophore), red (erythrophore), and nonpigment (leucophore and silver iridophore)

Blood profile analysis

After 56 days of rearing, as many of 10 samples of fish was collected to analyze the blood profile. Before that, the fish samples were anesthetized using phenoxyethanol. The blood samples were collected from caudal vein using a 26G×0.5 inch syringe which has been already drenched by anticoagulant. The blood sample was stored in an Eppendorf. The blood profile parameters were erythrocyte, leucocyte, lymphocyte, hematocrit, neutrophile, monocyte, and hemoglobin.

Antioxidant activity analysis

The antioxidant activity which analyzed in this study was superoxide dismutase (SOD) and malonyl dialdehyde (MDA). The SOD analysis was conducted based on the method by Mirsa and Fridovich (1972). As many of 1 g of the liver was crushed then added 2 mL of buffer phosphate. After that, it was centrifuged in 10000 rpm for 20 minutes. The supernatant (I) was poured into an Eppendorf and ready to be analyzed. As many of 0.25 mL of the supernatant was added by 0.4 mL of chloroform and ethanol (3:5), then centrifuged in 3000 rpm for 10 minutes. The supernatant (II) was collected as many of 100 µL and then it was added by 3 mL of buffer carbonate and 100 µL of epinephrine (0.05 mg/10 mL HCl 0.01 N). The mixture was analyzed using spectrophotometer in 480 nm of wavelength. The absorbance was noted in minute 1, 2, and 3 after epinephrine addition. HCl 0.01 N was used as the blank solution, while the control solution was 100 µL of aquadest added by 3 mL of buffer carbonate and 100 µL of epinephrine.

The MDA analysis was operated by grinding 0.5 g of the fresh liver in a chilled condition

Table 2. Growth, survival, and feed conversion ratio of Kurumoi rainbowfish

Parameters	Test fish						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
IW (g)	1.09 ± 0.12^{a}	1.08 ± 0.03^{a}	1.06 ± 0.06^{a}	1.11 ± 0.03^{a}	1.10 ± 0.02^{a}	1.05 ± 0.06^{a}	1.04 ± 0.12^{a}
FW (g)	1.74 ± 0.14^{a}	1.96 ± 0.10^{b}	1.87 ± 0.02^{ab}	1.75 ± 0.02^{a}	1.90 ± 0.05^{ab}	1.73 ± 0.01^{a}	1.80 ± 0.07^{ab}
IL (cm)	4.62 ± 0.17^{a}	4.56 ± 0.12^{a}	4.61 ± 0.04^{a}	4.52 ± 0.13^{a}	4.62 ± 0.17^{a}	4.44 ± 0.17^{a}	4.56 ± 0.15^{a}
FL (cm)	5.04 ± 0.08^{a}	5.26 ± 0.03^{b}	5.10 ± 0.12^{ab}	4.91 ± 0.13^{a}	5.20 ± 0.13^{ab}	4.91 ± 0.17^{a}	5.05 ± 0.09^{ab}
WG (g)	0.65 ± 0.07^{a}	0.87 ± 0.07^{c}	0.81 ± 0.06^{bc}	0.64 ± 0.05^{a}	0.80 ± 0.11^{bc}	0.69 ± 0.04^{ab}	0.76 ± 0.10^{abc}
RW (%)	60.16 ± 8.94^{a}	82.28 ± 4.58^{c}	76.04 ± 3.23^{bc}	57.70 ± 3.73^{a}	73.04 ± 9.42^{bc}	65.92 ± 1.89^{ab}	73.52 ± 11.29^{bc}
LG (cm)	0.43 ± 0.20^{a}	0.70 ± 0.17^{a}	0.49 ± 0.12^{a}	0.38 ± 0.11^{a}	0.58 ± 0.15^{a}	0.48 ± 0.08^{a}	0.49 ± 0.05^{a}
RL (%)	9.35 ± 4.62^{a}	15.55 ± 4.13^{a}	10.70 ± 2.58^{a}	8.45 ± 2.33^{a}	12.70 ± 3.51^{a}	10.78 ± 1.68^{a}	10.83 ± 1.03^{a}
SGR (%/day)	0.84 ± 0.10^{a}	1.07 ± 0.04^{c}	1.01 ± 0.03^{bc}	0.81 ± 0.04^{a}	0.98 ± 0.10^{bc}	0.90 ± 0.02^{ab}	0.98 ± 0.02^{bc}
SR (%)	80.00 ± 0.00^{a}	86.67 ± 2.89^{ab}	93.33 ± 5.77^{b}	85.00 ± 0.00^{ab}	91.67 ± 2.89^{ab}	86.67 ± 5.00^{ab}	88.33 ± 7.64^{ab}
FCR (%)	1.98 ± 0.28^{a}	1.56 ± 0.08^{a}	1.86 ± 0.11^{a}	1.93± 0.10^{a}	1.73 ± 0.24^{a}	1.97± 0.19^{a}	1.89 ± 0.43^{a}

Note : Initial weight (IW), final weight (FW), initial length (IL), final weight (FL), specific growth rate (SGR), weight gain (WG), relative weight (RW), length gain (LG), relative length (RL), survival (SR), feed conversion (FCR). Superscript letter following mean value (± standard deviation) in the same row indicates significant differences (P<0.05).

