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Original article

Sustainable aquafeed development: Incorporating select fruit wastes into Zebrafish diets using mathematical model-based approach

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A B S T R A C T

Expensive aquafeed is a major problem in aquaculture, creating the need for a low-cost feed that provides ideal nutritional requirement to maximize growth performances. This study aims to formulate and evaluate two new optimized Zebrafish feeds (F1 and F2) using linear programming mathematical model, one of which incorporates two pigment rich fruit wastes (Pitaya peel and Roselle calyx) in the formulation. The model represents nutritional content and cost of each ingredient into linear equations, with the goal of finding ideal combination that satisfies the specific nutrient requirements. By systematically evaluating ingredient proportions, linear programming ensures that Zebrafish receives adequate nutrients at the lowest possible cost, making the feed development process more efficient and cost-effective. The novel feed formulations derived from the mathematical model were tested on the growth and pigmentation of Zebrafish in comparison to a commercial feed (control). Feed intake of F1 and F2 were generally found to be similar to the control feed, indicating the acceptability of the formulated feeds by the Zebrafish. Body weight and length of Zebrafish fed with F1 and F2 were comparable to Zebrafish fed with control feed ($p > 0.05$). Similarly, Zebrafish fed with F1 and F2 showed no significant differences in pigment intensity compared to Zebrafish fed with control feed ($p > 0.05$). The survival rate of fishes in all feeding groups were greater than 70 % with no significant differences ($p > 0.05$). Results obtained in this study illustrated the potential of mathematic linear programming and effectiveness of utilising pigment-rich fruit wastes in formulating an optimized economic aquafeed.

1. Introduction

Ornamental fish cultivation is getting popular which received increasing demand. The growing of ornamental fish industry made it necessary to have a continuous supply of nutritionally proportioned and economical feed (Kasiri et al., 2011; Mandal et al., 2010). Feed cost and availability is a major limitation to aquaculture production (Edwards et al., 2000). Previous studies have reported that feed can contribute 40 to 66 % of yielding cost in a farm (De Silva & Hasan, 2007; Ofor, 2007). In addition, FAO (2009) stated that aquafeeds can account up to 50–70 % of total feed production costs. At times, if unsuitable feed is used, it can nullify economic profitability of a farm.

In ornamental fish markets, the vibrant colour in ornamental fish affects commercial acceptability and price in the commercial markets (Kaur & Shah, 2017; Saxena, 1994). Discoloration is a major negative effect in ornamental fish in culture (Kaur & Shah, 2017). As fishes are not able to synthesize their individual pigments *de novo*, fish need to secure these pigments source from dietary supplementation for enhancement (Mukherjee et al., 2009). Varieties of colouring agents are used in aquafeed industry

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to impart skin pigmentation of fishes such as astaxanthin, cantaxanthin and beta-carotene (Mirzaee et al., 2012; Stachowiak & Szulc, 2021; Yasir & Qin, 2010). Nonetheless, the use of certain synthetic colorants such as astaxanthin and cantaxanthin are pricey resulting in high price of feed. Synthetic colorants also have deteriorating consequence on the environment (Gubta et al., 2007). The use of pigments from natural sources in fish feed has been explored and reported in previous studies. Pérez-Escalante et al. (2012) reported that feed incorporated with 160 mg/kg pigment from Roselle were able to enhance the skin colour of Goldfish. While, Monica et al. (2019) disclosed that 400 mg/kg of anthocyanin extract can be used as one of the effective pigment enhancers to improve orange colour of Swordtail fish. These previous findings suggest successful use of plant pigments in aquafeed to enhance fish coloration, thus advocates the potential for utilizing pigment-rich plant wastes as an alternative to the pricey astaxanthin. Therefore, in this paper, we formulated zebrafish feed that incorporates select fruit wastes (Pitaya peel and Roselle calyx decoction residue) which contain natural pigments (betalains and anthocyanins) that could aid in enhancing the colour of Zebrafish as well as optimizing the growth and survival rate of fishes.

Developing low-cost effective feed is crucial to support optimal growth and quality of the aqua species. Researches have explored ways to cater feed formulation problems and found mathematical modelling to be a promising method in aquafeed formulation (Altun & Sahman, 2013; Nath & Talukdar, 2014; Pathumnakul et al., 2011; Rahman et al., 2017). The application of mathematical model in producing a single feed with reduced cost that can enhance growth is scarce (Aizam et al., 2018; Forsberg & Guttormsen, 2006; Nath & Talukdar, 2014). Furthermore, to our knowledge the use of mathematical model in formulating an optimal feed for Zebrafish have yet to be explored. Additionally, the utilization of Pitaya peel and Roselle calyx decoction residue as a pigment source in aquafeed is also scarce. These fruit wastes are commonly discarded during processing. However, betacyanin pigment in Pitaya peel (80.2 mg/g) (Chia & Chong, 2015) and anthocyanins in the Roselle calyx decoction residue (2.5 mg/g) (Amaya-Cruz et al., 2018) are valuable sources of natural colourant and antioxidant. Therefore, this study aims to formulate a low-cost Zebrafish feed which incorporates these select fruit-wastes, and investigate the effectiveness of the formulated feed on the growth and skin colour of Zebrafish.

