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Short communications: Effect of red spinach (*Amaranthus tricolor* L. var *Blitumbrum*) extract in the diet on color brightnes of guppy fish (*Poecilia reticulata*)

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ABSTRACT

Fish color brightness is influenced by food intake, but no prior reviews have explored the use of red spinach extract in fish feed to enhance color brightness and promote growth in guppy (Poecilia reticulata). Therefore, this study aims to determine the suitable concentration of red spinach extract Amaranthus tricolor color brightness, growth, and survival of guppy. The experiment followed a completely randomized design with three concentrations of red spinach extract (3%, 4%, and 5%) replicated three times at the UPTD Fish Breeding Center in North Binjai Regency, North Sumatra Province. Furthermore, red spinach extract was mixed with the commercial diet containing 41% crude protein and administered to fish at a rate of 3% of their body weight daily for 40 days. The results showed a significant effect of red spinach extract doses on guppy color brightness (P<0.05), but no significant influence on weight gain and fish survival (P>0.05). It was also discovered that the average color increase was 2,652 in feed mixed with 5% red spinach extract. Similarly, the highest fish weight gain was observed at the 5% concentration, which did not significantly differ from the other concentrations. Fish survival rates were high, with no significant differences among the treatments. Therefore, it was concluded that the optimal concentration of red spinach extract for enhancing guppy color brightness was 5%

Introduction

Guppy fish (*Poecilia reticulata*) is highly sought after in the ornamental fish market due to its appealing shape and vibrant color. Male guppy exhibit more visually striking morphological characteristics compared to females. This species holds significant commercial value in domestic and foreign markets, with premium guppy reaching prices of up to IDR 800.000 per fish. Therefore, guppy have immense potential in aquaculture, as indicated by the average annual growth rate of 64.8%, driven by the increasing demand for guppy fish in Indonesia. To meet this demand, it is essential to increase production due to the unique morphology and coloration of these fish (To'bungan, 2017; Hadijah *et al.*, 2020; Saputra *et al.*, 2017).

Color is vital in determining the quality of ornamental fish, as brighter hues often lead to higher selling prices. Fluctuations in pigment levels primarily drive color changes, and these levels can be influenced by environmental stressors, including sunlight, water quality, and carotene content in the diet. Therefore, it is crucial to provide feed that enhance vibrant color in ornament fish (Nazhira *et al.*, 2017; Lestari *et al.*, 2019).

One common technique to enhance color of these fish is by manipulating pigments through the addition of carotene to their diet. However, synthetic dyes in fish feed often yield less satisfactory results compared to natural pigments. Adding coloring substances to feed effectively stimulates an increase in color pigments within the body of fish. To enhance color brightness in

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ornamental fish, feed containing carotenoids are recommended (Prayogo *et al.,* 2012; Satyani and Sugito, 1997).

Orange-red vegetables such as carrots, pumpkins, peppers, sweet potatoes, corn, dragon fruit, and spinach are rich sources of carotene, acting as reliable indicators of its presence in plants. These vegetables are easily accessible in traditional and modern markets at affordable prices (Lestari *et al*., 2019), making them excellent natural sources of carotenoids.

Red spinach (*Amaranthus tricolor* L) is a highly nutritious vegetable, rich in protein, vitamin A, vitamin C, essential minerals, anthocyanins, carotenoids, and flavonoids, which possess antioxidant properties (Diansyah *et al*., 2019; Lestari *et al*., 2019). Spinach contains beta carotene as its primary carotenoid, along with chlorophyll. Also, flavonoids such as lutein and quercetin are present in spinach. Green and red spinach are two varieties available, and both are rich in vitamin C. However, green spinach has higher vitamin A levels, while red spinach is notably higher in iron content (Suwita *et et al*., 2011). This study aims to evaluate the impact of adding red spinach extract, a natural dye, on enhancing color brightness of guppy fish (*Poecilia reticulata*).

Materials and Methods

Time and place

This study was conducted between June and August 2021 at fish hatchery of UPTP Center for Fish Breeding in Binjai City, North Sumatra Province. The production of red spinach extract was carried out at the FMIPA Laboratory of the University of North Sumatra.

Experimental design

The treatment was conducted using a completely randomized design with three replications over a 40-day experimental period. It involved varying concentrations of red spinach extract at three levels, including 3%, 4%, and 5%.

Experimental fish

A total of 72 guppy fish, measuring 2 cm in length and weighing 0.2 grams, were obtained from an ornamental fish breeding facility in North Binjai Regency, North Sumatera. Before the experiment, fish were acclimatized in a container for four days.

Spinach extract preparation

A total of 500 g of red spinach was washed thoroughly with running water before being dried under the sun for two days. The dried leaves weighed 80 grams after the drying process. These leaves were then mashed into a flour-like consistency, resulting in a weight of 50 g. Extraction procedure followed the method outlined by Lestari *et al.* (2019). The yield of extract was determined by calculating the percentage of the dry extract obtained with the initial dry powder weight before extraction, multiplied by 100%.

Mixing red spinach extract into feed

Spinach extract were applied to the diet at concentrations of 4%, 5%, and 5%. Subsequently, the diet was air-dried at room temperature for 3 hours before being fed to the experimental fish twice daily for 40 days.

