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Effect of Different Enriched Crickets (*Gryllus sigilatus*) on Growth and Pigmentation of Asian Arowana (*Scleropages formosus*) Var. Super Red

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ABSTRACT

This study aimed to evaluate the effect of crickets enriched with a source of carotenoids on fish's growth, colour, and total carotenoid content in juvenile Asian Arowana (*Scleropages formosus*). Six juveniles were fed with different enriched crickets (G. sigilatus) based on the following treatments: (A) Crickets fed with fish feed without enrichment, (B) Crickets fed with fish feed containing 500 ppm of synthetic astaxanthin, and (C) Crickets fed with a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal. The results of the study revealed that The weight gain (WG), specific growth rate (SGR), feed efficiency (FE), length gain (LG), and survival rate (SR) for treatment A were 110 g, 1.16% head/day, 45.79%, 10.98 cm, and 100% respectively. There was also no significant difference (P>0.05) with the other treatments. However, treatment B had a positive effect on the redness (a*) of the Arowana tail with a value of 9.52, and treatment C increased the yellowness (b*) of the Arowana body with a value of 2.77 (P<0.05), which was different to the other treatments. There were no differences between any of the treatments concerning the effects on lightness (L*), chromaticity (C*), and hue (H*) on either the body or the tail. The astaxanthin enrichment produced the highest carotenoid content (P<0.05) with a value of 45.09 µg/g. Enriching crickets with astaxanthin influence colour and carotenoid content but not the growth of the Asian Arowana fish.

1. Introduction

Indonesia is listed among the world's top five ornamental fish-producing and exporting countries yearly. In 2019, the top five exporters of ornamental fish were Japan ($41.8M), Indonesia ($39.8M), Singapore ($35.4M), Thailand ($27M), and Malaysia ($18.2M), with 1.53% growth in export value from 2018 (OEC 2021). As a commodity, ornamental fish can be split into two categories: marine and freshwater. One study reported that Indonesia has more than 700 marine ornamental fish species (Suaib *et al.* 2018). Another report stated that approximately 400 species are freshwater ornamental fish (Satyani and Subamia 2014), while the Arowana (*Scleropages formosus*) is the most expensive freshwater fish species, especially the super red strain. Statistics Indonesia (2014) reported that this species contributed around 32.62% and 12.29% for super red Arowana and other Arowana, respectively, to the total value of exported Indonesian ornamental fish. Demand for Arowana has increased dramatically over the past three decades (Chang 2009; Setiawan 2018), especially for the golden Arowana and the super red strain (Yue *et al.* 2004). Outrageous performances, high quality and mythological subjects are the main factors driving the purchase of these fish as pets. Besides that, Arowana super red commands a very high price, ranging from US$ 170 to US$ 50,000 per fish (Sriyadi 2017), depending on its quality.

Sriyadi (2017) listed the following seven criteria for determining the super red Arowana: colour (30 points), body form (30 points), overall view performance (20 points), mouth and burble (5 points), eyes (5 points), pectoral fin (5 points), and

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dorsal, anal and caudal fin performances (5 points). Based on these criteria, colour is a priority for Arowana fish, with various scientists also stating that colour influences the price of ornamental fish (Ahilan *et al.* 2008; Gouveia and Rema 2005; Sun *et al.* 2012; Wang *et al.* 2006; Yang *et al.* 2012; Yuangsoi *et al.* 2010; Yue *et al.* 2020). The colour of a fish reflects the many pigments in its chromatophores cells, of which there are five types: melanophores, erythrophores, xanthophores, leucophores and iridophores (Fujii 1993, 2000; Goda *et al.* 2011; Sköld *et al.* 2016). Carotenoids are pigments in fish cells (Das 2016); however, fish cannot produce them in their body de novo. Therefore, the feed must meet their carotenoid requirements (Gouveia *et al.* 2003; Meyer 1994).

The study of nutrition in Asian Arowana research is relatively rare. However, Natalia *et al.* (2004) reported the characteristics of digestive enzymes in Arowana. Gomez *et al.* (2007) found that whole yellow mealworm (*Tenebrio molitor)* as feed for Arowana larvae negatively affected fish digestion, while various other studies have shown that Asian Arowana is carnivorous and specialised surface feeders (Dawes *et al.* 1999; Goh and Chua 1999). Therefore, many hobbyists give live feed such as freshwater prawns, chunks of fresh shrimp, cuts of fish, baby fish, insects (e.g. crickets, cockroaches, and centipedes), worms/caterpillars (e.g. silkworms, mealworms, earthworms, and blood worms) and frogs (Faber 2017). Based on hobbyist opinion, crickets are the favourite among these types of live feed, although there is no scientific evidence on the effect of crickets on Arowana fish. The nutrient composition of crickets can also be improved (Ogilvy *et al.* 2012), especially the carotenoid content, which can potentially increase the colour quality of Arowana fish. Therefore, this study principally explores the enrichment of crickets to increase the quality of Arowana juveniles. The study's objective is to evaluate the effect of cricket meal enriched with a carotenoid source on the growth and colour performance of super red Arowana juveniles (*Scleropages formosus*).

