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Research

Effects of Crab Shell Waste as Feed on Growth Performance and Colouration of Siamese Fighting Fish (*Betta splendens*)

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ABSTRACT

Betta splendens, Siamese fighting fish exhibit behavior, coloration, and morphology that make them popular aquarium fish in aquaculture. Coloration for this fish requires high-quality feeds rich in carotenoids. Crab shells, mostly dumped as agro-waste by the seafood industry contain carotenoids and protein that can be an alternative for carotenoids in fish feed. The objectives of this study were to analyze the proximate composition and carotenoid content in feed and determine the effect of crab shells as feedstuff on the growth performance and coloration of *Betta splendens*. A 40-day feeding trial using 45 tails of *B. splendens* with an average body weight, of 0.25 g was conducted by using five feed treatments namely TC (0% crab shell), T1 (25% crab shell), T2 (50% crab shell), T3 (75% crab shell) and T4 (100% crab shell) respectively. Sampling was done weekly. Analysis done were proximate composition and extraction of carotenoid while growth performance parameters such as body weight gain (BWG), specific growth rate (SGR), feed intake (FI), and feed conversion ratio (FCR) were also taken. Skin coloration and water quality data were collected every 10 days. All data were analyzed using One-Way ANOVA via SPSS. Results showed protein content for T1 (22.40±0.29) was slightly higher compared to all treatments. Carotenoid content in crab shells was lower compared to krill. Body weight from T1 (25% crab shell) showed a significant increase among all treatments. All treatments show no changes in coloration. In conclusion, T1 (25% crab shell waste) is the best treatment as it has a high protein content that improves body weight gain without any significant effect on coloration in all treatments.

Key words: *Betta splendens*, body weight, carotenoid, coloration, growth

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INTRODUCTION

The Siamese fighting fish (*Betta splendens*), commonly known as *Betta*, is a freshwater fish native to Southeast Asia. *Betta splendens* were called "*bettas*" due to their huge popularity and the best ornamental fish in the world (Fricke *et al*., 2019). This species belongs to the family Osphronemidae and is famously known for its body coloration, shape, and economy. *B. splendens* is native to Southern Asia such as the northern Malay Peninsula, central and eastern Thailand, and Southern Vietnam (Witte & Schmidt, 1992). The ornamental fish sectors are found to grow steadily in Malaysia. Various species can be found such as Siamese fighting fish (*Betta splendens*). *Betta splendens* export price can reach up to RM370 million per year even though Malaysia is not the biggest exporter of ornamental fish industry (Mohamad-Zuki *et al*., 2022). This fish also has opened another potential supporting market in ornamental fish feed based on the incremental growth of interest.

According to Monvises *et al*. (2009), local sales and export of breeding male *B. splendens* as ornamental fish had become increasingly lucrative. Ornamental fish that have various novel colors, color patterns, fins, and body shapes have high market demand market. Color pigment on *Bettas* body surface can also be found in various colors such as red, blue, green, and yellow. Melanins, carotenoids, xanthines, and pterins are the pigment compounds of the general structures whereas guanines and purine are the compounds of the scales and body surface. Colors can enhance fish's emotions and behavior (Martins, 2012). Three factors that can determine fish coloration are dietary supplements, genetics, and glandular factors including the nervous system (Kaur & Kumar, 2017). Tiewsoh *et al*. (2019) stated that to achieve their natural pigmentation, fish are dependent on the dietary supply of carotenoids to improve skin color as well as increase their market value.

In the seafood industry, discarded crab shell waste amounts to about 6 to 8 million tonnes per year globally and 1.5 million tonnes in Southeast Asia because only 40% of edible crab part will be taken while the remaining shells (60%) are discarded as waste (Su *et al*., 2019). Byproducts from crab's head and shell contain high protein, lipid, and chitin which chitin can contribute to upgrading shelf-life, safety, and quality of food formulation. Chitin and chitosan also contain antioxidants and antimicrobials. In ruminant feed, chitin from crab shells was used for microfloral adaptation in ruminant digestion, and protein from crab shells also has the potential as an additional protein supply for livestock. Extraction from crab shell waste can produce protein hydrolysate, polyunsaturated fatty acid (PUFA), and carotenoids (Mohamad-Zuki *et al*., 2022a; Mohamad-Zuki *et al*., 2022b). This waste can also be extracted to produce flavoring, fertilizer, quicklime, and filtration medium.