Table 3. Astaxanthin, canthaxanthin, lutein, vitamin A (retinol) in muscle tissues of *Melanotaenia parva* fed with diets containing carotenoids

Parameter (mg/kg)	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
Astaxanthin	n.d	0.67	0.84	n.d	n.d	n.d	n.d
Canthaxanthin	n.d	0.75	0.97	0.95	0.97	0.77	0.87
Lutein	1.06	1.11	1.35	n.d	n.d	1.08	1.11
Retinol	0.98	1.18	1.31	1.46	1.60	1.15	1.23

* n.d = not detected

Table 4. Astaxanthin, canthaxanthin, lutein, vitamin A (retinol) in skin tissues of *Melanotaenia parva* fed with diets containing carotenoids

Parameter (mg/kg)	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
Astaxanthin	n.d	0.76	0.71	n.d	n.d	n.d	n.d
Canthaxanthin	0.92	0.85	0.82	0.89	0.88	0.81	0.83
Lutein	1.63	1.66	1.65	n.d	n.d	1.66	1.67
Retinol	0.84	1.29	1.19	1.40	1.25	1.12	1.27

* n.d = not detected

Table 5. Astaxanthin, canthaxanthin, lutein, vitamin A (retinol) in fin tissues of *Melanotaenia parva* fed with diets containing carotenoids

Parameter (mg/kg)	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
Astaxanthin	n.d	11.17	13.06	n.d	n.d	n.d	n.d
Canthaxanthin	6.07	6.94	8.29	6.94	7.43	5.48	7.25
Lutein	1.36	1.58	1.57	n.d	n.d	1.59	1.70
Retinol	5.91	8.99	12.78	10.80	14.07	6.72	11.75

* n.d = not detected

in 1 ml of phosphate buffer saline (PBS). The homogenate was centrifuged in 10000 rpm for 20 minutes. As many of 0.5 mL of apparent supernatant was added 2 mL of mixture solution which contained 2.23 mL of concentrated HCl. A 10 g of TCA and TBA were added with 100 mL of aquadest. The mixture was incubated at 80°C for 1 hour. After that, it was centrifuged in 3000 rpm for 5 minutes. The supernatant was poured to the other tube and analyzed using spectrophotometer in 532 nm of wavelength. The tetraethoxypropane (TEP) was used as a standard solution (Singh *et al*., 2002).

Calcium level test

Calcium level test was conducted on the overall fish skeleton at the end of the experiment. The measurement was done using calcium chloride principal analysis which will turn into sediments in calcium oxalate form that dissolved in ammonium oxalate and acetate acid buffer solution (AOAC, 2005). Chapman reagent was prepared first. As many of 10 g of $(NH_4COO)_2$. H_2O, 42 g of NH_4Cl, 7 mL of concentrated CH_3COOH (96–97%), and 2 mL of Brom cresol green 0.1% were dissolved and diluted using H_2O into 1 L of volumetric flask.

The procedure was started by pouring 50 mL of HCl filtrate into a 25 mL of beaker glass. The Chapman reagent was added to the filtrate. After that, concentrated NH_4OH was added while stirred until it changed into a green colour. The pH level got higher so that the colour became blue. It would be green again when heated, then it would be as clear as water over time. The solution was incubated overnight. The sediment and filter paper was placed in the beaker glass. The solution was added with H_2SO_4 (1:4) and 150 mL of H_2O. It was heated until reached 80–90°C, then it analyzed using $KMnO_4$ 0.02 N or 0.05 N titer. The blank solution was made from 8.5 mL of HCl and it was diluted until 100 mL, then as many of 20 mL from the dilution was collected as a blank solution.

Data analysis

Fish growth, survival, feed conversion ratio, lightness, colourcolour density, hue, redness index, yellowish index, total carotenoids, percentage of chromatophores, blood profile, antioxidant activity, and calcium level test were statistically analysed using randomized complete design by IBM SPSS version 23. Significant differences between treatments were determined using a post-hoc Tukey test. Meanwhile, pigmentation and astaxanthin, canthaxanthin, and lutein deposition, and retinol content in muscle, fish skin, and fin were analyzed descriptively.

RESULTS AND DISCUSSIONS

Results

Growth, survival, and feed conversion ratio

There were no significant differences amongst treatments in initial weight and length (BAw & PAw), absolute length (PM), relative length (PR), and feed conversion ratio ($P>0.05$). Whereas, fish final weight and length (BAk & PAk), absolute weight and length (BM & BR), specific growth rate (LPS), and survival demonstrated significant differences ($P<0.05$) (Table 2).

Colour quality

Pigmentation and astaxanthin content in muscle, skin, and fin tissues were only detected on astaxanthin tested feed. The 260 mg/kg of astaxanthin treatment showed higher deposition compared to canthaxanthin tested feed, while the lutein content was not found in muscle, skin, and fin tissues in the basal feed (Table 3, 4, and 5). Carotenoids conversion into vitamin A (retinol) was established in all tissues that fed using basal and carotenoids tested feed (Table 3, 4, and 5).