2. Materials and Methods

2.1. Animals, Facilities, and System

A total of six Arowana fish (*Scleropages formosus*) of the super red strain with body lengths ranging from 12 to 14 cm and body weight of 17–19 g/fish were purchased from PT Arwana Citra Indonesia, West Java, Indonesia. The animals were acclimated to the experimental conditions for three days without being fed (fasted). Then, for seven days, the fish were fed daily (8 a.m.) with the control feed for this study until they reached apparent satiation. The fish were kept in 60 cm x 40 cm x 40 cm individual glass aquariums filled with aerated fresh water to a depth of around 20 cm. All fish tanks were equipped with physical filters and air stones connected to a blower to maintain dissolved oxygen content. A daily water change was performed (30%) by siphoning fish excretions to preserve the water quality. Data collection at the beginning of the experiment was conducted by measuring fish body length, body weight, and the colour quality of the body and caudal fin.

2.2. Experimental Diet and Fish Feeding

Experimental design. This study utilised three experimental diets, and each treatment used two Arowana fish kept in different aquariums as replication. The Arowana fish were fed with different enriched crickets (*G. sigilatus*), with the following treatments: (A) Crickets fed with fish feed only (without enrichment), (B) Crickets fed with fish feed containing 500 ppm of synthetic astaxanthin, and (C) Crickets fed with a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal.

The basal feed for cricket in this experiment was PF 500 (Commercial feed from PT Matahari Sakti Indonesia), containing 39% protein, 5% fat, 4% fibre, and 11% ash. The synthetic astaxanthin used was chlorophyll pink (DSM) with an astaxanthin content of 10 %. At the same time, sand crab (*Emerita* sp.) was obtained from Cilacap, Central Java, Indonesia. Sand crab flour was prepared by drying fresh sand crab using a freeze dryer and mincing. The meal was mixed with fish and marigold petals as cricket feed (the third diet). The nutritional content of the sand crab was 38% protein, 13% fat, 6% fibre and 23% ash.

Preparation of enriched cricket by feed and carotenoid sources. Around 300 crickets of uniform size were prepared every morning in different containers, with each container holding 100 crickets. The crickets in the first container were fed with basal feed (feed-1), which was then used as the control feed for the Arowana. The crickets in the second container were fed fish feed mixed with 500 mg synthetic astaxanthin per kg of feed (feed-2), while the crickets in the third container were fed a mixture of fish feed (50%) with sand crab meal (48%) and marigold petal meal (2%) as feed-3.

The crickets were fed with feeds-1, -2 and -3 for two hours. Then, after two hours, the Arowana juveniles ate the crickets. The fish were fed daily at 8.00 a.m. until they were fully satiated (ad-satiation). The crickets were counted and weighed before being given to the fish, and any leftover feed was also added to the feed intake calculation.

The proximate and carotenoid composition of cricket is presented in Table 1.

Table 1. Proximate and carotenoid composition of cricket (DM basis)

Nutrient content	Type of feed		
	A	B	C
Moisture (%)	7.44	7.86	6.01
Protein (%)	57.11	61.37	53.77
Fat (%)	22.81	17.40	26.61
Fibre (%)	7.00	5.97	7.66
Ash (%)	5.61	7.40	5.95
Total carotenoid (µg/g)	40.90	49.15	79.24

(A) Crickets (*G. sigilatus*) as a control diet (without enrichment), (B) Crickets enriched with 500 ppm of synthetic astaxanthin (carophyll pink, DSM), and (C) Crickets supplemented with formulated artificial feed, a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal

The main parameters of this research related to the fish body performance: length gain (LG), weight gain (WG), specific growth rate (SGR), feed intake (FI), and feed efficiency (FE), plus colour quality: lightness (L*), chromaticity (C*), hue or purity of colour (H*), redness (a*) and yellowness (b*); and the total carotenoid in the fish tissue.

Fish performance was measured three times: first during the initial plotting and rearing, then during the second month, and finally, in the fourth month. The data were calculated using the following formulas:

Length gain (LG)

Length gain = Final mean length (cm) – Initial mean length (cm)

Weight gain (WG)

Weight gain = Final mean weight *(g) – initial mean weight (g)

Specific growth rate (SGR)

SGR = ((ln final weight (g) – ln initial weight (g))/trial time (days))* 100

Feed intake (FI)

FI = (total feed offered (g)/number of fish)/ trial time (days)

% Feed efficiency (FE)

FE = (weight gain (g)*100) / feed consumed (g)

2.2.1. Colour Quality Parameter

Colour measurement was taken three times during the experiment, at the beginning, middle (second month) and end of the investigation (the fourth month).