Aanand (2021) and Caro (2021) stated that crab waste is enriched with coloration pigment like carotenoid while other parts such as viscera and un-extracted meat can be used as alternatives in animal feed especially for fish. Red, orange, and yellow colors can be found in the carotenoid group (Sathyaruban *et al*., 2021). Carotenoids can be classified into two groups such as xanthophylls (present –OH group) and carotene (absent –OH group) (Mohamad-Zuki *et al*., 2022). Xanthophylls are known as zeaxanthin and belong to a class of carotenoids. It is derived from a common color from *Zea mays* that provides yellow color pigment while astaxanthin provides the red color pigment that belongs to a group of oxygenated carotenoids that has a fat-soluble long chain molecular pigment found in shrimp, crab, lobster salmon, crayfish, and microalgae (Adeyemi *et al*. 2013).

Naguib (2000) stated that astaxanthin concentration synchronizes well with natural sources of color. In the ornamental fish industry, astaxanthin can improve fish skin coloration and immune stimulants including providing antioxidants and improving growth performances. In the aquaculture and ornamental industry, feed fed to fish must supply enough protein with admissible amounts of lipids, vitamins, and minerals to gain better growth and high productivity of fish. Extensive research has been carried out to discover locally sourced ingredients suitable as alternative ingredients in feed nutrition. Black soldier flies and Engkabang butter oil serves as illustrative examples of alternative feed ingredients that not only offer cost savings but also mitigate reliance on imported, expensive raw materials for fish feed production (Redhwan & Komilus, 2021; Zomorni *et al*., 2022; Redhwan *et al*., 2022a). Crab shell waste can be another protein and color source due to its carotenoid as a natural color pigment for ornamental fish. The objectives of this experiment were i) to analyze proximate composition in fish feed formulation and carotenoid content in a crab shell and ii) to inspect the effect of crab shell in fish feed on growth performance and coloration of *Betta splendens*.

MATERIALS AND METHODS

Sample preparation

7 kg of crab were purchased from the Malaysian Fisheries Development Authority (LKIM) Fisheries Complex Kuala Besut while krill were purchased from local stalls in the Besut area. All crabs were steamed at 100 °C for approximately 30 min and flesh was removed from the carapace. Carapaces considered as crab waste were then dried in an oven at 50 °C for 24 h. Dried shells and krill were then blended using Waring Commercial Bar Blender HGB25EK and sieved to obtain a powder. All samples were stored in a food container at -4 °C. Proximate analyses were conducted on formulated feed according to AOAC (2019).

Collection of *Betta splendens*

45 tails of *Betta splendens* with weights ranging from 0.1 g to 0.4 g were purchased and collected from Kuala Nerus, Terengganu. All fish were individually acclimatized for 7 days in plastic tanks with sizes 18 cm × 12 cm × 9 cm for one week (7 days) before the feeding trial. Fish were fed with Marubeni Nisshin no 3 twice daily.

Feeding trial

A 40-day feeding trial was conducted using 5 treatments in triplicates that consist of TC (0% crab shell waste & 100% krill), T1 (25% crab shell waste & 75% krill), T2 (50% crab shell waste & 50% krill), T3 (75% crab shell waste & 25% krill) and T4 (100% crab shell waste & 0% krill) respectively based on Table 1. Nine (9) tail fish were allocated in each treatment.

Table 1. Percentage of Fish Feed Treatment Formulation

Ingredient (g)	TC (0% CSW)	T1 (25% CSW)	T2 (50% CSW)	T3 (75% CSW)	T4 (100% CSW)
Crab shell waste	0.00	8.70	17.5	26.30	35.00
Krill	35.00	26.30	17.50	8.70	0.00
Wheat flour	15.00	15.00	15.00	15.00	15.00
Sago flour	15.00	15.00	15.00	15.00	15.00
Vitamin mix[1]	15.00	15.00	15.00	15.00	15.00
Mineral mix[2]	15.00	15.00	15.00	15.00	15.00
Palm Oil	5.00	5.00	5.00	5.00	5.00
Total (g)	100.00	100.00	100.00	100.00	100.00