Total carotenoids in the fish fed with 130 mg/kg of astaxanthin diet was significantly different with treatment 260 mg/kg of canthaxanthin, but both were not different significantly with the other treatment (Figure 1). The total carotenoids in muscle and skin tissues ranged from 1–2.8 mg/kg and 2–6 mg/kg, respectively, and there were no significant differences ($P>0.05$) (Figure 2). In the fin tissues, the highest carotenoids were obtained in 260 mg/kg of astaxanthin tested feed and it was significantly different with basal feed ($P<0.05$) (Figure 3).

The percentage of chromatophore closure (melanophore, xanthophore, erythrophore, and nonpigment cell) showed that the 260 mg/kg of carotenoids treatment produced extra melanophores compared to the 130 mg/kg of carotenoids treatment and basal feed. In the treatment of 130 mg/kg dosage, the one which produced more melanophore was astaxanthin tested feed. Xantophore in the fish integument was greatly produced by fish that fed using the three different kinds of carotenoids, while the basal feed treatment showed lower xanthophore production. The 260 mg/kg of astaxanthin treatment

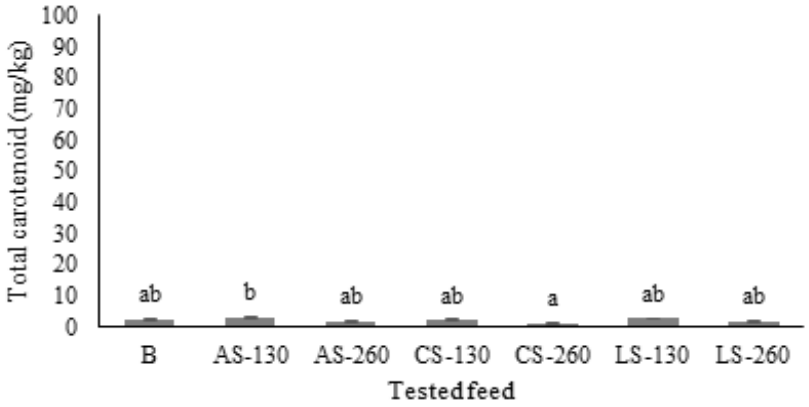

Figure 1. Total carotenoids in muscle tissues of *Melanotaenia parva*. Different letters above the bars indicate significantly different.

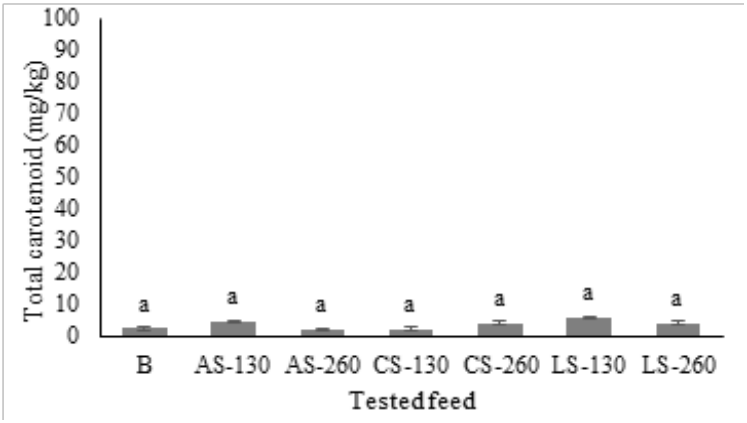

Figure 2. Total carotenoids in skin tissues of *Melanotaenia parva*. Different letters above the bars indicate significantly different

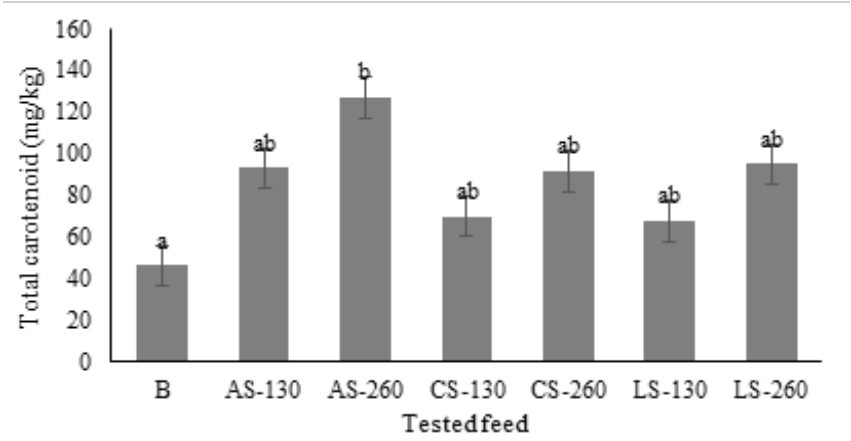

Figure 3. Total carotenoids in fin tissues of . Different letters above the bars indicate significantly different

presented a higher result in erythrophores in the fish integument. The percentage of the non-pigment cell was lower in carotenoids tested feed. Conversely, the basal feed was higher in the non-pigment cell (P<0.05) (Table 6).