A portable colourimeter (Minolta Chroma CR-400) was used to measure the skin colour quality in the fish, calibrated to a white plate standard (the original adjusted value of the white standard was L*= 97.40±0.01; a* = −0.10±0.01; b* = 1.92±0.01; C* = 1.92; H* = 93.8). During the measurement, the fish were anaesthetised with 2-phenoxyethanol (4 ml l^{-1}). The following fish parts were used as the measurement targets: (1) fish skin in the central body and (2) the central part of the caudal fin.

The following parameters were employed when evaluating the fish colouration: L for lightness (−100 black, +100 white), a* for redness or greenness (−100 green, +100 red), and b* for yellowness or blueness (−100 blue, +100 yellow), C* for chromaticity value (%) and H* for hue value or purity of the colour (0), respectively (Ayala-Silva *et al.* 2005; CIE 1976; Yilmaz and Ergün 2011; Yilmaz *et al.* 2013).

2.2.2. Total Carotenoid Content

The total carotenoid content of the fish tissue was determined using a modified method adopted by Tolasa *et al.* (2005). The study was conducted under yellow light. Ten grams of homogenised paste were extracted three times with 40 ml of a 0.05% acetonic BHT solution with ultra turax for 1 min. The samples were cooled during the examination to prevent warming. After each extraction, the samples were centrifuged at 4000 U/min for 5 min. Acetone extracts from the samples were then collected in a 250 ml separatory funnel. A 40 ml *n*-hexane, 100 ml water and 0.5 g salt (NaCl) was added to separate the water-soluble compounds. After the shaking process, approximately 20–30 min later, the entire white phase was taken. The upper layer was poured into a measuring cup and completed to 50 ml (raw extract).

Photometric valour was determined at a maximum of 470–474 nm (350–600 nm spectrum). The calculation of the total carotenoid concentration was rendered according to the standard curve of astaxanthin. Analysis of the samples was conducted three times (in triplicate).

Standard curve: Approximately 3 mg of standard Astaxanthin Call–E–Astaxanthin (Fa. Acros, 97–103%) and 100 mg of butyl-hydroxytoluene (BHT) were stored in a 10 ml volumetric flask and dissolved in dichloromethane (without acid) using an ultrasonic bath (stock solution). Then, 1 ml of this stock solution was diluted in a flask to 10 ml with n-hexane. Maximum absorbance was determined immediately following the solution production at the 350–600 nm spectrum interval. Later, the solution concentration was measured in maximum absorbance (approximately 472 nm).

The following formula was employed for the astaxanthin calculation:

$$\text{Call-E-Astaxanthin } (\mu g/ml \text{ or } mg/L) = \text{Absorbance} \times (10000/2100)$$

Where 2100 is E (1%, 1 cm) = standard absorbance of Call-E-Astaxanthin solution with 1% (w/v) in spectrophotometer with 1 cm in hexane at 470 nm, while 10,000 is the scale factor.

A 0.1, 0.5, 1.0, 1.5 and 2.0 ml of each diluted astaxanthin stock solution were put in a 10 ml flask using a pipette to prepare the standard curve. A ratio of n-Hexane: acetone: water (40:120:100) was completed to the marked level. Photometric tenacity was determined immediately after the solution production. A mixture of hexane, acetone and water was also treated as a blank.

An illustration of the standard curve for astaxanthin concentration is shown in Figure 1.

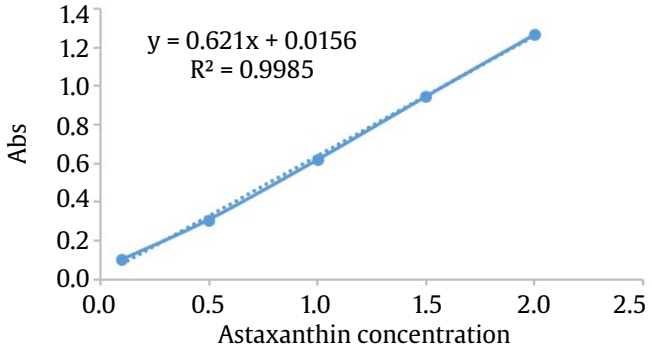

Figure 1. Standard curve of astaxanthin

2.3. Data Analysis

The data in this study are presented by the mean ± standard deviation (SD). Statistical analysis was performed using Minitab 17.0 statistical software. A two-way ANOVA was the method used for multiple comparisons. If the data had significant differences, Tukey's method analysed the level difference among treatments at $P<0.05$.