*CSW= crab shell waste

[1]Vitamin mix (g kg −1premix): vitamin A,0.05; vitamin D3 0.01; vitamin E 1.30; vitamin K3 0.01; vitamin B1 0.01; vitamin B2 0.016; vitamin B6 0.1; vitamin B12, 0.5; Niacin 0.2; $C_9H_{17}NO_5$, 0.056; $C_{19}H_{19}N_7O_6$ 0.008; biotin 0.5; Anticake 0.02.[2]Mineral mix (g kg −1 premix): Copper,7.5; Iron,125; Manganese,25; Zinc,125; Cobalt,0.5; Iodine,0.175; Selenium,0.3; and Anticake,10. Values are means ± SD of duplicate measurements. Treatment samples followed by a, b, and c in row were significantly different (p<0.05)

Fish were fed with a formulated diet at 3% of total body weight twice daily. Samplings were done every 10 days and data for growth performances like body weight gain, feed intake and feed conversion ratio, survival rate, and coloration were taken. This feeding trial was conducted at the Animal Feed Analysis Laboratory and Aquatic II Laboratory, Faculty of Bioresources and Food Industry (FBIM), Universiti Sultan Zainal Abidin (UniSZA).

Growth performance

The following growth performance parameters were evaluated: Body Weight Gain (BWG); Feed Feed Intake (FI), Feed Conversion Ratio (FCR), and Survival Rate (SR); according to Redhwan *et al*, (2022b).

$$Body\ Weight\ Gain\ (BWG) = \frac{Final\ weight - initial\ weight}{Initial\ weight} \times 100 \quad \text{– Equation 1}$$

$$Feed\ Intake\ (FI) = \frac{Feed\ Intake\ per\ replicate\ per\ week}{No. fish\ consumed\ feed\ daily\ during\ the\ week\ period} \quad \text{– Equation 2}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ Intake\ (g)}{Body\ Weight\ gain\ (g)} \quad \text{– Equation 3}$$

$$Survival\ Rate\ (SR) = \frac{Fish\ left}{total\ fish} \times 100 \quad \text{– Equation 4}$$

Carotenoid extraction

1 g of sample from crab shell, krill, and pure astaxanthin (standard curve) were mixed with 10 mL of ethanol in a separate beaker and filtered by using filter paper size Whatman No. 42 continually until the sample became colorless. The solution was quantified by using a *uv-vis* spectrophotometer ranging from 400 to 500 nm (de Carvalho *et al*., 2012). Carotenoid calculation formula:

$$Astaxanthin\ concentration\ (\mu g/g) = \frac{A \times V\ (mL) \times 10^4}{A_{1cm}^{1\%} \times P\ (g)} \quad \text{– Equation 5}$$

Where A = Absorbance, V= Total extract volume, P= Weight of sample, and $A_{1cm}^{1\%}$ = 2592

***Betta splendens* colouration**

Skin coloration of *Betta splendens* was determined by using the Toca Color Guide method (Fabanjo & Abdullah, 2021). Color changes of *Betta splendens* were recorded every 10 days by using a Vivo Y15 camera 13 MP, f/2.2, Phase Detection Auto Focus (PDAF) to compare skin color among treatments to determine the effects of carotenoid in crab shell waste on fish skin.

Statistical analysis

All the data collected were interpreted using One-way ANOVA IBM SPSS Statistics version 27 ((Workagegn *et al*., 2014; Komilus & Mufit, 2021).

RESULTS AND DISCUSSION

Proximate composition

The proximate analysis of a sample provides valuable insights into its composition and nutritional content. In this study, we aim to perform a proximate analysis of Crab waste to gain a better understanding of its nutritional composition and provide valuable insights for potential applications in the aquaculture industry.

Table 2. Proximate composition of Feed Treatment

Treatment	TC	T1	T2	T3	T4
Crude lipid	8.89±1.36[b]	6.66±0.10[ab]	6.10±0.05[a]	7.95±0.08[ab]	7.51±0.11[ab]
Crude Protein	20.95±0.38[d]	22.40±0.29[e]	18.04±0.49[c]	13.40±0.62[b]	11.04±0.08[a]
Crude Fiber	5.67±0.28[a]	4.86±0.06[ab]	6.505±0.19[ab]	6.66±0.30[bc]	7.44±0.35[c]
Ash	21.29±0.30[b]	18.27±0.43[a]	23.07±0.27[bc]	25.10±0.98[c]	28.17±1.29[d]
Moisture	11.42±0.42[a]	12.15±0.50[a]	14.28±0.12[b]	13.90±0.12[b]	11.52±0.20[a]

[a,b,c,d,e] shows the significant differences in proximate analysis for each feed

Proximate composition for TC (0% crab shell waste & 100% krill), T1 (25% crab shell waste & 75% krill), T2 (50% crab shell waste & 50% krill), T3 (75% crab shell waste & 25% krill) and T4 (100% crab shell waste & 0% krill) were shown in Table 2 respectively. The nutrient content such as moisture, protein, and ash showed significantly different ($P<0.05$) for all treatments. Protein content in T1 (22.40%) was slightly higher compared to the other treatments.