The brightness value of Kurumoi rainbow fish at the end of rearing period in the tested fish fed using carotenoids increased significantly (19–38%) from the beginning of the experiment compared to basal feed (P<0.05). The colour density of Kurumoi rainbowfish elevated as well. The highest to lowest colour density of all treatments was AS-260, CS-260, AS-130, CS-130, LS-260, and LS-130, respectively. The hue degree of the basal and tested feed, which ranged from 300–309º was also considerably higher than the basal feed treatment (P<0.05). The reddish value in the carotenoids feeds treatment tended

Table 6. The percentage of melanophore, xanthophore, erythrophore, and nonpigment chromatophore in *Melanotaenia parva*

Parameter (%)	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
Melanophore	8.92 ± 1.27^{a}	12.88 ± 1.46^{b}	13.22 ± 1.38^{b}	9.62 ± 1.04^{a}	13.73 ± 0.66^{b}	8.62 ± 0.46^{a}	14.71 ± 0.38^{b}
Xantophore	26.23 ± 4.01^{a}	33.56 ± 2.83^{b}	37.50 ± 1.86^{b}	36.24 ± 1.69^{b}	36.23 ± 1.49^{b}	34.65 ± 2.12^{b}	33.12 ± 1.17^{b}
Erythrophore	0.53 ± 0.33^{a}	2.71 ± 1.26^{bc}	3.69 ± 0.55^{c}	1.30 ± 0.17^{ab}	1.16 ± 0.33^{ab}	0.38 ± 0.66^{a}	2.32 ± 0.51^{bc}
Non-pigment cell	64.32 ± 3.53^{d}	50.84 ± 2.64^{abc}	45.59 ± 0.94^{a}	52.83 ± 1.51^{bc}	48.87 ± 2.03^{ab}	56.34 ± 2.84^{c}	49.84 ± 1.53^{ab}

Superscript letter following mean value (± standard deviation) in the same row indicates significant differences (P<0.05).

Table 7. Colour glimpse of Kurumoi rainbowfish during the 56 days of rearing

Parameter	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
LAw (%)	49.45 ± 0.76^{a}	46.45 ± 2.81^{a}	45.34 ± 0.70^{a}	44.71 ± 2.41^{a}	45.69 ± 3.61^{a}	48.34 ± 2.01^{a}	46.29 ± 1.88^{a}
LAk (%)	43.77 ± 3.12^{a}	58.57 ± 1.97^{bc}	59.67 ± 1.28^{bc}	61.70 ± 0.40^{c}	57.71 ± 1.66^{b}	57.51 ± 2.75^{b}	56.39 ± 2.39^{b}
CAw (%)	4.24 ± 0.49^{a}	4.33 ± 0.18^{a}	4.16 ± 0.27^{a}	4.06 ± 0.21^{a}	4.08 ± 0.50^{a}	4.06 ± 0.47^{a}	4.22 ± 0.51^{a}
CAk (%)	4.84 ± 0.05^{a}	6.03 ± 0.25^{c}	6.68 ± 0.17^{d}	5.64 ± 0.25^{bc}	6.20 ± 0.31^{cd}	5.37 ± 0.72^{ab}	5.87 ± 0.19^{bc}
HAw (º)	289.67 ± 0.11^{a}	289.67 ± 5.13^{a}	287.00 ± 1.00^{a}	290.00 ± 1.00^{a}	291.67 ± 7.37^{a}	291.00 ± 7.21^{a}	294.67 ± 0.58^{a}
HAk (º)	285.67 ± 1.53^{a}	308.67 ± 8.50^{b}	307.33 ± 4.73^{b}	301.00 ± 8.72^{b}	306.00 ± 6.56^{b}	304.33 ± 11.15^{b}	306.67 ± 3.06^{b}
a* Aw	1.44 ± 0.43^{a}	1.50 ± 0.42^{a}	1.26 ± 0.17^{a}	1.44 ± 0.10^{a}	1.48 ± 0.31^{a}	1.43 ± 0.38^{a}	1.60 ± 0.26^{a}
a* Ak	1.39 ± 0.22^{a}	3.78 ± 0.65^{bc}	4.11 ± 0.47^{c}	2.93 ± 0.66^{b}	3.65 ± 0.38^{bc}	2.97 ± 0.54^{b}	3.54 ± 0.44^{bc}
b* Aw	-3.98 ± 0.53^{a}	-3.99 ± 0.11^{a}	-3.98 ± 0.24^{a}	-3.81 ± 0.19^{a}	-3.79 ± 0.65^{a}	-3.78 ± 0.57^{a}	-3.83 ± 0.18^{a}
b* Ak	-4.59 ± 0.09^{a}	-4.60 ± 0.65^{a}	-5.30 ± 0.32^{a}	-4.80 ± 0.60^{a}	-5.01 ± 0.67^{a}	-4.42 ± 1.12^{a}	-4.70 ± 0.32^{a}
TCF Aw	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
TCF Ak	Light yellow	Orange	Reddish orange	Reddish orange	Reddish orange	Yellow	Reddish orange

Note : Initial lightness (LAw), final lightness (LAk), inial colour density (CAw), final colour density (CAk), initial hue (HAw), final hue (HAk), initial reddish value (a* Aw), final reddish value (a* Ak), initial yellowish value (b* Aw), final yellowish value (b* Ak), initial toca colour finder score (TCF Aw), final toca colour finder score (TCF Ak). Superscript letter following mean value (± standard deviation) in the same row indicates significant differences (P<0.05).

