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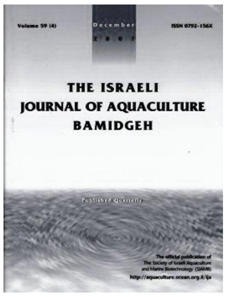

Vegetable Products as Dietary Pigment Sources for Juvenile Goldfish, *Carassius auratus*

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Key words: goldfish, *Carassius auratus*, plant ingredients, total carotenoids, pigmentation, growth performance

Abstract

Amaranthus leaf meal, moringa leaf meal, and red chili powder were chemically analyzed and evaluated as carotenoid sources in diets for juvenile goldfish, *Carassius auratus*. The fish readily accepted all test diets, indicating good palatability of the tested ingredients. In a 56-day growth trial, all growth performance indicators except survival significantly varied (p<0.05) between dietary treatments. The highest weight gain (1.19 g) was achieved by fish fed the diet containing krill meal (positive control diet), but this weight gain did not significantly differ from that of fish fed the diet containing red chili powder. Total carotenoids were significantly highest (p<0.05) in fish fed the diet containing red chili powder (2.07 µg/g) and very low in fish fed the diet containing no pigment supplement (negative control). Growth, skin coloration, and total carotenoids in the tissue were satisfactory in fish fed the diets containing amaranthus or moringa leaf meal.

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Introduction

Goldfish (*Carassius auratus*) is a widely cultured and commonly traded ornamental fish in the global aquarium business (Oliver, 2001). Color is a major factor that determines the quality and market value of goldfish (Saxena, 1994). Goldfish must appeal to the eye with its reddish-orange pigmentation to obtain consumer acceptance and an attractive price. Such skin pigmentation derives from carotenoids deposited in tissue chromatophores (Simpson et al., 1981). Skin colors in fish are predominantly dependent on carotenoids, a group of over 600 natural lipid-soluble pigments that are primarily produced in phytoplankton, algae, and plants. Carotenoids are absorbed from diets, transformed into other carotenoids if necessary, and incorporated into tissues. Carotenoids are vital for healthy growth, metabolism, and reproduction, as well as color, in fish (Miki, 1991). Like other animals, fish are unable to synthesize carotenoids *de novo* (Goodwin, 1984) and must obtain them from dietary sources.

When in captive culture conditions, fish skin often has faded coloration due to poor availability or absence of dietary pigments. The fish diet, therefore, must be supplemented with adequate carotenoids. Synthetic astaxanthin and canthaxanthin are widely used as supplements in diets for goldfish and other ornamental fishes to induce the desired coloration. Considering cost, sustainability, and poor digestibility of the synthetic colorings, natural compounds from organic sources such as red yeast (*Phaffia rhodozyma*; Bon et al., 1997; Xu et al., 2006), the marine bacteria *Agrobacterium aurantiacum* (Yokoyama and Miki, 1995), and spirulina (Alagappan et al., 2004) have been suggested as cheaper sources of dietary carotenoids for fish. Vegetable products such as red pepper (*Capsicum annum*), marigold flower (*Tagetes erecta*), leafy greens, *Amaranthus* sp., carrots (*Dacus carota*), and moringa leaf meal have rich and abundant carotenoid pigments and could be alternative sources of pigments for fish (Boonyaratpalin and Unprasert, 1989; Yanar et al., 1997, 2007; De La Mora et al., 2006; Aguirre-Hinojosa et al., 2012). Farmed vegetable products can serve as cost-effective sustainable sources of pigments for large scale production of goldfish. In this study, we evaluate the potential of amaranthus leaf meal, moringa leaf meal, and red chili powder as feed ingredients and sources of pigments in diets for the goldfish, *C. auratus*.

Materials and Methods

Test ingredients. Test ingredients were collected from a local market and proximate composition, gross energy, and total carotenoid contents were analyzed. Except for red chili, the ingredients were dried in a convection hot air oven for 15 h at 40°C, finely ground in a dry masala grinder, passed through a 200-μ sieve, and stored in airtight containers in a refrigerator until use.