This result showed it was closest to the optimum protein requirement for *Betta splendens* which is 35% (Watson *et al*., 2019). T1 (6.66%) and T2 (6.10%) have lower lipid content compared to the other treatments. Wang *et al*. (2021) stated that high lipids given to fish might damage the health of the fish also can cause fatty liver and fat deposition to the fish as well. Crude fibre in T4 is 7.44% which is higher than all treatments. Fish growth and digestibility just need 3% to 5% only, more fiber could lead to an efficiency ratio of the other nutrients and shorten the fish digestibility.

Carotenoid analysis

Astaxanthin standard control was sourced from *Heamatococcos pluvialis* for carotenoid analysis to measure astaxanthin content in crab shell waste and krill. Results showed that astaxanthin content in krill was slightly higher than in crab shell waste. Wavelength for carotenoid absorbance used ranged between 400 nm to 500 nm using a *uv vis* spectrophotometer (Sindhu & Sherief, 2011; de Carvalho *et al*., 2012). The study found that the higher reading for krill but lower crab shell waste 400nm wavelength.

Table 3. Carotenoid Content

Carotenoid (nm)	400	450	500
Astaxanthin	142.6312	108.5648	135.0694
Krill	7.407407	6.790123	4.899691
Crab Shell Waste	5.632716	3.433642	2.391975

Growth Performance

The weight gain of T1 gradually increased from day 10 until day 40, followed by T2, T3, and T4 which significantly lower the growth rate from day 10 until day 40. Protein is the main component for fish muscle development and repair of damaged tissue (Watson *et al*., 2019). A study by James and Sampath (2003) claimed that animal and plant protein dietary at 10%, 15%, 25%, 25%, and 45% respectively for *Betta splendens* proved that the maximum protein requirement for *Betta splendens* for faster growth is 35% indicating a suitable ratio for adequate nutritional for *Betta.* The high inclusion of 45% protein proved to be unsuitable for *Betta*. The poor growth was due to high protein which may have led to an increase in ammonia production and energy expenditure on protein catabolism. This feeding trial observed that 22.40% of crude protein in T1 may be the closest amount to the maximum protein requirement for *Betta splendens*.

Fish from all treatments were fed based on 3% of body weight. Growth performance is the most important indicator to measure by daily feed intake of fish (Eriegha & Ekokotu, 2017). It is observed that the daily feed intake of *Betta* in this trial is inconsistent due to nutrient utilization. TC feed intake for the first 10 days was quite high at 0.012 g but slowly decreased during days 20 and 30. Similar patterns were observed in other treatments. T3 and T4 feed consumed by *Betta splendens* were quite low compared to other treatments due to low protein content in feed in T3 (13.40% crude protein) and T4 (11.04% crude protein). Low protein content in both treatments was unable to provide enough protein for fish growth. This observation is the first report regarding the effect of crab shell waste dietary for *Betta splendens* feed intake.

High amounts of water-soluble chitosan (WSC), a by-product from marine exoskeletons like marine crab shell waste may enhance high water-soluble chitosan (WSC) in freshwater diets (Al Shaqsi *et al*., 2020). A dietary supplement that contains chitosan from crab shell waste significantly increased the growth performance of Nile tilapia. In comparison, *Betta splendens* may not be able to digest crab shell protein as the inclusion of more than 25% caused extremely slow growth as shown in Figure 1. This could be caused by the inability of *Betta splendens's* intestinal microbial to absorb water-soluble chitosan from crab shells. Further studies need to be undertaken in the future to determine the effect of chitosan concentration on digestibility in *Betta*.

