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Diverse Levels of *Amaranthus dubiusleaves* on Growth and Carotenoid Improvements in Fresh Water Ornamental Fish Peacock Cichlid (*Aulonocara hansbaenschi)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Attractive coloration and growth of ornamental fishes are important quality criteria in the aquarium fish industry. Colour-producing carotenoids cannot be synthesized by ornamental fish, so they must rely on dietary carotenoids in natural or synthetic forms to achieve their color pigmentations. The

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aim of this review is to compileand summarize recent investigations into different carotenoid sources used in ornamental fish feed formulations and to highlight the research gaps and investigation needs in the field of aquaculture. An attempt has been made to evaluate the effects of diets containing 0%, 2.5%, 5%, 7.5%, 10% *Amaranthus dub* the growth and carotenoid changes in peacock cichlid (*Aulnocara hansbaenschi*).This isonitrous (40% protein) diet supplemented with *Amaranthus dubius* leaves diet was prepared and offered ad libitum to the experimental fish in 5% body weight for 45 days. The results indicated that in the skin and muscle tissue of experimental fish, the growth and carotenoid contents were found to vary between control diets and also during the time intervals of experimental period. The results indicated that incorporation of 10% diet *A. dubiusleaves* in the diet was found to enhance the growth and coloration of peacock cichlid's (*Aulnocara hansbaenschi).* This is considered the optimum dietary level to enhance feed intake growth and coloration.

Keywords: Carotenoid; Amaranths dubius leaves; Peacockcichlid; Aulonocara hansbaenschi.

1. INTRODUCTION

"The ornamental fish sector is a widespread and global component of international trade, fisheries, aquaculture, and socio-economic development. Since 1985, the international trade in exports of ornamental fish, which usually takes place in the majority of developing countries, has followed an increasing trend with an average growth rate of approximately 14% per year. Successful rearing of larval stages of aquatic organisms is a challenge for aquarium hobbyists, as well as an aim and a necessity for the success of aqua culturists. All these specialists will agree that the primary problem in any type of larval rearing is that of food. Ideally, one would prefer to give larvae their natural diet, which is characterized by a wide diversity of nutritious live organisms. Live feed is an essential food source for the fry of cultured species; especially those without a fully developed digestive system" (Arulrasu and Munuswamy, 2009). Ornamental fish also derive the name "life jewels" because of their ornamental values. That means it must be beautiful and decorative, as it helps to beautify our living environment. "In other words, the coloration of ornamental fish plays the most important role. The pigment of ornamental fish is one of the most important quality criteria dictating their market value. Like other animals, fish are unable to perform de novo synthesis of carotenoids (dietary carotenoids)" [1]. "*Amaranthus dubius* is one of the most widely cultivated plants, and it contains one of the most concentrated natural sources of nutrition for all mammals. Early interest in the *Amarathus* species focused mainly on its potential as a source of protein and vitamins" [2]. "The composition of dried *A. dubius leaves* powered has 34% protein. 16% vitamin A, 1% vitamin C, and 6% calcium 4% iron, 3% vitamin B6, carotinoids, and so on. *Amaranthus* species leaves contain the essential amino acid lysine, which is deficient in wheat and barley. Several studies have shown that amaranth leaves may be of benefit for those with hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels while improving antioxidant status and some immune parameters" [3]. "In this regard, *Amaranthus dubius* holds promise as a possible substitute for fish meal or feed additive along with fish meal; however, there is no feed intake, growth, coloration, leucocyte count, or reproduction in ornamental fishes. Hence, the present investigation has been carried out to analyse the impact of different levels of the *Amaranthus dubus* diet on growth, coloration, leucocyte count, and reproduction in peacock cichlids (*Aulonocara hansbaenschi*). *Amaranthus* species are a highly popular group of vegetables that belong to different species" [4], (Grubbens, 1977; Grubbens and van Sloten, 1981; Sauer, 1976). "The vegetable amaranth has been used in China for over 400 years, yet is commonly found in the Caribbean and Africa; grain amaranth was cultivated and revered by the Aztecs in Mexico, the Mayas in Central America, and the Inca in South America" (O'Brien and Price, 2008).The present study was to study the different levels of *A. dubius* leaves on growth and carotenoid Improvements in fresh water ornamental fish peacock cichlid.