Diet preparation. The computer program, Feed Soft Professional (Version 3; Feedsoft Corp., USA), was used to formulate five isoproteic test diets adapted to cyprinids: (a) a diet without supplementary pigments (negative control), (b) a diet with krill meal, which has been proven as a supplementary pigment source (positive control), and diets containing (c) amaranthus leaf meal, (d) moringa leaf meal, or (e) red chili powder (Table 1). All ingredients except fish oil and the vitamin/mineral premixes were blended and finely ground to particle sizes less than 200 micron. These were mixed with an adequate amount of water to produce a dough. The dough was cooked in a laboratory autoclave for 15 min at 100ºC, cooled, blended with the fish oil and premixes in a kitchen food processer, and pelletized with a 2-mm die kitchen noodle maker. The pellets were dried in a convection oven dryer at 55ºC for 4 h, broken into crumbles of 1-1.8 mm diameter/length, and stored in plastic containers under refrigerated conditions until use.

Proximate and total carotenoid analyses. Proximate compositions and gross energy of the test ingredients and diets were determined according to procedures described in AOAC (2007). Samples were analyzed in triplicate. Total carotenoids of the ingredients and feeds were estimated according to the method described by Cyanotech (2002). Dried test ingredients of 250 mg, extracted using acetone and absorbance, were read spectrophotometrically at 480 nm. Amaranthus had the highest protein contents, while red chili powder had the highest crude lipid and crude fiber (Table 2).

Table 1. Ingredients and proximate compositions of diets containing supplementary pigments for juvenile goldfish (*Carassius auratus*).

	Diet (supplementary pigment)				
	No supplement (negative control	*Krill meal (positive control)*	*Amaranthus leaf meal*	*Moringa leaf meal*	*Red chili meal*
Ingredient (g/kg)					
Fishmeal	40.90	37.34	39.56	39.36	42.15
Soybean meal	10.00	10.89	14.50	18.93	12.25
Peanut meal	8.00	12.94	8.00	8.00	6.00
Rice (broken)	15.16	5.00	5.10	5.00	10.00
Corn	9.42	10.00	4.00	4.50	1.00
Wheat flour	10.00	5.00	4.00	4.00	4.40
Fish oil	1.00	1.00	1.00	1.00	1.00
Sodium alginate	1.00	1.00	1.00	1.00	1.00
Vitamin premix	2.03	1.00	2.48	1.81	0.47
Mineral premix	0.50	0.50	0.50	0.50	0.50
Salt	2.00	2.00	2.00	2.00	2.00
Krill meal	0	13.33	0	0	0
Amaranthus leaf meal	0	0	17.86	0	0
Moringa leaf meal	0	0	0	13.89	0
Red chili meal	0	0	0	0	19.23
Proximate composition (%)					
Dry matter	94.29	96.68	96.19	96.59	94.86
Crude protein	44.29	44.99	45.66	45.54	44.97
Crude fiber	3.46	1.84	2.71	2.42	4.8
Ether extract	4.56	5.33	6.35	4.1	6.52
Total ash	9.4	11.43	12.1	12.25	10.42
Nitrogen free extract	38.29	36.41	33.18	35.69	33.29
Gross energy (kcal/kg)	100	100	100	100	100
Carotenoids total (mg/g)	Negligible	0.56	0.48	0.52	0.59

Experimental design. The feeding trial was carried out in an indoor system consisting of 15 circular 30-l plastic tanks. A red variety of goldfish fry was obtained from a local commercial breeder, kept under quarantine for three weeks, then acclimatized to the experimental conditions for two weeks before the trial. During this period, the fish were fed the control diet without supplementary pigments *ad libitum*.