2. Materials and methods

2.1. Formulation of Zebrafish experimental diet

Two feed formulation were developed, the first is a formulation without inclusion of fruit wastes (F1) and the second with inclusion of fruit wastes (F2). The fruit wastes used in this study included Pitaya peel and Roselle calyx decoction residue which is a by-product from Roselle beverage processing. In formulating the least-cost feed formulation, data such as nutrient requirement of the fish, prices of ingredients and the nutritional composition of the feed ingredients were required to be used as constraints in the Linear Programming mathematical modelling.

The feed formulation combined the basic ingredients in commercial feed (rice bran, soybean meal, fish meal, anchovy waste and mixed vitamin), as well as the inclusion of Pitaya peel and Roselle calyx decoction residue (in F2 only) which contains natural pigment for potential colour enhancement of the fish. Specific amount of each ingredient used in the formulation is based on the optimal results provided from the mathematical model constructed by Linear Programming. This feed formulation has been filed for intellectual property right (copyright) with the Intellectual Property Corporation of Malaysia (CRLY00017229), and some details are therefore not disclosed in this manuscript.

In order to obtain a complete nutrient in the feed formulation, data on nutrient required by the Zebrafish is essential. As reported by Velasco-Santamaría and Corredor-Santamaría (2011), protein, carbohydrate and lipid are the main nutrients required for fish energy, growth and bodily functions. However, a standard reference of Zebrafish nutrient requirement is limitedly known (Lawrence, 2007; Kaushik et al., 2011; Farias & Certal, 2016). Nevertheless, Zebrafish are reared with nutrient requirement of fish from a similar family which is Cyprinus such as carp (Kaushik et al., 2011; Farias & Certal, 2016). The minimum nutrient requirement of carp (38 % protein, 6 % lipid, 26 % carbohydrate) was thus made a reference and adapted for the feed formulation of zebrafish since this information is much more available from the literature (Nath & Talukdar, 2014; Fernandes et al., 2016; Quinlivan & Farber, 2017; Rasool et al., 2018). This minimum nutrient requirement was included as parameter input in the mathematical model for the formulation of zebrafish feed in this study.

Information on price of each ingredient used in the formulation was obtained from the literature and market survey. The nutritional composition of all feed ingredients was determined by performing standard method proximate analysis according to Association of Analytical Chemists (AOAC, 2000). Both data on price and nutritional composition of ingredients were included as parameters input in the mathematical model for the formulation of Zebrafish feed development. As mentioned previously, the feed formulation was registered for intellectual property right. Therefore, the information on ingredients is not enclosed in this manuscript.

The linear programming model for feed formulation is briefly explained below. In this model, the decision variables x_j are the amount of each ingredient needed in 100 kg of fish feed and converted to weight based on the bag size of one (1) kg. The list of ingredients included in the model for selection are amongst the commonly used ingredients in commercial feeds. Example of these ingredients are rice bran, soybean meal, fish meal, anchovy waste and mixed vitamin. Additional ingredients incorporated in the model for the purpose of colour enhancement of the fish are Pitaya peel and Roselle calyx decoction residue that can be easily found locally. These two additional elements that contains natural pigment for potential colour enhancement are included in the list of ingredients for F2 feed formulation.

Linear Programming:
Minimize $Z = \sum_{j=1}^{n} c_j x_j$
Subject to

$$\sum_{j=1}^{n} a_{ij} x_j \le b_i,\ \forall_i = 1, \cdots, m$$

$$\sum_{j=1}^{n} x_j = d$$

$$x_j \ge 0$$

where

Z = the total price of the ingredients (basic ingredients, mixed vitamin and natural wastes) used to formulate the fish feed.

x_j = weight of ingredients j per kg (for $j = 1, 2, \cdots, n$) in the feed mixture.

c_j = unit price of each feed ingredients j per kg listed.

b_i = dietary nutrient requirement by zebrafish (for.$i = 1, 2, \cdots, m$)

a_{ij} = amount of nutrient content i contain in different ingredients.j

d = total weight of feed (in kg).

j = feed ingredients with $j = 1, 2, \ldots, n$.
I = feed nutrient compositions with $i = 1, 2, \ldots, m$.

Linear programming (LP) is a strategy for optimizing a linear objective function of a set of decision variables shown in equation (1) that is constrained by linear equality and inequality requirements, which in this case are the nutritional requirements for the zebrafish; proteins, carbohydrates, fibers and vitamins. These requirements are presented by equation (2). Other constraint considered is the preference type of requirement, such as the total amount of feed to be produced that can be presented in equation (3). Equation (4) represents the logical requirement of anticipating a return of non-negativity values for each variable, x_j. It is aimed that the mathematical model developed based on the necessity requirements and preferences attains the minimum cost production whilst ensuring the nutritional demands of the zebrafish are met. The feed is formed with the best combination of selected ingredients.