3. Results

3.1. Body Performance of Arowana Fish

The growth and development of the Arowana juveniles' bodies are presented in Table 2. There was no difference in WG, SGR, FE, LG and SR among the treatments ($P>0.05$). This means that cricket enrichment did not affect the growth of the Arowana juveniles even though those parameters had lower values in treatment C (cricket enriched with sand crab meal and marigold petal meal) compared to the other groups.

3.2. Colour of Arowana Juveniles

As presented in Table 2 and Figure 2, the enriched crickets affected the colour of the Arowana juveniles. Both types of enriched crickets, whether enriched with 500 ppm astaxanthin or with a formulated meal (combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal), were found to have

Table 2. Body performance of Arowana juveniles (*Scleropages formosus*) treated with different enriched crickets during the four-month rearing period

Parameter	Experimental diet			p-value
	A	B	C	
Initial body weight (g/head)	19.15±3.45	21.37±1.10	17.18±1.31	0.540
Body weight at 2nd month (g/head)	65.30±4.95	65.50±1.27	58.50±10.18	0.196
Final body weight (4th month) (g/head)	130.05±14.7	127.94±3.20	111.09±26.10	0.243
Weight grain (g)	110.9±11.25	106.6±4.32	93.9±24.79	0.196
Specific growth rate (%/head/day)	1.60±0.05	1.49±0.06	1.55±0.13	0.562
Feed intake (g/head/day)	2.38±0.03	2.37±0.01	2.29±0.10	0.087
Feed efficiency (%)	45.79±5.27	44.11±1.64	39.94±8.89	0.296
Initial body length (cm)	11.53±0.47	11.33±0.23	10.69±0.65	0.409
Body length at 2nd month (cm)	17.50±0.71	17.25±0.35	16.45±1.48	0.223
Final body length (4th month) (cm)	22.50±2.12	21.25±0.35	19.25±3.89	0.219
Length gain (cm)	10.98±1.65	9.90±0.30	8.56±3.01	0.229
Survival rate (%)	100.00±0.00	100.00±0.00	100.00±0.00	-

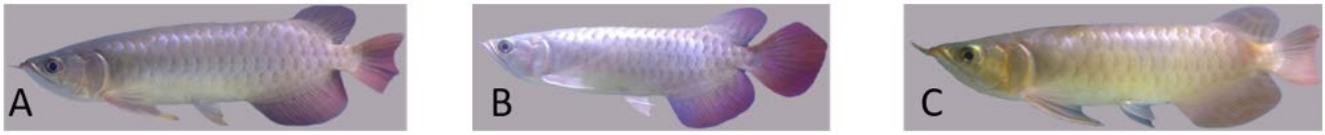

Figure 2. Arowana juveniles' performance after being treated with different cricket meals. (A) Control, (B) crickets enriched with 500 ppm astaxanthin, (C) crickets supplemented with formulated feed (a combination of 50% fish feed, 48% sand crab and 2% marigold petal meal)

influenced the Arowana juveniles' colouration after feeding for two or four months. After two months of treatment, there was no difference (P>0.05) in the body colour of the Arowana juveniles in terms of L* (lightness), C* (chroma) and a* (redness) among all treatments. In contrast, the body colour of the Arowana juveniles treated with formulated feed (treatment C) in terms of b* (yellowness) showed a higher value than for the other treatments (P<0.05). At the same time, the crickets enriched with astaxanthin produced the lowest b* (yellowness) compared to the other treatments. Another value was the body H* (hue), where the body hue showed a similar trend among all treatments during the second and fourth months of the rearing period. The highest value was obtained by treatment B, where the crickets were enriched with astaxanthin, followed by treatment A (non-enriched crickets) and treatment C (crickets supplemented with formulated feed).

After four months of rearing, treatment B showed the best body a* (redness) in the Arowana juveniles (crickets enriched with 500 ppm astaxanthin). However, this was significantly different from the other groups (P<0.05). While the highest body b* (yellowness) was shown in the Arowana juveniles that were fed crickets enriched with formulated feed (a combination of 50% fish feed, 48% sand crab meal and 2 % marigold petal meal) (treatment C) compared to the other treatments (P<0.05). Based on Table 2, we can see that the body a* (redness) of the Arowana juveniles showed a negative value in both the second and fourth months after the feeding period in all treatments. In contrast, the body b* (yellowness) of the Arowana juveniles showed a positive value on treatment C (crickets enriched with formulated meal). While treatment B (crickets enriched with astaxanthin) showed a different result, where the amount of b* (yellowness) in the Arowana juvenile body was negative.

The enriched crickets affected fish colouration, notably in the caudal fins (tails) as opposed to the bodies of the Arowana juveniles. In addition, the crickets enriched with synthetic astaxanthin had the best effect on the C* (chroma), a* (redness) and b* (yellowness) values compared to the other treatments, not only for the second month but also at the end of the rearing period (the fourth month). At the same time, treatment C (crickets enriched with formulated meal) had the highest h* (hue) value in comparison to the other treatments (P<0.05). Moreover, none of the treatments influenced the L* (lightness) value of the caudal fin colour in the second and final months of rearing.