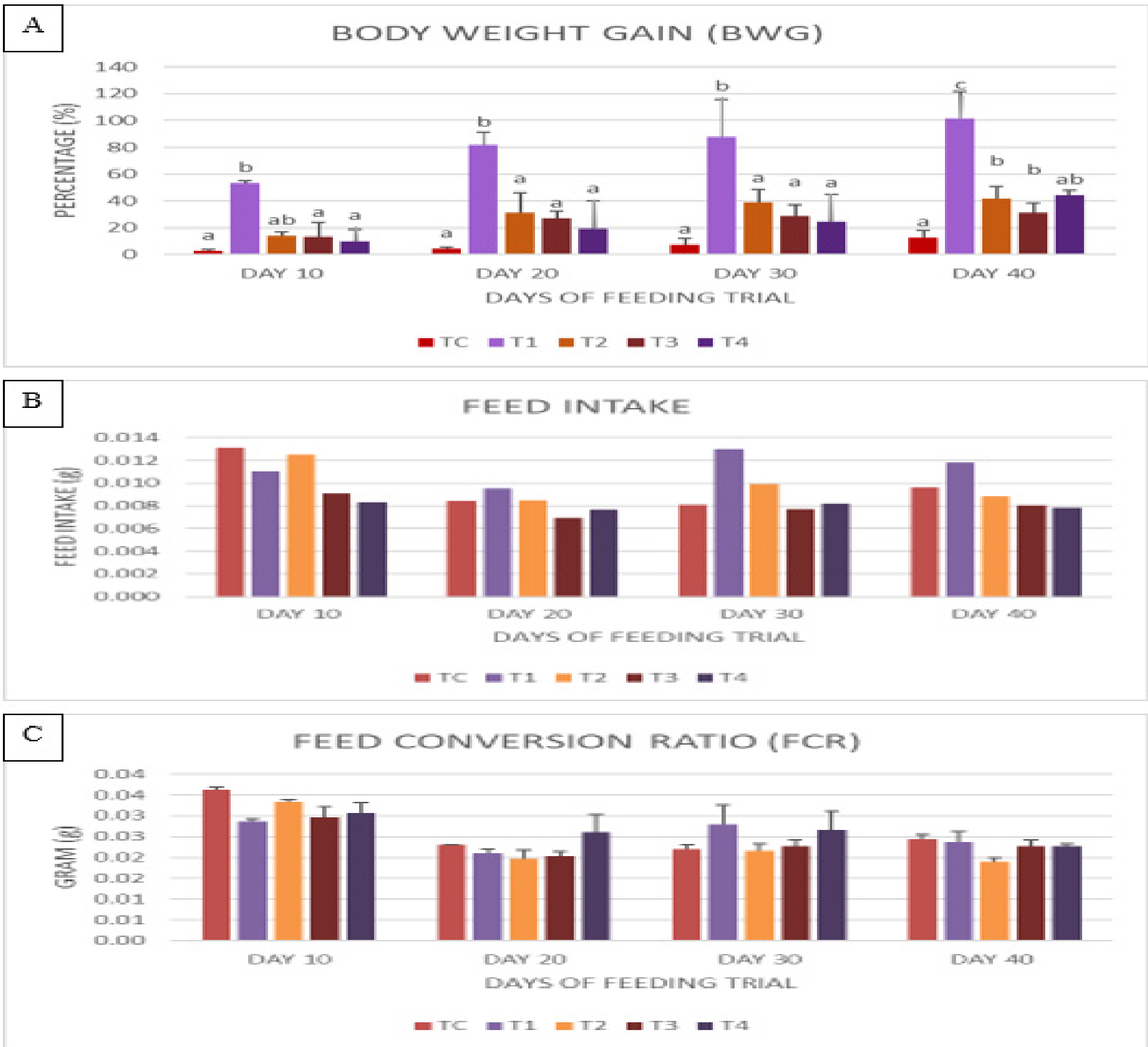

Fig. 1. A= Body Weight Gain (BWG), B=Feed Intake (FI), C=Feed Conversion Ratio (FCR) in 5 treatments: TC (0% crab SW & 100% krill), T1 (25% crab SW & 75% krill), T2 (50% crab SW & 50% krill), T3 (75% crab SW & 25% krill) and T4 (100% crab SW & 0% krill).

In wild habitats, *Betta splendens* consume live feed such as tubifex worm, Daphnia, mosquito larvae, and small aquatic insects (James & Sampath, 2003) while in captivity, commercial diets are usually given as feed. As observed, the concentration of chitosan from crab shell waste needs to be studied in depth to determine a suitable amount to be included in the commercial diet for *Betta*.

Feed conversion ratio (FCR) is the relation or ratio between feed given over fish weight gain in a certain period. Feed is considered more efficient when it converts to weight gain when the FCR value is low (Charles Bai *et al*., 2022). T2 at day 40 showed low FCR compared to other treatments. Feed intake for TC is high but body weight gain is lower indicating that formulation for TC is not efficient to improve fish growth. The feed conversion ratio for T1 is slightly higher and decreases with incremental feed intake. The growth rate for T1 showed a steady increase every 10 days while other treatments showed a slow growth rate. The lowest FCR is T2 at day 40 is 0.02. This result proved that T4 with 100% crab shell has the potential to improve growth although the protein content in this diet is 11.04%, lower than other treatments.