Maintenance of fish

Guppy were stocked in 27-liter containers at a density of 8 fish per container. The fish were fed twice daily at 9 AM and 4 PM, with an amount equal to 3% of their body weight, for 40 days. Water quality parameters such as temperature, pH, and dissolved oxygen content were measured at 10-day intervals.

Fish color observation

Color brightness in guppy were assessed by a panel of 5 individuals with normal color vision. Each panelist conducted color observations every 10 days using the Toca color finder. The original color of fish on a rated paper was visually compared, and scores from 1 to 30 were assigned, representing color gradations from light orange to dark red.

Parameters

The daily weight gain was calculated using the methods proposed by Yusni (2006) as follows:

Daily weight gain (g/day) = (w2 -W1 /t)

where W2 is the body weight of fish at the beginning of the experiment, W1 is the body weight of fish at the end of the experiment, t= experiment duration (days)

The final percentage of fish was compared to the initial population using the formula proposed by Muchlisin *et al.* (2016) as follows:

SR (%) = (No-Nt/No) x100

where, SR = Survival rate of fish (%), No = Number of fish at the beginning of the experiment, Nt = Number of death during the experiment

Data analysis

Data were analyzed using Analysis of Variant (ANOVA), followed by the multiple range analysis of Duncan.

Results

The results showed a significant effect of red spinach extract on color brightness ($P<0.05$), while weight gain and fish survival were not significantly

affected (P<0.05). After 40 days, the highest color brightness was observed in fish fed with a 5% concentration of red spinach extract, which differed significantly from the other concentrations. Similarly, the 5% concentration resulted in the highest weight gain, but it was not significantly different from the 3% and 4% concentrations. The survival rate exceeded 95% in all treatment groups (Table 2).

Table 1. Mean and standard error of color brightness of guppy fish *Poecilia reticulata* during 40 days of the experiment.

Red spinach con.	Day-10	Day-20	Day-30	Day-40
3%	1.417±0.103[a]	1.708±0.112[a]	2.417±0.158[a]	2.087±0.165[b]
4%	1.417±0.119[a]	2.542±0.190[b]	2.375±0.168[a]	1.783±0.153[a]
4%	1.625±0.118[a]	2.792±0.262[b]	2.458±0.134[a]	2.652±0.256[c]

Table 2. Weight gain and survival rate of guppy fish *Poecilia reticulata* fed experimental diet for 40 days experiment

Red spinach concentration	Weigh gain (g day^{-1})	Survival rate (%)
3%	0.011±0.013[a]	95.8
4%	0.021±0.001[a]	95.8
5%	0.022±0.001[a]	95.8

Discussion

This study shows the significant influence of red spinach extract on color brightness of guppy fish (*Poecilia reticulata)* due to its carotenoid pigment content. Sholichin *et al.* (2018) noted that providing feed containing carotenoids enhances fish color brightness. Red spinach extract contains beta-carotene and betacyanin, which are known to impact coloration of guppy. This finding aligns with Saputra *et al.* (2017) who observed increased color brightness in goldfish (*Carrasius auratus)* fed with red spinach flour.

The study found no significant influence of red spinach extract on the weight gain and survival rate of guppy fish, indicating that carotenoids may not play a significant role in fish growth. This could be due to carotenoids not serving as an efficient energy source for guppy fish. However, Bjerkeng *et al.* (1992) reported a contradictory finding, showing that the addition of carotenoids to salmon feed improved growth performance compared to feed without carotenoids. Therefore, this study shows effect of carotenoids may vary depending on the species or be influenced by other substances present in spinach extract, such as anti-nutrients, which could adversely affect fish growth.

Prayogo *et al.* (2012) highlighted the importance of nutrition in fish growth. Therefore, this study shows that adding extract to the nutrient-enriched feed significantly increased fish body weight. Consistent with Barus *et al.* (2014), natural feed did not significantly affect carp growth. For ornamental fish, incorporating feed with carotenoids is more likely to enhance body coloration.

The water quality during the study remained within acceptable limits for fish survival, with temperatures ranging from 26-29 ^{0}C, pH levels between 6.9 to 7.3, and Dissolved Oxygen concentrations of 3.4 to 3.6 ppm. Nixon *et al.* (2004) reported that maintaining guppy fish in high-pH conditions (above 7) can enhance their appetite, promoting faster growth and improved tail development. However, extreme pH values, such as 5 or 9, can be fatal for fish (Fitriana *et al.*, 2023). Temperature plays a crucial role in supporting growth and survival of fish including guppy, as sudden fluctuations can negatively impact their metabolism and lead to mortality (Nur *et al.*, 2020). This finding is consistent with the observations of Istuanto *et al.* (2015) and Amir *et al.* (2022), indicating the significance of physicochemical water parameters as critical factors in fish growth.

Conclusion

The results of this study showed that adding a 0.02ml dose of red spinach extract to commercial feed affected coloration significantly. Additionally, mixing feed with 3% red spinach extract and 48% protein content had a notable impact on weight compared to other feed variations.

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