3.3. Total Carotenoid Content in the Caudal Fins of Arowana Juveniles

Figure 3 illustrates the total carotenoid content in the caudal fins of the Arowana juveniles. During the second month of the rearing period, treatment B yielded the best total carotenoid value, where the fish were fed with astaxanthin-enriched crickets (P<0.05). While for the other treatments, such as treatment C, the effect was not significant (P>0.05). At the end of the rearing period, treatments B, C and A had the highest to lowest values for total carotenoid content, respectively (P<0.05). At the same time, the total carotenoid content in the caudal fins of the Arowana juveniles fed with astaxanthin-enriched crickets was relatively stable during the second and fourth months of rearing, while in the other groups, the values increased slightly over the rearing period. In other words, the longer the rearing time, the larger the rise in the total carotenoid value; this was evident, for example, in the increment of the total carotenoid value in treatment C.

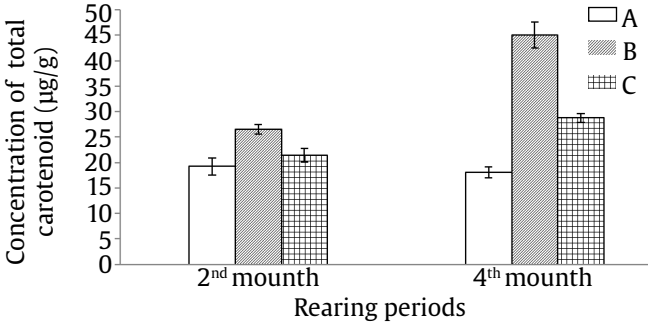

Figure 3. Total carotenoid content in caudal fin of Arowana juveniles treated with different crickets during the four-month rearing period. (A) control, (B) crickets enriched with 500 ppm astaxanthin, (c) crickets supplemented with formulated feed (a combination of 50% fish feed, 48% sand crab and 2% marigold petal meal)

4. Discussion

4.1. Effect of Treatment on the Body Performance of Arowana Juveniles

Crickets are one of several live feed types frequently given to the Asian Arowana (Faber 2017). However, the effect of this type of feed on fish performance has been poorly studied. Tran *et al.* (2015), Jayanegara *et al.* (2017), Taufek *et al.* (2018a), Fialho *et al.* (2021), Jeong *et al.* (2021) and Pastell *et al.* (2021) reported that cricket contains more than 50% protein, a high level of lysine, and methionine. However, while these are the most limited amino acids in fish feed, fish still need them for physical development (NRC 2011; Taufek *et al.*2018a). The nutritional value of crickets can also be improved (Bawa *et al.* 2020; Finke 2003, 2015; Finke *et al.* 2020), which includes enhancing its carotenoid content (Ogilvy *et al.* 2012).

This study evaluates the effect of crickets enriched with a source of carotenoids on the performance of Arowana juveniles. The results show that the super red Arowana juveniles treated with enriched crickets had a similar body performance in all treatments (e.g. WG, SGR, FE, LG, SR). Therefore, it can be stated that exogenous pigment sources such as astaxanthin and carotenoid did not affect the growth, FE and SR of the Arowana juveniles. These results correspond to other studies which reported that the supplementation of astaxanthin in daily feed <300 mg kg^{-1} did not influence the growth, SR, feed conversion FCR or FE of goldfish (Dananjaya *et al.* 2017; Paripatananont *et al.* 2006; Yeşilayer *et al.* 2011), gilthead sea bream Sparus aurata (Gomes *et al.* 2002), red porgy Pagrus pagrus (Chatzifotis *et al.* 2005; Kalinowski *et al.* 2005), Japanese koi carp (Sun *et al.* 2012) and large yellow croaker Larimichthys crocea (Yi *et al.* 2014). In comparison, Song *et al.* (2017) reported that astaxanthin supplementation was indicated to reduce growth in Discus fish.

Table 2 shows that fish performance (e.g. WG, SGR, FE, LG) was no different from the control groups (P>0.05). This means that astaxanthin supplementation (500 ppm) is safe in the daily feeding regime of super red Arowana. However, unlike astaxanthin treatment, for which many similar studies have been conducted, there is relatively little information about using sand crab flour (*Emerita* sp.) as fish feed. As such, this study of cricket enrichment using a formulated meal (a combination of fish feed, sand crab meal and marigold petal meal) as feed for Arowana juveniles can be considered a novelty. Conversely, marigold petal meal's effect as a fish supplement has been well documented. Some studies have reported that adding up to 8% marigold petal meal to feed did not affect the growth of Swordtail fish, *Xiphophorus helleri* (Pailan *et al.* 2015) and rosy barb fish, *Puntius conchonius* (Jagadeesh *et al* .2015). While in other studies, various scientists reported that adding carotenoids to feed could increase fish growth (Goda *et al.* 2018; Meilisza *et al.* 2017).