Survival Rate (SR) shows no mortality rate was recorded during the first 30 days for all treatments. On day 40, mortality was recorded in TC and T2. Several conditions like environment, water quality, and stress behavior could affect the survival of fish throughout the feeding trial. As *Betta splendens* is a territorial and aggressive species, it can easily become stressed especially male *Betta splendens* (Pleeging & Moons, 2017). Temperature changes, dissolved oxygen, and pH water in rearing tanks may also affect the survival rate of fish. Suitable water quality should be 25.8 °C to 26.5 °C, 6.0 mg/L to 8 mg/L, and 5.5 to 6.0 for temperature, dissolved oxygen, and pH respectively. The wild habitat condition as highlighted by Watson *et al*. (2019) should be applied to *Betta splendens* reared in this trial.

Colouration analysis

There were no changes in colors in all treatments throughout the 40-day feeding trial (Figure 2). This may be due to the ineffectiveness of color changes within a short rearing period. Short feeding trials may influence the skin color of the fish. Fabanjo and Abdullah's (2021) previous study using the Toca Color Guide stated that to make a fish color change, a longer period of at least 4 months is required. Fish genetics, the nervous system, and the glandular are the factors that may affect fish coloration as well as fish diets (Kaur & Kumar, 2017). Fish color during the experiment did not change due to the low carotenoid (astaxanthin) content in crab shell waste and krill. *Betta splendens* coloration depends on their diets and live feed to enhance their skin body color since it cannot synthesize de novo which means the fish cannot produce their color continuously without coloration from feed (Tiewsoh *et al*., 2019).

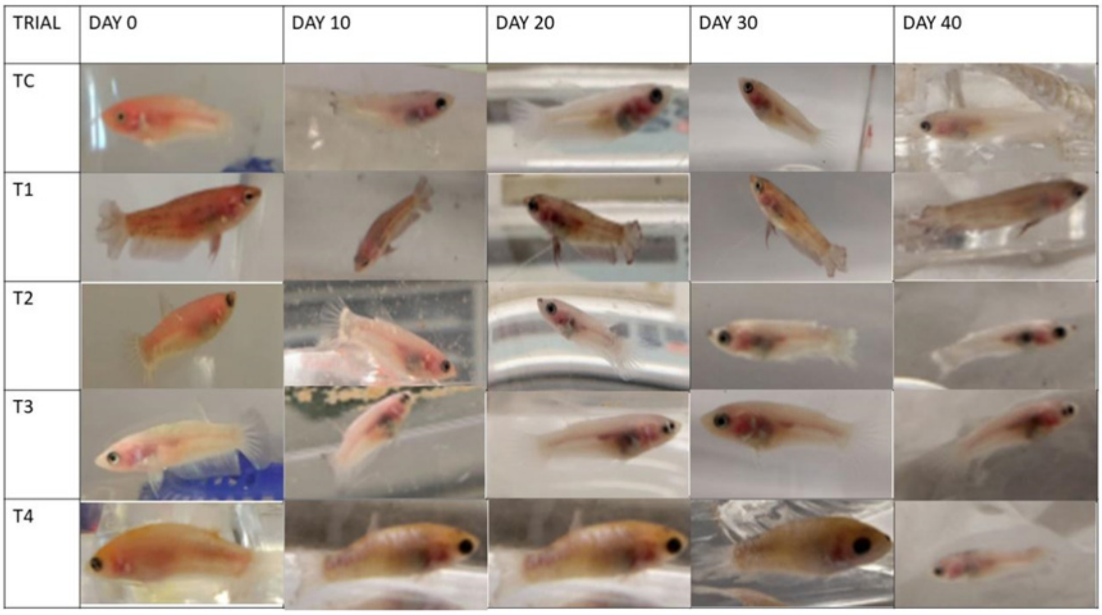

Fig. 2. Fish color changes during feeding trial

CONCLUSION

Proximate composition indicated that T1 (25% crab shell) is the best feed with the highest crude protein and low crude lipid content. Body weight gain for T1 also gave the best uniform growth every 10 days compared to other treatments. The feed conversion ratio (FCR) for T2 (50% crab shell) is the lowest at 0.02 while the survival rate is only 88.9%. Coloration for fish skin and body did not change due to the low content of carotenoid (astaxanthin) in crab shell waste and krill. It is recommended that further research needs to be undertaken to determine the effect of culture period on the coloration of *Betta splendens*.