Fish of the same size, color hue, and initial weight (0.5 g) were selected. All were about 45 days of age. Each tank was stocked with 10 fish, randomly allotted to triplicate tanks for each dietary treatment. The fish were fed by hand twice daily (08:00 and 17:00) at 5% of their body weight, evenly divided into two rations, for 56 days.

Fish were sampled fortnightly to assess survival, growth, and general healthiness. Aeration was supplied by airstone. Water was exchanged at 30% each alternate day. Uneaten feed and feces were siphoned out daily. Temperature, pH, and dissolved oxygen were measured daily. Temperature ranged 24-25°C, dissolved oxygen was kept above 4.3 mg/l, and pH was around 7.2. The photoperiod was kept constant by fluorescent lamps (12 h L:12 h D).

Total carotene estimation of fish tissue. At the start and end of the trial, total carotenoids of whole fish tissue were extracted and estimated as described by Olson (1979). One gram of fish tissue was extracted with chloroform. Total carotenoids were analyzed by reading the color intensity of the extract spectrophotometrically at 450 nm.

Statistical analysis. Data on growth performance and carotenoid contents were analyzed by one-way ANOVA. Significant differences (if present) were ranked with Duncan's multiple comparison test at a 5% significance level using the computer program SigmaPlot 12 for Windows.

Table 2. Proximate composition (%), gross energy, and total carotenoids of the dietary vegetable ingredients in diets for goldfish (*Carassius auratus*).

	Ingredient		
	Amaranthus leaf meal	*Moringa leaf meal*	*Red chili powder*
Dry matter	90.49±0.03	93.48±0.02	91.28±0.02
Crude protein	27.95±0.02	21.66±0.17	12.35±0.03
Crude fiber	6.86±0.04	8.3±0.05	24.34±0.02
Ether extract	2.22±0.03	6.95±0.04	10.58±0.01
Ash	15.54±0.01	12.48±0.01	5.15±0.01
Nitrogen free extract	47.43±0.03	50.69±0.14	47.59±0.05
Gross energy (kcal/kg)	3579.67±1.53	3976.67±2.08	4225.00±3.00
Total carotenoids (mg/g)	1.68±0.02	2.16±0.03	1.56±0.04

Results

Fish readily accepted all five diets, indicating good palatability of the test ingredients. After 56 days, survival was 100% in all treatments. Fish fed the positive control (krill meal) had the highest final weight,

weight gain, and specific growth rate (SGR), and the lowest feed conversion ratio (FCR; Table 3). Differences in skin color were significant (Fig. 1).

Table 3. Growth performance and feed efficiency of goldfish fed diets containing various pigment sources.

	Diet (supplementary pigment)				
	No supplement (negative control)	*Krill meal (positive control)*	*Amaranthus leaf meal*	*Moringa leaf meal*	*Red chili meal*
Initial wt (g)	0.52±0.02	0.54±0.03	0.55±0.02	0.54±0.03	0.54±0.02
Final wt (g)	1.40±0.04[a]	1.73±0.03[c]	1.62±0.05[b]	1.58±0.07[b]	1.72±0.04[c]
Wt gain (g)	0.88±0.06[a]	1.19 ±0.05[c]	1.07±0.03[b]	1.04±0.09[b]	1.18±0.02[c]
Specific growth rate (%)	2.20±0.15[a]	2.60±0.15[b]	2.41±0.03[ab]	2.40±0.19[ab]	2.58±0.05[b]
Feed consumed (g)	1.61±0.05[a]	1.81±0.04[b]	1.86±0.03[b]	1.84±0.01[b]	1.88±0.06[b]
Feed conversion ratio	1.84±0.17[c]	1.52±0.10[a]	1.73±0.03[abc]	1.78±0.16[bc]	1.59±0.05[ab]
Protein efficiency ratio	1.23±0.11[a]	1.46±0.10[b]	1.27±0.02[a]	1.24±0.11[a]	1.40±0.04[ab]