2.2. Pellet production and analysis of nutrient composition of feed

Based on the formulation developed from the mathematical model, all ingredients were weighed accordingly using weighing balance and mixed thoroughly in a feed mixer in order to obtain a homogenous mass. The dough mixture was moistened by adding 50 % (w/v) of water, and then extruded through diet mixer pelleting machines. A strand of feed diets was formed which later were grinded into small size and sieved to obtain suitable size for Zebrafish. The pellets were dried in oven at 40 °C for 24 h and stored in an airtight container at room temperature prior to usage.

The prepared feeds were then subjected to proximate analysis (AOAC, 2000). Crude protein was determined using the Kjeldahl method by measuring nitrogen which acts as the precursor for protein of a substances (N × 6.25). Crude lipid was determined using petroleum ether based on Soxhlet method (AOAC, 2000), where petroleum ether (40 to 60 °C boiling point) was used. The differential method was used to determine carbohydrate value of diet samples.

2.3. Feeding trial

2.3.1. Experimental site and trial conditions

A total of 90 late juvenile Zebrafish with the initial average body weight of 0.03 ± 0.00 g and length of 1.30 ± 0.50 cm were randomly distributed over nine experiment tanks, which represents triplicates for each feeding groups, at capacity of 7L filled with 5L water with a density of 10 fishes per 5L (1 fish per 0.5L) following completely randomized design. Experiment diets consists of two formulated feeds (F1) formulation without inclusion of Pitaya peel and Roselle calyx decoction residue and (F2) formulation with inclusion of Pitaya peel and Roselle calyx decoction residue (F2) with a commercial ornamental pellet (C). A commercial feed contained 27 % digestible protein composed of mixture of fish meal, wheat meal, yeast, germ meal, shrimp meal, vitamin and mineral supplementation. Fish were fed twice a day for 60 days (2 % of body weight). The photoperiod was set at 12:12 h (light: dark) with water pH and temperature maintained between 6.5 and 7.8 and 26.6 ± 0.1 °C, respectively. Total ammonia, nitrite and nitrate were monitored weekly by Sera test kit (Sera GnH, D52518 Heinsberg, Germany) (ammonia 0 ppm, nitrite and nitrate below 10 ppm). All procedures implemented in this experiment were approved by the Research Animal Care Ethics Committee (UMT/JKEPHT/2019/42).

2.3.2. Assessment of growth performance

The initial weight and length of all fishes were measured individually at the beginning of the feeding trial period. Weight and length of fish were measured thereafter at day 30 and day 60 of feeding. The length of the fish (cm) was measured using ruler, while electronic weighing balance was used to record the weight of fish (mg) (Rasool et al., 2018). Feeding intake were recorded from the remaining feed at the end of each feeding day, and subsequently averaged from the total number of feeding days. Percentage of body weight, body length and survival rate were calculated with the following equation:

$$Body\ weight = \frac{(final\ body\ weight - initial\ body\ weight)}{initial\ body\ weight} \times 100$$

$$Body\ length = \frac{(final\ body\ length - initial\ body\ length)}{initial\ body\ length} \times 100$$

$$Survival\ rate(\%) = \frac{number\ of\ fish\ at\ day\ 30\ and\ 60}{number\ of\ fish\ at\ the\ begining\ of\ the\ trial} \times 100$$

2.3.3. Assessment of body pigmentation

Skin pigment of fish was assessed at day-30 and day-60. At day-30, determination of red index using Adobe® Photoshop CS6 software (Adobe Systems Incorporated, United States) was performed. No scarified required with this method and allow fish to survive until the end of the feeding trial. Pictures of each single fish from each feeding group was captured individually using a camera with 16MP and 3x magnification. The pictures were then analyzed for red index using the software using the following formula, as described by Svensson et al. (2011).

$$Red\ index(R_i) = \frac{R}{(R + G + B)}$$

At the end of the feeding trial (day-60), a* value (redness) was measured using Chromameter CR-400 (Konica Minolta, Inc., Japan). The measuring head of the chromameter was applied directly on the surface of the fish body to measure the redness value.

2.4. Statistical analysis

All data are given as mean ± standard error. Data on growth performance which includes assessment on body weight, body length, survival rate and skin colour of zebrafish were analysed by one-way analysis of variance (ANOVA) followed by Tukey's test. Survival rate data was analysed with chi-square test. The analyses were performed to determine whether there are any statistically significant differences between the feed tested in the trial. Statistical significance was accepted at $p < 0.05$. All the analyses were performed by SPSS software (Version 20.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Nutrient composition of diet

Pellet formed based on the formulation given by the mathematical model was subjected to analysis of nutrient composition and the results are tabulated in Table 1. The analysis shows that the F1 Zebrafish feed formulation contains 37.42 %, 5.83 %, 33.52 %, 14.25 %, 3.80 % and 5.19 % of protein, lipid, carbohydrate, ash, fibre and moisture respectively. F2 feed formulation shows values of 38.41 %, 5.91 %, 32.29 %, 13.95 %, 3.94 % and 5.5 % of protein, lipid and carbohydrate, respectively. Considering the focus on macronutrients needed by fish which are carbohydrate, lipid and protein which provides energy and are crucial for fish growth, the formulated diets obtained in this study fit to the nutrient requirement for zebrafish, where the values obtained were in the range of zebrafish nutrient needs.