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ETHICAL STATEMENT

This research obtained its approval from UniSZA Animal and Plant Research Ethics (UAPREC/07/014).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adeyemi, O.S., Otuechere, C.A., Atolani, O. & Ekor, M. 2013. Chemopreventive and Therapuetic Benefits of Lycopene, Zeaxanthin, Lutein and Astaxanthin. International E. Publication pp. 1-39.

Al Shaqsi, N.H.K., Al Hoqan, H.A.S., Hossain, M.A. & AlSibani M.A. 2020. Isolation, characterization and standardization of demineralization process for chitin polymer and minerals from the crabs waste of Portunidae segnis. Advances in Biomarker Sciences and Technology, 2: 45-58. https://doi.org/10.1016/j.abst.2020.10.002

AOAC. 2019. Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington DC. https://www.aoac.org/resources/official-methods-of-analysis-revisions-to-21st-edition/ (accessed 15.09.22)

Caro, T. 2021. When animal coloration is a poor match. Evolutionary Ecology, 35(1): 1-13.

Charles, B.S., Ronald, W. & Hamidoghli, A. 2022. Diet Analysis and Evaluation. Fish Nutrition. 4th Edition. Academic Press. pp. 709-743. https://doi.org/10.1016/B978-0-12-819587-1.00010-0

De Carvalho, L.M., Gomes, P.B., Godoy, R.L., Pacheco, S., Do Monte, P.H., De Carvalho, J.L., Nutti, M.R., Neves, A.C., Vieira, A.C. & Ramos, S.R. 2012. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata Duch*): A preliminary study. Food Research International, 47(2): 337–340. https://doi.org/10.1016/j.foodres.2011.07.040

Eriegha, O. & Ekokotu, P.A. 2017. Factors affecting feed intake in cultured fish species: A review. Animal Research International, 14(2): 2697–2709.

Fabanjo M.A., & Abdullah, N. 2021. Effect of addition of pro-enzymes and Spirulina flour on growth and color intensity of betta fish (*Betta* sp.). IOP Conference Series: Earth and Environmental Science, 890(1). https://doi.org/10.1088/17551315/890/1/012025.

Fricke, R., Eschmeyer, W.N. & Van der Laan, R. 2018. Catalog of fishes: genera, species,. references. California Academy of Sciences, San Francisco, CA, USA http://www.researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. (accessed 15.09.2022).

James, R. & Sampath, K. 2003. Effect of Animal and Plant Protein Diets on Growth and Fecundity in Ornamental Fish *Betta splendens* (Regan). The Israel Journal of Aquaculture – Bamidgeh 55(1): 39-52.

Kaur, R. & Shah, T.K. 2017. Role of feed additives in pigmentation of ornamental fishes. International Journal of Fisheries and Aquatic Studies, 5(2): 684-686.

Komilus, C.F. & Mufit, N.M.M. 2021. Dried *Acetes* as growth promoter for guppy (*Poecilia reticulata*) nutrition. IOP Conference Series: Earth and Environmental Science, 919(1): 012049. https://doi.org/10.1088/1755-1315/919/1/012049

Martins, C.I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J., Carter, T., Planellas, S.R. & Kristiansen, T. 2012. Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38: 17-41. https://doi.org/10.1007/s10695-011-9518-8

Monvises, A., Nuangsaeng, B., Sriwattanarothai, N., & Panijpan, B. 2009. The Siamese fighting fish: well-known generally but little-known scientifically. ScienceAsia, 35(1): 8-16. https://doi.org/10.2306/scienceasia1513-1874.2009.35.008

Mohamad-Zuki, N.A., Redhwan, A.I., Rashid, Z.M., Nguang, S.I., Kamu, A., Traifalgar, R.F.M. & Komilus, C.F. 2022a. Crustacean Shell Waste as a Potential Feed Material. Bioscience Research, 2022 19(SI-1): 360-372.

Mohamad-Zuki, N.A., Aminudin, N.H., Redhwan, A.I., Mohd-Khairulnizam, N.A.N. & Komilus, C.F. 2022b. Effects of crab shell waste as feed on growth performance and coloration of Siamese Fighting Fish (*Betta splendens*). In e-Proceeding of the International Conference on Fisheries and Animal Science, 1(T2): 45.