This study provides new information that super red Arowana juveniles have a feed consumption of approximately 11.00 to 13.37% body weight per day, an SGR ranging from 1.49 to 1.60% per day, and a FE for crickets of between 39.94% and 45.79%. As a comparison, the SGR of the super red Arowana (cricket feeding) was lower than that of the silver Arowana (*Osteoglossum bicirrhosum*) at 2.05% per day (Armando *et al.* 2018), African catfish (*Clarias gariepinus*) at 2.32% per day (Taufek *et al.* 2018b) and the tilapia at 3.3% per day (Alfaro *et al.* 2019). These different SGR values occur because of the varying responses of different fish based on the species, type of feed and effect on growth. Furthermore, the FE of the cricket on super red Arowana juveniles is also very low. Therefore, it can be indicated that Arowana cannot digest the entire protein content of crickets. On the other hand, most insects have a high chitin content; for example, a whole cricket contains 7.7 % chitin (Jayanegara *et al.* 2017). Several studies have reported that chitin may disrupt animal nutrient absorption (Fuah *et al.* 2016). Karlsen *et al.* (2015) also found that chitin content >1% in a diet negatively affected growth and nutrient utilisation in Atlantic salmon. The presence of chitin may also have influenced the availability and digestibility of the nutrients concerning the growth performance of juvenile turbot Psetta maxima (Kroeckel *et al.* 2012).

4.2. Effect of Treatment on the Colour of Super Red Arowana Juveniles

When a colour is described in the CIELAB colour diagram, L^* defines lightness and a^* denotes red if the value is positive and green if the value is negative. At the same time, b^* represents yellow if the value is positive and blue if the value is negative (CIE 1976). Based on the study results, cricket enriched with astaxanthin did not improve the body colour's redness (a^*) of the super red Arowana juveniles after four months of treatment. This result relates to the

amount of astaxanthin in the crickets. As shown in Table 1, the total carotenoid content in the crickets enriched with astaxanthin was only 49.15 mg kg^{-1}, which is lower than the recommendation. Song *et al.* (2017) reported that 200 mg kg^{-1} of astaxanthin is required in feed for Discus *Symphysodon* spp. to show an improvement in skin and body redness (a*). Various studies have recommended minimum carotenoid contents in feed, notably 100 mg kg^{-1} for flame-red male dwarf gourami *Colisa lalia*, Atlantic salmon, red porgy *Pagrus pagrus* L., Arctic charr *Salvelinus alpinus* L., Japanese koi *Cyprinus carpio* and Olive flounder (Baron *et al.* 2008; Bjerkeng *et al.* 1996; Chatzifotis *et al.* 2005; Hatlen *et al.* 1995; Nguyen *et al.* 2014; Pham *et al.* 2014).

In Europe, the general recommended dosage for canthaxanthin is 80 mg kg^{-1} and 100 mg kg^{-1} for astaxanthin (Choubert 2001). There are wide variations in the recommended carotenoid doses in feed, particularly for ornamental fish. Previous studies have reported that clownfish, Amphiprion occelaris require a carotenoid dosage of 19.3–75.5 mg kg^{-1} (Ho *et al.* 2013), goldfish require 36–232 mg kg^{-1} (Paripatananont *et al.* 1999; Sukarman and Hirnawati 2014; Villar-Martinez *et al.* 2013; Yeşilayer *et al.* 2011) and Japanese koi carp require 50–173 mg kg^{-1} (Boonyapakdee *et al.* 2015; Gouveia *et al.* 2003; Sun *et al.* 2012; Yuangsoi *et al.* 2010; Yuangsoi *et al.* 2011). In contrast, the ideal dosage for super red Arowana is not well known. The efficacy of carotenoids on the external colouration of fish also depends on the availability of the carotenoid compounds (NCR 2011) and the duration of the administration (Ho *et al.* 2013).