Table 1
Nutrient composition of Zebrafish feed formulations produced by linear programming method. Formulation without inclusion of Pitaya peel and Roselle calyx decoction residue (F1), formulation with inclusion of Pitaya peel and Roselle calyx decoction residue (F2).

Feed	Nutrient					
	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	Fibre (%)	Moisture (%)
F1	37.42	5.83	33.52	14.25	3.80	5.19
F2	38.41	5.91	32.29	13.95	3.94	5.5

3.2. Growth performance

Fig. 1 illustrates the feed intake for all feeding groups at day-30 and day-60 of feeding trial. F1 was found to be significantly more favoured by the Zebrafish than the control feed ($p < 0.05$) at day-30 of trial. F2 also showed a tendency for higher rate of intake when compared to control at day-30, but was not significantly different. Meanwhile, at day-60 of feeding trial, F1 intake was significantly higher when compared to F2, but was no different with control feed.

Fig. 2 illustrates the body weight of Zebrafish from different feeding groups at day-30 and day-60 of feeding trial. Body weight of Zebrafish fed with F1 and F2 were comparable to zebrafish fed with control feed at day-30 of the trial ($p > 0.05$). Albeit the tendency for a slightly higher body weight in zebrafish fed with F1 at day-30, but this was not statistically significant when compared to the other two feeding groups. Overall, body weight of zebrafish in all groups were maintained relatively stable with no significant difference ($p > 0.05$).

Fig. 3 shows the body length of Zebrafish fed with different feed formulation in different feeding groups at day-30 and day-60 of feeding trial. In term of body length improvement showed that all zebrafish were grown relatively comparable with no significant difference noticed ($p > 0.05$).

Fig. 4 illustrates the survival rate of Zebrafish fed with different feeds at day-30 and day-60 of feeding. The survival rate of zebrafish at day-30 of feeding were recorded at 85.71 %, 90 % and 80 % for control, F1 and F2 respectively with no significant difference ($p > 0.05$). In addition, the survival rate of zebrafish at day-60 of feeding were noted at 85.71 %, 90 % and 70 % for C, F1 and F2 respectively with no significant difference ($p > 0.05$). This denotes that fish fed with formulated feeds is comparable with commercial feed (C), and suggests that the formulated feeds are sufficient to provide a cheaper alternative to rear ornamental Zebrafish.

3.3. Skin colour of zebrafish

Fig. 5 illustrates the skin colour of Zebrafish from different feeding groups at day-30 and day-60 of feeding trial. The skin pigment of Zebrafish in all feeding groups were comparable at both day-30 and day-60 of feeding ($p > 0.05$). However, there was a tendency for a slightly higher a^* value which was detected with better redness in Zebrafish fed with F2.

4. Discussion

This study has shown the ability of linear programming (LP) in allocating various sources of ingredients that includes the fruit waste to substitute the expensive pigment ingredient in the effort to produce an optimal aquafeed formulation. The capability of LP as a mathematical technique in solving resources allocation has also been reported by Panik (2018). LP method takes into consideration the cost and the nutritional composition of each ingredient to produce feeds with reduced cost that meets the minimum nutrient requirement for Zebrafish. The method therefore provided solution to select cheapest ingredients to develop the feed that is capable to fulfil nutrition requirement for Zebrafish, while reduce reliance on expensive ingredients. The formulation gained from this LP model in this study produced a low-cost feed which is 70 % lower in price than the tested commercial feed. The formulation thus can potentially be a replacement to a much more expensive commercial feed in the market.

The formulated feed was thereafter validated through a feeding trial in assessing the effectiveness with the comparison to commercial feed. There are several factors that influence the feed intake such as feed acceptability, palatability and digestibility which can differ with the broad ingredients and feed quality. According to Kasumyan and Doving (2003), the feed taste attributes a prominent implication inciting feed intake and growth of fish. In this study, feed intake for F1 and F2-fed Zebrafish compared to the Zebrafish fed with commercial feed was slightly higher. This