Table 8. The blood profile of Kurumoi rainbowfish.

Parameter	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
SDM (10^6 cell/mm^3)	1.15 ± 0.06^{a}	1.54 ± 0.13^{ab}	1.51 ± 0.27^{ab}	1.59 ± 0.18^{ab}	2.08 ± 0.04^{b}	1.16 ± 0.49^{a}	1.15 ± 0.06^{a}
SDP (10^4 cell/mm^3)	5.09 ± 0.26^{a}	8.45 ± 0.63^{c}	8.78 ± 0.91^{c}	8.32 ± 0.61^{c}	9.31 ± 1.09^{c}	5.38 ± 0.44^{ab}	6.41 ± 0.17^{b}
Hematocrit (%)	11.30 ± 1.48^{ab}	14.70 ± 1.20^{b}	19.65 ± 1.45^{c}	14.75 ± 1.85^{b}	20.50 ± 2.17^{c}	9.20 ± 1.00^{a}	12.15 ± 1.35^{ab}
Lymphocyte (%)	89.00 ± 1.00^{a}	87.00 ± 1.00^{a}	92.50 ± 1.50^{a}	91.50 ± 1.50^{a}	91.50 ± 2.50^{a}	88.50 ± 0.50^{a}	82.50 ± 2.50^{a}
Neutrophil (%)	3.00 ± 0.58^{a}	6.00 ± 1.00^{ab}	8.00 ± 2.31^{ab}	5.67 ± 1.15^{ab}	7.33 ± 1.71^{ab}	6.00 ± 1.00^{ab}	11.00 ± 2.00^{b}
Monocyte (%)	5.33 ± 0.58^{a}	7.00 ± 0.58^{a}	6.00 ± 0.71^{a}	5.50 ± 1.50^{a}	7.33 ± 0.94^{a}	5.50 ± 0.50^{a}	6.50 ± 0.50^{a}
Hb (%)	2.50 ± 0.10^{a}	2.93 ± 0.23^{b}	3.00 ± 0.12^{b}	2.93 ± 0.12^{b}	3.40 ± 0.20^{c}	2.60 ± 0.20^{ab}	2.93 ± 0.23^{b}

Superscript letter following mean value (± standard deviation) in the same row indicates significant differences (P<0.05).

Table 9. Antioxidant activity (SOD and MDA) of Kurumoi rainbowfish

	Tested feed						
	Basal	AS-130	AS-260	CS-130	CS-260	LS-130	LS-260
SOD (unit/mg protein)	3.29 ± 0.14^{bc}	2.24 ± 0.35^{a}	2.71 ± 0.24^{ab}	3.32 ± 0.41^{bc}	3.58 ± 0.66^{c}	3.94 ± 0.53^{c}	3.46 ± 0.93^{bc}
MDA (unit/mg protein)	10.96 ± 2.27^{b}	7.03 ± 0.11^{a}	8.28 ± 1.75^{a}	6.46 ± 0.47^{a}	7.65 ± 1.20^{a}	6.25 ± 0.74^{a}	8.48 ± 2.08^{a}

Superscript letter following mean value (± standard deviation) in the same row indicates significant differences (P<0.05).

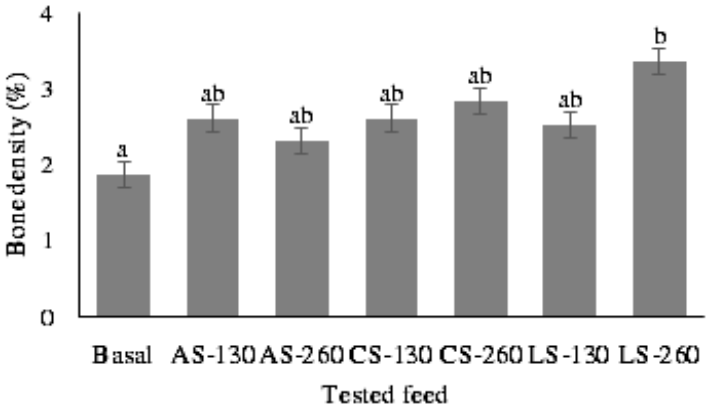

Figure 4. Bone density of Kurumoi rainbowfish

to raise at the end of the treatment, while the basal feed treatment was not. The minus value of yellowish value indicated that the tested fish were likely more bluish at the end of the experiment. Visually, the tested fished presented the best colour which is orange because of the astaxanthin supply (Table 7).