2. MATERIALS AND METHODS

2.1 Experimental Fish

Peacock Cichlid (*A. hansbaenschi*) juveniles in fresh water have a mean body length of 1.97± 0.12 cm and a mean body weight of 0.17± 0.01 g. were divided into five groups, each consisting of 15 individuals. Throughout the experiment, they were housed in 35-liter water-capacity troughs with 25-liter water. Three replicate troughs were randomly assigned to dietetic supplementation of *Amaranthus dubius* feed intake and Peacock Cichlid (*Aulonocara hansbaenschi*) growth coloration.

2.2 Collection and Preparation Ingredient

Fresh leaves of *A. dubius* were collected from the Kulathur agricultural area. The leaves were washed with tap water to remove them, and other debris was drained properly and dried at room temperature (30°C - 32°C). These were ground with a kitchen blender to powder and kept in an airtight container until needed.

2.3 Diet Formulation and Preparation

“The feed formulation was done by the square method (Hardy et al. 1980), and a 40% basal protein diet was prepared using ingredients like Fish meal, rice bran, ground nut oil cake, and wheat. First, dried and powdered ingredients were blended to make a homogenous mixture. Subsequently, the feed ingredients were mixed with an aliquot of boiled water and then cooked in steam for 20 minutes. Five diets were prepared with different levels of *A. dubius* leaves: 0%, 2.5%, 5%, 7.5%, and 10%. The pellets (2 mm in size) were made with a hand-operated pellet press and sun-dried. The dried diets were prepared and stored in a refrigerator until use”. Lowers et al. (1951) used a spectrophotometer to determine the protein and lipid content of experimental diets.

2.4 Temperature

Although the fish can grow and culture in a wide range of water temperatures, the ideal temperature for breeding is between 25°C and 280°C Temperature.

2.5 Oxygen & P^H

The dissolved oxygen levels in water should be maintained above 5 mg/l; they will tolerate much lower dissolved oxygen for a short period of time. The ph should be kept as close to 7 as possible, though fish may tolerate a P^H range of 7 to 8.5.

Table 1a. Formulation and percentage composition of experimental diets

Ingredients (g/100gdiets) Levels of *Amaranthus dubius* (g)	**Levels of *Amaranthus dubius*** 0	2.5	5	7.5	10
Fish meal	38.25	36.25	38.25	38.67	38.23
Groundnut oilcake(g)	26.75	25.75	24.75	23.75	22.75
Rice bran (g)	14.25	13.25	12.25	11.25	10.25
Tabico powder (g)	12.25	10.25	9.25	8.25	7.25
Wheat flour	9.23	13.23	12.29	11.25	12.25

Table 1b. Proximate composition

Protein (%)	39.08±0.11	38.86±1.98	38.25±1.23	37.92±2.22	37.08+1.53
Carbohydrate	14.67e±1.01	14.01 ±0.86	13.48±1.41	12.76 ±0.11	14.63±0.33
lipid	7.86±1.05	8.43±1.11	8.31 ±0.96	7.20±1.43	7.11±1.48
ash	18.37±2.23	17.7±2.11	16.96±2.03	15.12±1.88	16.18±1.98

Biological Evaluations:

Feeding rate (Mg/g live fish /day) $=\frac{\text{Total feed consumped (MG)}}{\text{intial weight of fish (g)XNo Of days}}$

Mean body weight (g) $=\frac{\text{Wet Weight of fish(MG)}}{\text{NO.of fish in the tank}}$

Specific growth rate SGR%== $\frac{\text{In Wt1} - \text{in wt0}}{\text{t1}}$

Feed conservation ratio (FCR) $=\frac{\text{feed consumed (g)}}{\text{wet weight gained (g)}}$

2.6 Chemical Analysis

2.6.1 Estimation of carbohydrates

"The colorimetric method described by DuBois et al. [5] is the most used to date for the determination of the concentration of carbohydrates in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives".