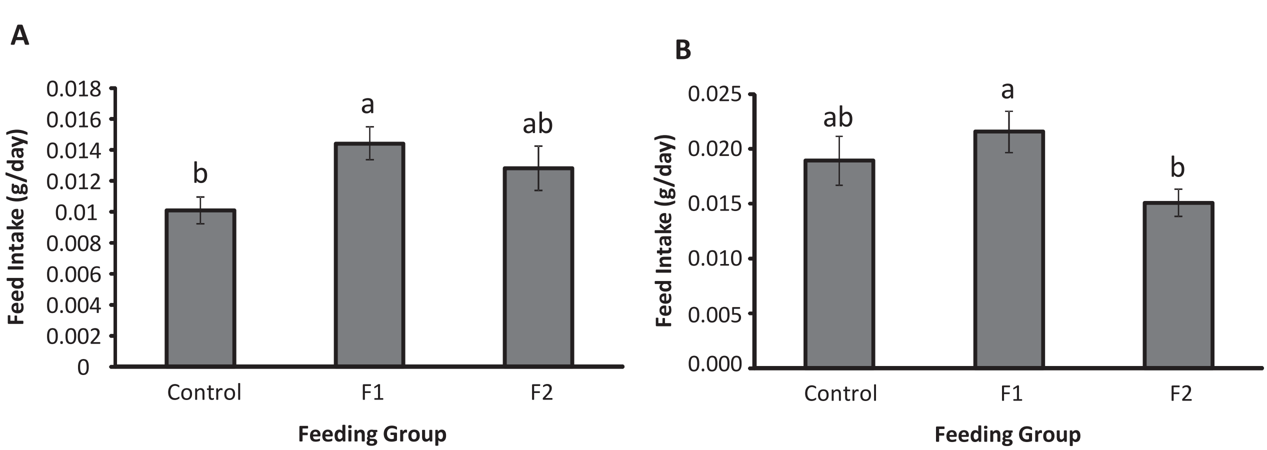

Fig. 1. Feed intake of zebrafish from different feeding groups at day-30 (A) and day-60 (B) of feeding trial. Formulation without inclusion of pitaya peel and roselle calyx decoction residue (F1), formulation with inclusion of pitaya peel and roselle calyx decoction residue (F2). Data is represented as mean ± standard error. Columns with different letter indicates significant difference ($p < 0.05$).

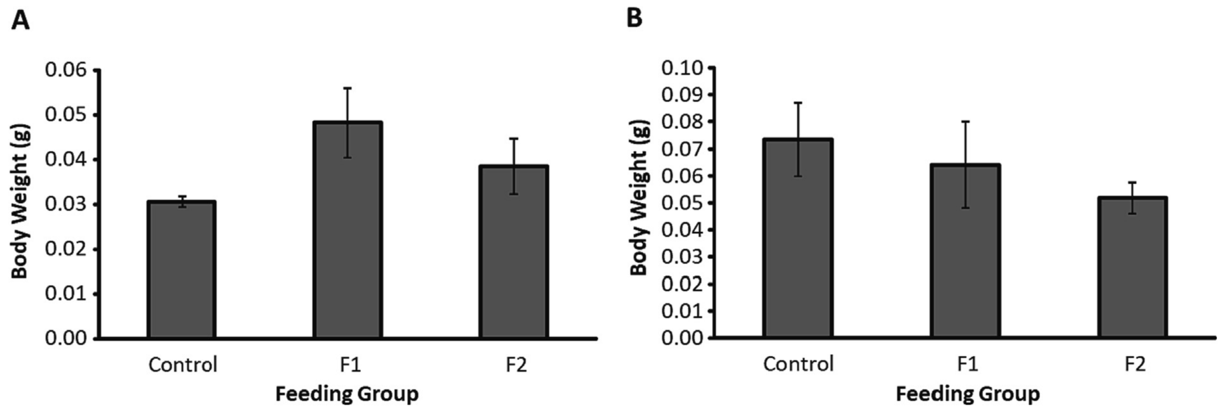

Fig. 2. Body weight of zebrafish from different feeding groups at day-30 (A) and day-60 (B) of feeding trial. Formulation without inclusion of pitaya peel and roselle calyx decoction residue (F1), formulation with inclusion of pitaya peel and roselle calyx decoction residue (F2). Data is represented as mean ± standard error.

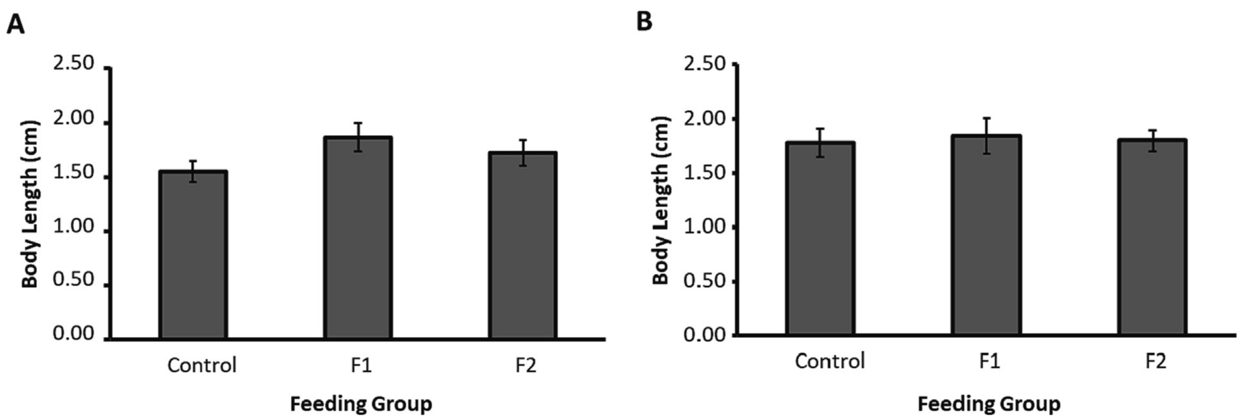

Fig. 3. Body length of zebrafish from different feeding groups at day-30 (A) and day-60 (B) of feeding trial. Formulation without inclusion of pitaya peel and roselle calyx decoction residue (F1), formulation with inclusion of pitaya peel and roselle calyx decoction residue (F2). Data is represented as mean ± standard error.