Blood profile

The parameters of the blood profile were leucocyte (SDP), erythrocyte (SDM), hematocrit, lymphocyte, neutrophil, monocyte, and hemoglobin (Hb). The result of the blood profile analysis was shown in Table 8.

The carotenoids tested feed significantly affected blood profile as well, especially erythrocyte, leucocyte, hematocrit, neutrophils, and hemoglobin ($P<0.05$). The other blood profile parameters such as lymphocyte and monocyte showed no significant differences amongst treatments ($P>0.05$).

Antioxidant activity

The superoxide dismutase (SOD) and malondialdehyde (MDA) were observed and the result was presented in Table 9.

The carotenoids dietary reatment showed a significant result on SOD and MDA activity ($P<0.05$). The carotenoids tested feed treatment was significantly lower compared to basal feed treatment on MDA activity ($P<0.05$). The lower activity of MDA was presented in carotenoids tested feed compared to basal feed treatment. The 260 mg/kg of astaxanthin feed treatment resulted in lower SDA activity than the other kinds of carotenoids and control ($P<0.05$).

Bone density

The bone density was observed in percent (%) unit. The result was shown in Figure 4. The higher level of bone density was obtained in the carotenoids tested feed treatment. The most prominent result was obtained by the 260 mg/kg of lutein tested feed treatment. It was significantly higher than the basal feed treatment, although there was no difference between the other carotenoids tested feed treatment.

Discussions

The result in Table 2 showed that carotenoids tested feed directly affected the growth and survival rate of the Kurumoi rainbowfish (Meilisza *et al.*, 2017). Niu *et al.* (2017) and Parisanti *et al.* (2011) reported similar results. The high level of ash content in the tested feed did not alter the result. It was proved by the result of feed conversion which not differs significantly. In a conventional culture of Kurumoi rainbowfish, the survival rate commonly ranged from 40–50 % (Kadarini *et al.*, 2015; Yuliyana *et al.*, 2016) and the disease prevalence 34% (Sholichah, 2014).

The carotenoids tested feed treatment obtained improved colour appearance than the basal feed treatment, especially the fin. There were differences in carotenoids deposition in several tissues of Kurumoi rainbowfish. The clownfish *Amphiprion ocellaris* showed greater pigmentation in skin and fin tissues after fed using carotenoids tested feed (Ho *et al.*, 2013; Seyedi *et al*. 2013). The total carotenoids in muscle tissues were fairly low (1–3 mg/kg) than in skin tissues (Figure 1 and 2). The carotenoids content of Japanese koi carp ranged from 30–35 mg/kg with 30 and 80 mg/kg of astaxanthin feed additive and more than 45 mg/kg using 110 mg/kg of canthaxanthin addition in the feed. A lower dosage of astaxanthin was adequate to increase the colour appearance of guppy and rainbow trout (Mirzaee *et al*. 2012; Teimouri *et al.* (2013).

The highest carotenoids content was in fin

tissues was astaxanthin. The carotenoids dosage also affected the total deposition of carotenoids in fish tissues. The 260 mg/kg of astaxanthin showed greater result than 130 mg/kg, the same thing happened in both other treatment (canthaxanthin and lutein) (Figure 1, 2, and 3). The blood parrot fish and bivalve presented that the accumulation of total carotenoids could be higher than the quantity it got into the tissues (Zheng *et al.*, 2010; Sun *et al.,* 2012). In this experiment, astaxanthin was more effective than canthaxanthin and lutein. A similar result was also reported by Pham *et al.* (2014) that astaxanthin was found more effective to deepen the pigmentation in olive flounder *Paralichthys olivaceus* compared to the other kinds of carotenoids. In Showa koi, the pigmentation could be boosted using 150 mg/kg canthaxanthin (Sun *et al.*, 2012). The astaxanthin was discovered to be absorbed quicker than lutein in goldfish *Cyprinus auratus* (Yuangsoi *et al*. 2010) and the retention coefficient was 1.3 times higher than canthaxanthin (Choubert & Storebakken, 1996).

The measurement of astaxanthin in the muscle, skin, and fin tissues indicated that the astaxanthin was only formed from the feed which contained astaxanthin (Table 3, 4, and 5). On the contrary, Yuangsoi *et al*. (2010) described that *Carassius auratus* was able to transform lutein to astaxanthin. Common carp have the ability to oxidate 4 and 4' position of the ionine ring and potentially converts lutein into astaxanthin (Dharmaraj & Dhevendaran, 2011). A similar result was also achieved when canthaxanthin was delivered to the tested fish because there was no astaxanthin production with canthaxanthin as the precursors (Table 3, 4, and 5). Canthaxanthin was retained in similar form and detected in each kind of tested feed.