2.6.2 Estimation of protein

Lower et al. [6] used the following method to calculate the protein content of the experiment diets. A known weight of about 100 mg of tissues was homogenized in 2 ml of 10% TCA and 8 ml of distilled water and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. The precipitate was dissolved in 5 mL of sodium hydroxide and incubated for 10 minutes at 60°C-70°C. 0.5 ml of the solution was taken in a test tube, and 4.5 ml of reagent D was added and mixed well. The mixture to stand for 10 minutes. To the sample, 0.5 ml of Folin-Ciocalteau reagent was added. The test tubes were incubated for 30 minutes at 37°C and the optical density was measured at 640 nm. Then the concentration of protein in 1 gram of tissue was calculated.

2.6.3 Estimation of lipid

This method was used to calculate the lipid content of the experiment diets (Bragdon, 1951). A known weight of about 15 mg of the sample tissue was grounded well with a few ml of chloroform, and the solution was centrifuged. The superant was evaporated to dryness, and then 3 ml of 2% potassium dichromate in concentrated sulfuric acid was added, followed by 3 ml of distilled water. The developed colour was read in a spectrophotometer using a 640 nm filter

2.6.4 Estimation of Carotenoid content

Total carotenoids in the fins, skin, and muscle of the peacock cichlid (*A. hansbaenschi*) were analysed. The skin and muscle were kept separately in small packets and stored in a deep freezer. Tissues were cut into small pieces and homogenized three times in one hour in an issue homogenizer, with 20 ml of acetone-carotene content measured in a UV spectrophotometer [7].

2.7 Statistical Analysis

The results obtained in the present study were subjected to statistical analysis (Mean ± SD) SPSS 13.5 was used for statistical analysis. A one-way ANOVA was applied to find out the significant differences among average values of total carotenoid content, and the difference between the mean treatments was tested with the turkey's test (Zar et al. 1986).

3. RESULTS

An increase in *A. dubius* leaves with a base diet of 40% protein increased the protein contents, while lipid ash and nitrogen-free extract showed the opposite trend.In general, the diet's water stability decreased over time (Table 2). Diet variation was found to influence the growth responses of peacock cichlids (*A. hansbaenschi*) fed with control and experimental diets at 0 and 45 days. However, the overall growth registered a definite trend. The mean body length and weight of Peacock Cichlid (*A. hansbaenschi*) increased with an increase in *A. dubius* leaves at the 0%, 2.5%, and 5% 7.5% and 10%dites were 74.46%, 76.43%, 76.93%, 77.66%, and 77.67% for 1 hour, respectively (Table 2).The average stability of experimental diets was 77.46% in the 7.5% and 10% diets, which appeared to be more stable than other diet levels (0% - 5%) (Table 2). The mean body length and weight, weight gain, and specific growth rate of peacock cichlids (*A. hansbaenschi*) with increased dubious levels up to 5% and declined thereafter. Fish fed the 5% *A. dubius* diet had greater mean body length and weight, weight gain, and specific growth rate than fish fed other *A. dubius* diets. (0,2.5,7.5,10). However, the growth parameters of fish fed with a 5% *A. dubius* diet did not differ significantly ($P > 0.05$) as compared to fish fed with 7.5 and 10% *A. dubius* diets, but they significantly (P 0.05) differed with 0 % and 2.5% *A. dubius* diets (Table 3). Fish fed with 7.5 and 10% *A. dubius* diets exhibited the maximum feed consumption. Feed consumption increased with the increase in *A. dubius* levels. An opposite trend was observed in the feed conversion ratio. However, feed consumption of fish fed with a 5% *A. dubius* diet did not differ significantly ($P > 0.05$) as compared to fish fed with a 7.5% and 10% *A. dubius* diet, but significantly (P 0.05) differed with a 0% and 2.5% of *A. dubius* diet. The protein and ash contents increased in Peacock Cichlid (*A. hansbaenschi*) with *A. dubius* levels in the diet. However, the trend was reversed in lipid content. The drop in lipid content was greatest in fish fed a