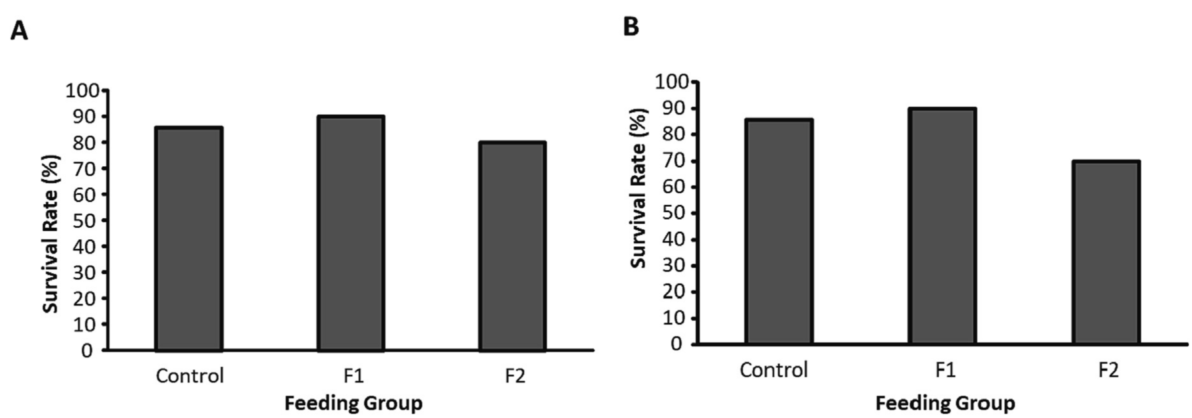

Fig. 4. Percentage of zebrafish survival rate at day 30 (A) and day 60 (B) of feeding trial. Formulation without inclusion of pitaya peel and roselle calyx decoction residue (F1), formulation with inclusion of pitaya peel and roselle calyx decoction residue (F2).

indicates that the present diet formulation is acceptable by Zebrafish. Feed intake of F1 was found slightly higher than F2 at day-60 of feeding suggesting that this feed was more desirable to the zebrafish. Feed preference is attainable through the observation on portion of feed consumed. Therefore, the acceptability of our formulated feeds as shown in the feed intake data illustrates their potential to be used for rearing zebrafish.

The principal nutrient requirement for carp (Cyprinus) was used in this experiment due to limited information on the requirement for the zebrafish (Lawrence, 2007; Kaushik et al., 2011, Farias & Certal, 2016). The minimum protein requirement for this species was stated to be at 38 %. Thus, the LP model employed in formulating F1 and F2 considers selection of ingredients to produce a feed that meets the stated minimal protein requirement as much as possible. Hence, the formulated F1 and F2 had higher protein content (37.42 % and 38.41 % respectively) compared to the commercial diet (27 %). Despite the higher protein content in the two formulated feeds, the growth of fishes in all feeding groups were

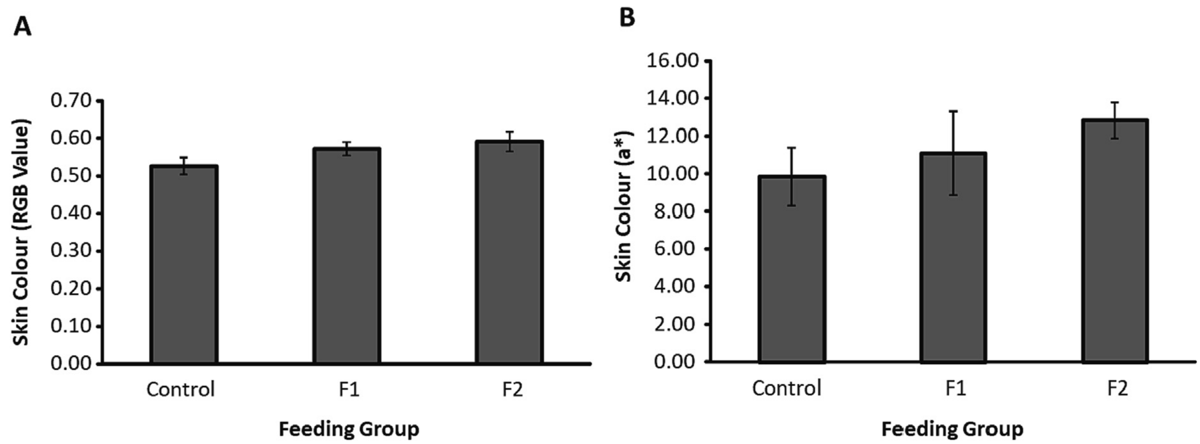

Fig. 5. Skin colour of zebrafish from different feeding groups at day-30 of feeding trial measured using Adobe® Photoshop CS6 (A) and day-60 of feeding trial measured using Chroma meter CR-400 (B). Formulation without inclusion of pitaya peel and roselle calyx decoction residue (F1), formulation with inclusion of pitaya peel and roselle calyx decoction residue (F2). Data is represented as mean ± standard error.

comparable. The feeds formulated from LP model, F1 and F2 showed insignificant difference of body weight and body length when compared with the commercial feed (control) at day-30 and day-60 of feeding ($p > 0.05$).