In our case, crickets that had been enriched with the formulated meal (a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal) affected the body colour b* (yellowness) of the Arowana juveniles at the second and fourth months after treatment ($P<0.05$), and its value was positive. This means that the body hue (H*) was yellower ($P<0.05$) than in the other treatments. These results correlate with the crickets' total carotenoid content and the types of carotenoid in the crickets' feed. The total carotenoid content in the treatment C crickets (a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal) was approximately 79.24 mg/kg derived from basic ingredients such as the sand crab and marigold petal meal. As we know, sand crabs are crustaceans that live in Indonesian waters (Wardiatno *et al.* 2015). We also understand that the main carotenoid in these crustaceans is astaxanthin (Meyer and Latscha 1997) as the red pigment (Amaya and Nickell 2015). At the same time, the most abundant carotenoid type in marigold petal meal is lutein as the yellow pigment (Cantrill and Dessipri 2016; Kusmiati *et al.* 2015; Šivel *et al.* 2014). Those pigments affected the colour of the Arowana juveniles' bodies, which turned a yellowish colour. These results are similar to those from NRC (2011), which stated that the visual yellow colour intensity score was high in fillets from catfish fed with lutein, followed by astaxanthin and canthaxanthin. However, the author did not recommend this treatment for super red Arowana farmers or hobbyists because it ceases to be attractive if it has a yellowish body colour. Additionally, the price will be lower.

The astaxanthin treatment did not affect the body colour of the Arowana juveniles. However, it did affect the caudal fin colour, where the value was dramatically increased. The caudal fin colours C* (chroma), a* (redness) and b* (yellowness) were higher compared to the other groups ($P<0.05$), not only in the second month but also in the fourth month after feeding with crickets. The rise in the chroma value indicated that astaxanthin accumulation in the Arowana caudal fin had increased. Choubert (2001) reported that chroma increases in line with the concentration of canthaxanthin in fish flesh as a rise in the chroma value are positively correlated with the carotenoid concentration in fish tissue (Sukarman *et al.* 2014). These results indicate that the astaxanthin content in crickets was sufficient for treating pigmentation in the fin. Thus, the first pigmentation occurred in the caudal fins of the Arowana fish, followed by pigmentation on the body scales of the Arowana juveniles. However, there was an increase in the colour variations of the fish treated with enriched crickets in treatment C, the redness (a*) of the caudal fin was lower than in the astaxanthin treatment ($P<0.05$). It indicated that not all carotenoids were deposited in the caudal fin; some were deposited in the body scales.

Carotenoids are deposited in different forms, depending on the tissue (Choubert 2001). Free forms of astaxanthin are deposited in fish flesh, esterified astaxanthin is deposited in fish skin, and certain complex caroteno-proteins and carote-lipoproteins are deposited in the skeletons of crustaceans (Choubert 2001; NRC 2011; Meyer and Latscha 1997). Some fish also store other carotenoids as astaxanthin,

without modification. Based on the colour parameters, either a* (redness) or b* (yellowness) in the body and caudal fin (Table 3) indicated that the super red Arowana stored carotenoid astaxanthin in the caudal fin along with other, non-modified carotenoids in their body scales. This is why the body colour hue of the Arowana juveniles turned a yellowish colour after being treated with the enriched crickets in treatment C (a combination of fish feed, sand crab meal and marigold petal meal). Latscha (1990) found that carnivorous fish stored lutein, zeaxanthin and canthaxanthin in their original form or converted a few pigments into astaxanthin. Since the bodies of Arowana are covered in scales, the body colour accumulates on the scales and not on the skin. However, certain studies have reported lower carotenoid deposition on scales than on skin (Liang *et al.* 2012; Ninwichian *et al.* 2020), which has implications for the pigmentation step of Arowanas.

4.3. Effect of Treatment on Carotenoid Concentration in the Caudal Fin of Arowana Juveniles

Carotenoid concentrations in aquatic animals vary; in Caridae and Penaeidae they range from 60 mg kg^{-1} to 499 mg kg^{-1}, but in wild shrimp bodies, the range is 80 to 200 mg kg-1 (Meyer and Latscha 1997). The carotenoid concentration in salmon flesh ranges from 0.63 to 3.95 mg kg^{-1} if given feed containing different levels of *Spirulina platensis* (Teimouri *et al.* 2013) and can be improved to a range of 8 to 12 mg kg^{-1} (Choubert 2001). Recent studies have conducted carotenoid analysis in the caudal fins of Arowana fish. While the caudal fin is only a small part of the whole body, it is suspected to have a high carotenoid content. After two months of the feeding trial, the carotenoid concentrations from highest to lowest were 26.51, 21.45 and 19.25 mg kg^{-1} for astaxanthin, the combination of sand crab meal and marigold

Table 3. Body and caudal fin colour of Arowana juveniles (*Scleropages formosus*) fed with different enriched crickets during the four-month rearing period