10% *A. dubius* diet versus those fed other diets (7.5%, 5%, 2.5%, and 0%).However, the lipid levels of the fish fed with 10% *A. dubius* declined but did not differ significantly (P > 0.05) from those of fish fed with other levels of 0-7.5% *A. dubius*. The nitrogen-free extract of fish fed with 7.5 % *A. dubius* was lower as compared to other levels (0 %, 2.5 %, 7.5 %,10% and *A. dubius* diets).The total carotenoid content in the skin and muscle of the Peacock Cichlid (*A. hansbaenschi*) increased as *A. dubius* levels increased (Table 4). Maximum carotenoid content was observed in fish consuming 10% *A.dubius* in all two tissues (skin and muscle), while the control group elicited low carotenoid content. Duncan's multiple range Tests showed that carotenoid content between *A. dubius* treatments differed significantly (p 0.05), with better values in fish fed with 10% *A. dubius* diets. However, the carotenoid content of fish fed with a 10% *A. dubius* diet was significantly (P 0.05) different as compared to fish fed with other levels (0, 2.5, 5, and 7.5%) of *A. dubius* diets.

Table 2. Water Stability (%) of Experimental Diets

Time minutes	***Amaranthus dubius* levels (%)**				
	0	**2.5**	**5**	**7.5**	**10**
10	92.56a ± 2.23	92.85a ± 2.04	92.89a ± 2.42	92.92ab ± 1.20	92.97 ab± 1.33
20	91.05a ± 1.86	91.68ab ± 0.84	92.83b ± 1.22	92.89b ± 1.43	93.02b ± 2.04
30	87.05a ± 2.25	90.18b ± 1.52	91.26bc ± 1.66	91.43c ± 2.11	91.86c ± 1.33
40	81.15a ± 2.10	85.11b ± 0.76	85.67b ± 1.66	85.92bc ± 2.01	85.90bc ± 1.66
50	78.74a ± 1.23	80.26b ± 0.63	81.24c ± 1.01	81.66c ± 0.81	82.02cd ± 2.60
60	74.46a ± 2.01	76.43b ± 1.66	76.93c ± 0.94	77.24c ± 1.11	77.67c ± 1.04

Values (Mean ±SD) with different superscripts in the same row are significantly different (p>0.05)

Table 3. Effect of different levels of *Amaranthus dubius leaves* on growth Peacock Cichlid (*Aulonocara hansbaenschi*)

Levels of *Amaranthus dubius* leaves	Mean body length (cm)		Mean body weight (g)		Weight gain wet weight	Specific growth rate (SGR) %day
	Initial 1 st day	Final 45th day	Initial 1 st day	Final 45th day		
0	2.1±0.34	3.6a±0.86	0.17±0.04	0.89a±0.09	1.33a±0.25	0.505a±0.11
2.5	2.1±0.34	3.9a±0.16	0.17±0.04	0.95ab+0.08	1.46b±0.14	1.066b±.0.05
5	2.1±0.34	4.8b±0.89	0.17±0.04	0.99b±0.26	1.86c±0.40	1.213c±0.12
7.5	2.1±0.34	4.7b±0.86	0.17±0.04	0.94ab±0.28	1.51b±0.21	2.406d±0.23
10	2.1±0.34	4.8b±0.68	0.17±0.04	0.92a±0.22	1.48b±0.16	3.198e±0.23