Naguib Y.M. 2000. Antioxidant activities of astaxanthin and related carotenoids. Journal of Agricultural and Food Chemistry, 48(4): 1150-1154.

Pleeging, C.C.F. & Moon, C.P.H. 2017. Potential Welfare Issues of The Siamese Fighting Fish (*Betta spelndens*) At the Retailer and In the Hobbyist Aquarium. Vlaams Diergeneeskundig Tijdschrift 86(4): 213223.

Redhwan, A.I. & Komilus, C.F. 2021. Mahsheers in Malaysia: A Review on Feed for *Tor tambroides* (Empurau) Cultured in Captivity. In 1st Postgraduate Seminar on Agriculture and Forestry 2021 (PSAF 2021). p. 64.

Redhwan, A.I., Khairulnizam, N.A.N.M., Mohamad-Zuki, N.A., Aminudin, N.H. & Komilus, C.F. 2022a. Effects of Engkabang butter oil (*Shorea macrophylla*) As lipid source on growth performance of

Javan Mahseer Fingerlings (*Tor tambra*). In e-Proceeding of the International Conference on Fisheries and Animal Science 2022. p. 22.

Redhwan A.I., Rao S.N., Mohamad-Zuki N.A., Kari A., Kamarudin A.S., Ismail N., Nguang S.I., Ha H. C., Yong W.S., Yong F.H. & Komilus C.F. 2022b. Mahseer in Malaysia: A Review of Feed for Cultured *Tor tambroides* and *Tor tambra.* Bioscience Research, 2022 19(SI-1):349-359.

Sathyaruban, S., Uluwaduge, D.I., Yohi, S. & Kuganathan, S. 2021. Potential natural carotenoid sources for the colouration of ornamental fish: A review. Aquaculture International, 29(4): 1507–1528. https://doi.org/10.1007/s10499-021-00689-3

Sindhu, S. & Sherief, P.M. 2011. Extraction, characterization, antioxidant and anti-inflammatory properties of carotenoids from the shell waste of Arabian red shrimp Aristeus alcocki, Ramadan 1938. The Open Conference Proceedings Journal, 2(1).

Su, W., Yu, S., Wu, D., Xia, M., Wen, Z., Yao, Z., Tang, J. & Wu, W. 2019. A critical review of cast-off crab shell recycling from the perspective of functional and versatile biomaterials. Environmental Science and Pollution Research, 26: 31581-31591 https://doi.org/10.1007/s11356-019-06318-0/

Tiewsoh, W., Singh, E., Nath, R., Surnar, S.R. & Priyadarshini, A. 2019. Effect of Carotenoids in Growth and Colour Enhancement in Gold Fish, *Carassius auratus* (L.). Journal of Experimental Zoology India, 22(2).

Wang, J., Liu, T., Zheng, P., Xu, H., Su, H., Tao, H. & Yang, Y. 2021. Effect of dietary lipid levels on growth performance, body composition, and feed utilization of juvenile spotted knifejaw *Oplegnathus punctatus*. Aquaculture Reports, 21: 100797. https://doi.org/10.1016/j.aqrep.2021.100797

Watson, C.A., DiMaggio, M., Hill, J.E., Tuckett, Q.M., & Yanong, R.P. 2019. Evolution, culture, and Care for Betta splendens. Edis, 2019(2): FA212

Witte, K. & Schmidt, J. 1992. *Betta brownorum*, a new species of *anabantoid* (Teleostei: *Belontiidae*) from northwestern Borneo, with a key to the genus. Ichthyological Exploration of Freshwaters 2(4): 305-330.

Workagegn, K.B., Ababboa, E.D., Yimer, G.T. & Amare, T.A. 2014. Growth performance of the Nile tilapia (*Oreochromis niloticus* L.) fed different types of diets formulated from varieties of feed ingredients. Journal of Aquaculture Research and Development, 5(3). https://doi.org/10.4172/2155-9546.1000235

Zomorni, M.S., Hai, C.T., Redhwan, A.I., & Komilus, C.F. 2022. Efficacy of Black Soldier Fly (*Hermetia illucens*) Larvae Meal as Feed on Growth Performance for Juvenile Javan Mahseer (*Tor tambra*). Journal Of Agrobiotechnology, 13(1S): 118-130. https://doi.org/10.37231/jab.2022.13.1S.321