The deposition of astaxanthin was quite low. The results indicated that the effectiveness of pigmentation from astaxanthin was low. It was even lower than the study by Garner *et al*. (2010) showed that the pigmentation in Chinook salmon *Oncorhynchus tshawytscha* fed using astaxanthin feed was 24% and 16% in muscle and skin tissues, respectively. Even though the astaxanthin deposition was pretty low, the total carotenoids were fairly high. It described that there might be several kinds of carotenoids besides astaxanthin which not measured in this study. Lutein was disclosed in every feed treatment, except canthaxanthin feed (Table 3, 4, and 5). The astaxanthin provision was able to be retained as lutein which has been reported by Li *et al.* (2016) in blood parrot fish (*Cichlasoma synspilum* × *Cichlasoma citrinellum*). It also showed there was no lutein retained in the canthaxanthin feed treatment.

According to the classification by Schiedt *et al.* (1985), Kurumoi rainbowfish is classified to salmonid or carnivorous species which can preserve lutein, canthaxanthin, and other kinds of carotenoids in similar molecules, without converting into astaxanthin. It is renewal information of Kurumoi rainbowfish and it added scientific updates in fish classification according to how it converts one kins of carotenoids into astaxanthin as a major fish pigment.

Carotenoid functioned as a precursor of vitamin A (retinoid) (Schiedt *et al*. 1985). According to Garner *et al*. (2010), retinoid in Chinook salmon *Oncorhynchus tshawytscha* egg fed using 50 mg/kg of astaxanthin extended up to 36%. The concentration of retinol in muscle and skin tissues of Kurumoi rainbowfish ranged from 1.0–1.6%. The higher the concentration get, the retinol concentration will be higher, although the effect will be lower in Kurumoi rainbow fish.

The measurement of colour quality was conducted based on the percentage of chromatophore closure in the integument tissues (Table 6). Several former studies showed that carotenoids were reserved as xanthophore and erythrophore (Mills & Patterson, 2009; Chatzifotis *et al*., 2011; Sefc *et al*., 2014). The study also described that feed contained carotenoids was able to boost xanthophore pigment. The tested fish fed using 260 mg/kg of astaxanthin were considered to have an intense red pigment. The basal feed treatment obtained more non pigmented cells (Table 6). It was in line with Yang *et al*. (2016) in blood parrot fish which showed xanthophores and erythrophores escalation when fed using *Pomacea canaliculata* eggs.

Yang *et al.* (2016) also stated that feed contained different kinds of carotenoids would generate colour differences. The percentage of chromatophore showed that carotenoids addition affected the chromatophore appearance in the integument of Kurumoi rainbowfish. The melanophore closure in 130 mg/kg of astaxanthin was greatly different than the canthaxanthin and lutein in the same dosage, while in the higher dosage (260 mg/kg) both canthaxanthin and lutein has no differences with astaxanthin. In the integument, carotenoids were preserved in the xanthophore dan erythrophore (Braasch *et al*., 2007). Similar to this study, it was known that carotenoids addition stimulated erythrophore

forming in the Kurumoi rainbowfish integument. This result was also supported by reddish value (a*) and colour density (Table 7). The 260 mg/kg of astaxanthin tested feed treatment presented more vibrant and dense red pigment. Yedier *et al.* (2014) also reported that red zebra cichlid *Maylandia estherae* showed orange-reddish colour, while *Spirulina* resulted in orange and yellow pigment. meningkatkan warna jingga dan kuning. Furthermore, toca colour finder (TCF) presented a more attractive colour on Kurumoi rainbowfish (orange reddish) which has become a major preference of ornamental fish enthusiasts.

Nutrition is of the factor that directly affected blood profile which described health status (Campbell, 2015). Carotenoids also contributed to enhancing fish health status (Anbazahan *et al.,* 2014; Ibrahim & Banaee, 2014). Chow *et al.* (2016) mentioned that a hybrid catfish performed higher leucocyte, hematocrit, and hemoglobin and it was supported the result of this study in Kurumoi rainbowfish. The result of Kurumoi rainbowfish blood profile was presented in Table 8. The erythrocyte, leucocyte, hematocrit, neutrophils, and hemoglobin were significantly higher compared with basal feed treatment ($P<0.05$). It is understood that carotenoids feed treatment presented better health status compared to basal feed treatment. The related result was also obtained in *Osphronemus goramy* and *Clarias gariepinus* (Minaka *et al.,* 2012; Ziyadaturrohmah *et al.,* 2013).

The application of astaxanthin and canthaxanthin feed in a dosage of 130 and 260 mg/kg acquired similar result in leucocyte content, whereas lutein obtained lower leucocyte. The 130 mg/kg of lutein resulted in low leucocyte content and it was not different significantly with the basal feed treatment, while the higher dosage (260 mg/kg) resulted in the adequate level of leucocyte. It was considered that a lower dosage of lutein could not support Kurumoi rainbowfish health status, while the higher dose could (Table 8). It was assumed that lutein absorption was lower than two other carotenoids. The statement was supported by the study by Yuangsoi & Jintasathaporn (2011) and Meilisza *et al.* (2017)

The health status of the tested fish was discovered through blood profiles. The erythrocyte content of a healthy teleost species generally ranges in $1.05–3.0\times10^6$ cell/mm^3 (Robert, 2012). Based on the statement, it was considered that the erythrocyte of Kurumoi rainbowfish was classified in normal condition. The canthaxanthin (260 mg/kg) treatment was quite higher and it was significantly different with basal feed treatment ($P<0.05$).