Values (mean± SD) with different suprscriptes in the same coloumn are significantly different (p<0.05) between same days

Table 4. Effect off feeding different levels of *Amaranthus dubius* on carotenoid contents skin and muscle of the peacock cichlid species

Levels of *Amaranthus dubius* leaves	**Total Carotenoid**			
	Skin		**Muscle**	
	Initial (1st day)	**Final (45th day)**	**Initial (1st day)**	**Final (45th day)**
0	0.011±0.001	0.022a±0.001	0.003±0.001	0.009a±0.001
2.5	0.011±0.001	0.026a±0.004	0.003±0.001	0.016b±0.003
5	0.011±0.001	0.047b±0.013	0.003±0.001	0.024c±0.008
7.5	0.011±0.001	0.062c±0.011	0.003±0.001	0.038d±0.008
10	0.011±0.001	0.071cd±0.008	0.003±0.001	0.044d±0.011

4. DISCUSSION

The present investigation reveals that fish fed with a 5% *A. dubius* diet elicited the maximum. Feeding parameters (feed consumption and feeding rate) and growth parameters mean body length and weight, weight gain, and specific growth rate) may be due to the highest amount of 40.96 and the stimulatory effect of the *A. dubius* diet. The overall stability of experimental diets was averaged. 77.46% during the 1 hr. of feeding. The results of the present study in the case of *A. dubius* indicate that there are more suitable binders for this type of feed, and pellets prepared with these binders have shown good water stability for a period of 1 hour. The protein and ash contents of species increased with the addition of *A. dubius* leaves to the diet. It suggests that *A. dubious* contains a high concentration of essential amino acids, which are concentrated in peacock cichlid bodies. (Gopalan 2007). “It is plausible that the anti-nutritional factors impaired the absorption of some essential amino acids in the diets containing ALPC, thus depressing fish growth at high levels of dietary ALPC inclusion. It has also been observed that is the major phosphorus storage compound in leafy vegetables and chelates multivalent metal ions such as Zn, Ca, and Fe, thus affecting their bioavailability” [8]. “A higher stocking density of fish resulted in a pH decrease in the aquaporin system, which is detrimental. Autotrophic bacteria were said to have an optimum growth rate in the pH range of 7.2 and 8.5, while a pH of 7 is suitable for the seed germination of *Amaranthus* species” [9]. “Plant-parasitic nematodes can be reduced by poor or non-host cover crops when applied in soil as green manure, intercrop or rotation crop. It is therefore important to assess host suitability since certain cover crops can enhance PPN densities. An ideal cover crop should not affect yield of the host plant. In addition, cropping practices, amount of cover crop, environmental and edaphic factors should also be considered” [10]. “The inclusion of amaranth in diets affected the proximate composition of *Biceps femoris* meat. Thus, higher moisture values corresponded to the A0 treatment, while CP was higher in A16 and A32 diets including *A. dubius*. Higher ash content corresponded to the A16 treatment, while higher fat content was observed in the A32 diet. Differences observed in the proximate composition of meat could be mainly related to the proportion of fat in the diets” Fernández and Fraga [11,12-23].

5. CONCLUSION

Based on the result of the present investigation, it was observed that among the binding materials, 2.45% tapiaco with 5, 7.5, and 10% *Amaranthus dubius* leaves meals were found to have good binding capacity, but the 5% *A. dubius* diet produced more growth. In other words, carotenoids may help to integrate the empirical and theoretical perspectives underlying the bewildering diversity of colourful fishes, especially cichlids. Also, one of the greatest challenges in the ornamental fish industry is to replicate the accurate natural color of the fish in a captive environment. Numerous operations have been tailed in order to successfully market the fish due to faded colour. Various products have been introduced to alleviate this problem, but none has performed as effectively and consistently as carotenoid pigment. Varieties of carotenoid pigments are used in fish diets for colour enhancement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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