The ornamental fish pellet available in the market is usually designed to all common fishes, but no specific formulation for Zebrafish is available. Information on the minimum nutrient requirement for Zebrafish is also limitedly known. The observation on similar growth across all feeding groups in this study suggest that Zebrafish require a lesser protein content in the feed than the carp, which was used as a reference. The protein content in the experimental feed can therefore still be reduced to the minimum required amount of Zebrafish, thus lowering the existing minimal cost of the formulated experimental feed.

Generally, fish meal is used as core protein supply in fish feed yet it also imposes a higher cost compared to the rest of ingredients. The developed formulation derived from the LP method can reduce the dependence on costly ingredients but still able to meet the nutrient requirements of zebrafish. Therefore, there is possibility to reduce protein levels as in control feed with stable growth performance. Other sustainable source of protein for instance insect (Sogari et al., 2019; Wachira et al., 2021) could also be incorporated into the LP model in the future to further establish a much lower cost of feed.

Previous study has demonstrated that the inclusion of 0.15 % of Roselle as dietary additive in feed gave the highest mean weight gain of *Clarias gariepinus* (African sharptooth catfish) (Adewole, 2014). Another study also mentioned that the inclusion of 1 % of Roselle calyx in feed showed the highest growth performance in Nile tilapia (El Mesallamy et al., 2016). Phenolic and flavonoid compounds in roselle with a range of biological activities such as antioxidant and antimicrobial (Hapsari et al., 2021) can promote good growth through the regulation of husbandry-related stressor in culture environment (Adewole, 2014). However, the different results obtained from these researches may be related to species-specific factors such as the diverse intestinal microbiota and morphology, as well as the experimental condition. The observation of our study is consistent with Baron et al. (2008) which reported that betalain and anthocyanin dietary pigments that are found in Pitaya peel and Roselle calyx decoction residue respectively, had no significant effect on growth of flame-red dwarf gourami.

This study also observed no significant difference in survival rate of fish fed with either F1 or F2 compared with commercial feed. The presence of betalains and anthocyanins pigments did not negatively affect survival of fish. This tally with a previous study where various carotenoid sources did not affect survival of fish as well as growth (Ramamoorthy et al., 2010; Nhan et al., 2019). Albeit the facts, fish growth performance in this study displayed significant difference than those of the above-mentioned researchers. The result of the least-cost feed formulation produced by LP model met the minimum requirement of protein (38 %), lipid (6 %) and carbohydrate (26 %) that was considered for Zebrafish. This possibly explains the observation where the survival rate was comparable with commercial feed.

In this study, we observed a tendency for higher intensity of redness in Zebrafish fed with F1 and F2 formulation at day-60, which was not significantly different compared with the Zebrafish fed with commercial feed. Interestingly, Zebrafish skin redness was observed to be slightly more intense on Zebrafish fed with F1 and F2 than commercial feed. In aquaculture industry, fish are regularly exposed to a variety of stressful circumstances, which will cause an oxidative disorder and consequently impact their quality and health. The preservation of fish's vibrant hues is an essential challenge in the ornamental industry (Tamaru and Ako, 1999; Abbas et al., 2020) and providing feed enhanced with colourant is one of the potential remedies to solve this problem. Oftentimes synthetic astaxanthin, a factory-made carotenoid is used as a feed supplement for pigmentation in most of the ornamental fish feeds. Astaxanthin is reported to successfully engage in intensifying the skin redness on many species (Buttle et al., 2001; Gouveia et al., 2002; Booth et al., 2004). Studies on the utilisation of natural pigment sources as an alternative to synthetic carotenoid sources in fish feed have been conducted in recent years due to substantial consumer awareness on healthier and safer food source. Some studies have been conducted on the use of natural carotenoids as an alternative to synthetic carotenoid sources in fish feed such as beet root, carrot peel and tomato peel powder on common carp, spirulina powder on dwarf gourami, carrot and spinach on swordtails fish, citrus peels on goldfish, carrot meal on clown loaches, dietary oleoresins on ocellaris clownfish, freshwater microalga rich in astaxanthin on golden pompano (silverfish), and *Lantana camara*; a flowering plant native to the American tropics on green swordtail (Maiti et al., 2017; Bakshi et al., 2018; Wagde et al., 2018; Abbas et al., 2020; Andriani, Priyadi, & Firdaus, 2020; Ebeneezar et al., 2020; Xie et al., 2020; Zutshi & Madiyappa, 2020). Fish obtains pigment sources such as carotenoid or astaxanthin through dietary intake (Furr & Clark, 1997; Gupta et al., 2007) and are delivered through the blood by serum lipoproteins (Bowen et al., 2002). It is then metabolically oxidized into another structure (Katayama et al., 1973) and then projected into chromatophores, a specialized skin cells (Chatzifotis et al., 2005) which can give visual effect of skin colour.