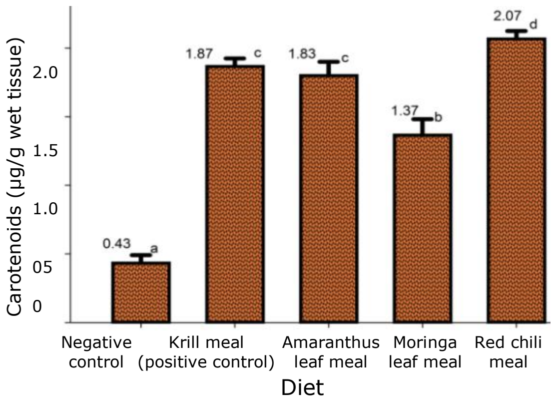

Fig. 1. Total carotenoids in whole tissue of juvenile goldfish fed diets containing different pigment sources for 56 days.

Discussion

The present study demonstrates that the selected plants are rich in carotenoids and their dietary inclusion can influence growth and skin pigmentation in the goldfish *C. auratus.* In any food chain, plants and other photosynthetic organisms such as algae, bacteria, and fungi produce carotenoids that supply the needs of animals in higher trophic levels (Armstrong and Hearst, 1996). Accordingly, marine products such as shrimp meal (Diler and Gökoglu, 2004) and krill meal have been evaluated as pigment sources in diets for ornamental fish (Yılmaz S. and S. Ergün, 2011) and trout (Roncarati et al., 2011; Hernández et al., 2012).

In addition, the red yeast *Phaffia rhodozyma* (Bjerkeng et al., 2007), the marine bacteria *Agrobacterium aurantiacum* (Yokoyama and Miki, 1995), the green algae *Haematococcus pluvialis* (Harker et al., 1996), spirulina powder (Yeşilayer et al., 2010), red pepper (Yılmaz and Ergün, 2011), and marigold flower (Yanar et al., 2007) have been tested as dietary sources for pigmentation of the skin and/or muscle with different results. These sources are natural in origin, are not grown in an industrial manner, and have yet to be priced competitively. Red chili is produced in enormous quantities, and grows in all but the coldest regions. Other plant materials that are rich in carotenoids could be considered as alternatives to the above ingredients, providing flexibility to farmers and feed manufacturers in choosing ingredients for feed formulation.

The differences in growth performance and skin pigmentation could be attributed to different levels of total carotenoids in the test diets. Differences in skin coloration may be due to interactions of the fish to the supplements. The skin of goldfish contains a variety of pigments including esters of xanthophylls, astaxanthin, lutein, and zeaxanthin in different proportions; fish interact differently with each substance, displaying unique color patterns (Hata and Hata, 1971). In this study fish fed krill meal or red chili meal had a brilliant skin color and a higher total carotenoid carcass content. The better performance of krill was expected, since it is a well-documented aquafeed ingredient in terms of attraction, palatability (Shimizu et al., 1990), pigmentation (Storebakken, 1988; Yoshitomi et al., 2006), and growth performance (Hansen et al., 2011). However, the study of red chili as a source of carotenoids is limited with respect to goldfish although red pepper in fish feeds shows promising results (Yanar et al., 1997; De La Mora et al., 2006).

Color development in fishes is directly related to the amount and nature of carotenoids in the dietary ingredients (Boonyaratpalin and Unprasert, 1989). In our study, skin pigmentation and total carotenoid content of the whole fish tissue were strongly related. As all the test diets were formulated to contain a similar carotenoid level, the similar skin color and tissue carotenoid in fish fed the krill meal or red chili meal indicate that expensive krill meal can be replaced by inexpensive whole red chili meal. The better color development in these two treatments could be attributed to a good combination of pigments and/or better availability of the pigments to the fish. Amaranthus and moringa leaf meals are even less expensive sources of protein and carotenoids. Thus, further research into these leaf meals is warranted, even though the growth and pigmentation in fish fed these feeds were moderate.

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