Parameter	Experimental diet			p-value
	A	B	C	
Body colour				
2nd month				
Lightness (L*)	63.70±2.88	58.64±1.17	59.24±4.60	0.356
Chroma (C*)	6.83±0.22	5.88±0.21	6.10±0.07	0.147
Redness (a*)	-6.83±0.23	-5.39±0.46	-6.59±0.53	0.050
Yellowness (b*)	0.15±0.07^{b}	-2.35±0.11^{c}	0.83±0.18^{a}	0.000
Hue (H*)	178.73±0.63^{b}	203.57±0.32^{a}	172.86±1.01^{c}	0.000
4nd month				
Lightness (L*)	60.57±1.29	52.31±3.42	56.45±6.16	0.281
Chroma (C*)	6.78±0.15^{b}	5.84±0.07^{a}	6.79±0.08^{b}	0.000
Redness (a*)	-6.78±0.15^{c}	-5.56±0.10^{a}	-6.21±0.12^{b}	0.006
Yellowness (b*)	0.50±0.04^{b}	-1.78±0.06^{c}	2.77±0.06^{a}	0.000
Hue (H*)	175.76±0.45^{b}	197.71±0.89^{a}	156.05±0.90^{c}	0.000
Caudal fin colour				
2nd month				
Lightness (L*)	29.69±2.85	33.78±2.54	29.03±3.20	0.337
Chroma (C*)	13.41±0.41^{b}	16.54±0.00^{a}	13.40±0.49^{b}	0.005
Redness (a*)	7.80±0.28^{b}	9.45±0.52^{a}	8.13±0.18^{ab}	0.037
Yellowness (b*)	10.92±0.30^{b}	13.57±0.37^{a}	10.65±0.48^{b}	0.009
Hue (H*)	54.47±0.20	55.15±2.22	52.63±0.62	0.292
4nd month				
Lightness (L*)	26.10±5.79	29.14±1.68	26.19±1.21	0.662
Chroma (C*)	11.23±0.14^{b}	13.18±0.20^{a}	11.99±0.25^{b}	0.005
Redness (a*)	7.75±0.35^{b}	9.52±0.06^{a}	7.57±0.28^{b}	0.012
Yellowness (b*)	7.04±0.21^{b}	9.13±0.22^{a}	9.30±0.09^{a}	0.002
Hue (H*)	42.25±1.97^{b}	43.80±0.50^{b}	50.85±0.77^{a}	0.006

petal meal, and the control treatment, respectively. The carotenoid concentration was almost doubled in the caudal fins of the Arowana juveniles treated with astaxanthin in their feed. After four months of feeding, the value increased by approximately 30% compared to the treatment comprising a combination of sand crab meal and marigold petal meal (Figure 2).

The carotenoid concentrations in the fins in the first and second treatments were significantly different compared to the control treatment ($P<0.05$). These results align with those of previous studies, which reported carotenoid concentrations in yellow croaker fish skin in the range of 37–138 mg kg^{-1} (Yi *et al.* 2015), 5–59 mg kg^{-1} in goldfish skin (Sukarman and Hirnawati 2014), 3–171 mg kg^{-1} in the skin of Discus fish (Song *et al.* 2017), 33.75 mg kg^{-1} in the skin of *Melanotaenia parva* (Meilisza *et al.* 2017), 4.33–27.7mg kg^{-1} in red porgy fish skin (Chatzifotis *et al.* 2005), and in the skin of juvenile Olive flounder in the range of 5–18 mg kg^{-1} (Pham *et al.* 2014). The variations in carotenoid concentration in aquatic animal tissue depend on species, genetics, development stage, metabolism, sex, target tissue, moulting, amount of pigment in feed, the structure of pigment, the form of pigment, stability, bioavailability, administration period, feed quality, environment and disease (Choubert 2001; Meyer and Latscha 1997). In this study, the carotenoid treatment administration period was 120 days longer than in other studies that looked at different species. Our analysis also indicated that astaxanthin was superior to other carotenoid sources for pigmentation in the caudal fins of Arowana fish. Still, in level, a dosage of approximately 50 mg kg^{-1} was not enough for pigmentation in the body.

Given the low level of understanding regarding the pigmentation of super red Arowana, farmers or hobbyists should provide crickets with a high carotenoid content as a first step before feeding those crickets to Arowana. The authors suggest finding other live feed types with high carotenoid content, such as shrimp and some red hue insects.

In conclusion, feeding with enriched crickets for astaxanthin and a formulated meal combination (sand crab and marigold petal meal) was not found to influence the growth of super-red Arowana juveniles. Furthermore, the body colour of the Arowana juveniles was not affected by crickets containing 500 ppm of astaxanthin. Nevertheless, there was a positive effect on the red hue (a^*) of the Arowana tail fin with a value of 9.52. At the same time, crickets enriched with a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal produced an increase in the yellowness (b^*) of the Arowana body by 2.77 but did not influence the colour of the tail fin. The highest carotenoid content in fish tails resulted from the astaxanthin crickets, with a value of 45.09 µg/g.

Ethics Statement

This manuscript does not require ethical approval.

Data Availability Statement

Data availability within the article or its supplementary materials. The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Conflict of Interest

The authors confirm that there are no potential financial and competing interests related to this manuscript that needs to be stated.

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