According to Jahazi et al. (2020), polyphenols, which are significant antioxidant and immunomodulator substances, are found naturally in plant tissues. Polyphenols are classified into numerous classes, and anthocyanins and betalains are two of them that cause plant coloration. The health benefits of anthocyanins and betalain, which are present in a wide range of plants, include antioxidant, antibacterial, immunomodulatory, hypolipidemic, and hypoglycaemic actions in animals (Chen et al., 2018; Choung & Lim, 2012; Cisowska et al., 2011).

Anthocyanin and betalaine present in Roselle calyx decoction residue and Pitaya peel, are water-soluble pigments, and they may lack in ability to be delivered in the bloodstream by serum lipoprotein (Bowen et al., 2002). Despite that, Roselle calyx reported to have high antioxidant activity owing to the anthocyanins and other polyphenols as well as ascorbic acid (Tsai et al., 2002; Escribano-Bailón et al., 2006; Wu et al., 2018). Betalain that is richly found in Pitaya peel and other plants such as Beetroot has also been attributed for its high antioxidant activity (Lee et al., 2005; Mello et al., 2014; Belhadj Slimen et al., 2017). Vitamins A, C, and E, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, carotene, and phyto-alphabumin are among the bioactive substances found in Pitaya peel (Fitria, 2021).

There are several studies that focused on the application of anthocyanin from Roselle in fish feed. Based on study done by Vanegas-Espinoze et al. (2019), the microencapsulation of Roselle's anthocyanin provides natural pigments that increase fantail goldfish growth and pigmentation. Not only limited to elevation of fish skin pigmentation, Roselle extract has been shown to improve goldfish development performance (Pérez Escalante et al., 2012), as well as feed efficiency, immunological response, disease resistance, and hematological parameters in Nile tilapia (El Mesallamy et al., 2016). The treatment of in vitro Roselle extract resulted in hepatoprotective and antioxidant effects on the liver cells of common carp, *Cyprinus carpio* (Yin et al., 2011). Recent study also reported that the inclusion of Roselle calyx in feed of rainbow trout gave higher growth and lower mortality rates (Hoseini et al., 2021).

Various study has been conducted to optimised the potential of Pitaya peel as colour pigments in fish feed for examples their addition in feed had affected the colour quality of comet and clown fish (Udjan et al., 2023; Barros et al., 2023). Pitaya peel treatment in feed of goldfish and chef carp can influence higher brightness in their colour (Wijaya et al., 2021; Rani et al., 2022). Hence, even though the water-soluble pigments possibly cannot directly be delivered into the skin and do not greatly intensify skin pigmentation, they could still offer antioxidant attributes to secure existing skin carotenoids against the oxidation which explains the growth and survival that were comparable with commercial feed. The antioxidant activity in these fruit wastes can also potentially protect labile nutrients such as inhibition of lipid oxidation in the feed ingredients (Aklakur, 2018), which in turn helps retain good growth for the fish. Therefore, the feed formulations produced in this study is not only cost-effective, but also successful in meeting the requirements for fish development.

The agri-food industry produces vast amounts of fruit or vegetable wastes, and because of their high moisture content and microbial load, they have the potential to seriously harm the environment. Feeding fish with plant waste could potentially result in negative impact on the growth performance (Kuebutornye et al., 2023). This phenomenon may be influenced by a number of factors, including the type of plant waste utilised in the trials, the concentrations of plant waste administered to supplement the fish diet, the phytochemical composition of the plant waste, its nutritional profile and antinutritional factors (Larrosa et al., 2021; Bertocci & Mannino, 2022). In order to determine the ideal concentration to prevent the adverse effects, it is recommended that experimental effects for the growth of fish fed with agri-food waste be thoroughly investigated on a small scale before being employed for medium- to large-scale production.

5. Conclusion

In conclusion, linear programming method is proven as a model that can be used in solving problems in Zebrafish feed formulation with optimal solutions since all the nutrient required by the tested species are met at minimum cost compared to commercial feed. Diets containing natural pigments from Roselle calyx decoction residue and Pitaya peel provided comparable growth performances and pigment enhancement on zebrafish with commercial feed. The formulated feeds are therefore found to be applicable for rearing ornamental zebrafish, and can be an efficient alternative to the commercial feed. Further assessment on the skin coloration using skin biopsy can be done in the future to further understand the impact of the formulated feed on ornamental fish. In addition, nanoencapsulation of natural pigments such as anthocyanin and betalaine for incorporation into fish feed is also worth to be investigated.

6. Declaration of competing interest

The authors declare there is an issued copyright for the feed formulation that is relevant to the work in the manuscript (#Copyright- CRL Y00017229).

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CRediT authorship contribution statement

Rabiatul Adawiyah Ibrahim: Investigation, Formal analysis, Validation, Writing – original draft. **Nur Aidya Hanum Aizam:** Conceptualization, Investigation, Project administration, Supervision, Resources, Writing – review & editing. **Hon Jung Liew:** Investigation, Project administration, Supervision, Writing – review & editing. **Nurul Sakinah Din:** Investigation, Formal analysis, Validation, Writing – original draft. **Aidilla Mubarak:** Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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