The leucocyte functioned as the immune protector from any infections (Chow *et al.*, 2016). Related studies showed that leucocyte level was depended on its species. Spotted rose snapper *Lutjanus guttatus* leucocyte ranged from $2.5\times10^4–11.1\times10^4$ cell/mm^3, the bonnethead shark *Sphyrna tiburo* leucocyte ranged from $3.5\times10^4–8.3\times10^4$ cell/mm^3, and wild lake sturgeon *Acipenser fulvescens* leucocyte ranged from $0.3\times10^4–2.3\times10^4$ cell/mm^3 (Del Rio-Zaragoza *et al*., 2011; Haman *et al*., 2012; DiVincenti *et al*., 2013). The leucocyte of Kurumoi rainbowfish ranged from $5.1–9.3\times10^4$ cell/mm^3 and overall, the carotenoids feed treatment presented higher leucocyte content compared to basal feed treatment ($P<0.05$).

The highest hematocrit (approximately 20%) of Kurumoi rainbowfish was observed in AS-260 and CS-260. Both were significantly different from the other carotenoids treatment and basal feed treatment. Related studies showed a different range of hematocrit percentage. Ferreira *et al.* (2010) reported that smooth dogfish *Mustelus canis* produced 20–32% of hematocrit. Atlantic sharpnose *Rhizoprionodon terraenovae* hematocrit ranged from 18.9–30.8% (Haman *et al.,* 2012), while wild lake sturgeon was within 17–38 % (DiVincenti *et al.,* 2013). The result of this study was in line with those several former studies so that the hematocrit of Kurumoi rainbowfish was considered in normal condition.

Normally, the lymphocyte percentage in a healthy fish is 20%–99% of total leucocyte (Robert, 2002). It is in line with the lymphocyte of Kurumoi rainbowfish so that it was categorized as normal. The lymphocyte percentage presented that the tested fish were in good condition and it did not differ between treatments. Monocyte percentage was normally 5% of the total leucocyte (Campbell & Ellis, 2013). In this study, all of the tested fish, either carotenoids or basal feed treatment, were considered as normal because the monocyte ranged from 5.3–7.3% and it did not differ significantly ($P<0.05$). Neutrophil holds a particular role in the inflammatory process. According to Roberts (2012), a normal fish will produce relatively 6–8% of the total proportion of leucocyte. The carotenoids feed treatments produced 6–11% of the total leucocyte. The most significantly different was LS-260 which was higher (11%) than control (3%) ($P<0.05$).

The blood profile od Kurumoi rainbowfish in each carotenoid feed treatment showed distinctive superiority. Generally, the CS-260 and AS-260 resulted in improved result of blood profile compared with the other treatments. It was possibly caused by the higher level of palatability and absorption rate of the astaxanthin and canthaxanthin. Therefore, both of them were well-utilized in metabolism (Niu *et al.,* 2009; Yuangsoi & Jintasathaporn, 2011).

The behavior of carotenoids as a secondary antioxidant was discovered in several former studies (Kiokias *et al*. 2008; Mekkawy *et al.,* 2011; Mekkawy *et al.,* 2013). In this study, the primary antioxidant effects (SOD and MDA) of the carotenoids feed treatments compared with basal feed treatment (Table 9). Similar results were also discovered in different species that the activity of SOD enzyme with carotenoids addition was lower than basal feed. It was also described by Pham *et al.* (2014) using olive flounder and Choi *et al.* (2016) and Rahman *et al.* (2016) using rainbow trout which presented lower SOD activity in carotenoids feed treatment compared with basal feed treatment.

Pham *et al.* (2014) reported that generally, the antioxidant activity of astaxanthin was more efficient than the other kind of carotenoid. It was caused by the parallel orientation of astaxanthin polar in hydrocarbon chain and it trapped free radical. Moreover, it would prevent oxidative destruction in cellular macromolecules (Liu & Osawa, 2007). In brief, the low level of SOD and MDA activity showed that carotenoids feed treatment became an indication of lessening the requirement to detox reactive oxygen species (ROS). The accumulation of carotenoids in the liver showed intense prevention against hydroxyl and superoxide radical.

Carotenoids also held a major role in bone density (Bovier & Hammond, 2017). Carotenoid, especially lutein, is able to improve bone density (Takeda *et al.*, 2017). The elevation of calcium content was discovered in LS-260 (Figure 2). The number of studies about carotenoids as bone density precursor was limited, but this study particularly became initial scientific prove.

CONCLUSION

Carotenoids potentially elevated colour appearance, growth, and health status of Kurumoi rainbowfish. The best treatment was astaxanthin in a dosage of 260 mg/kg. It increased growth performance, survival rate, and health status of Kurumoi rainbowfish, compared to the other carotenoid feed treatment. This study also classified Kurumoi rainbowfish as salmonids which only reserved carotenoids as one stable form and has no ability to convert the other kind of carotenoids to astaxanthin.

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