

Research Article

Effects of Feeding Diets Containing Different Levels of Shrimp Shell Waste Meal on Growth and Colouration of Koi Carp (*Cyprinus rubrofuscus* Lacepède, 1803)

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Abstract

Astaxanthin is a xanthophyll carotenoid which is found in various microorganisms and marine animals. The shell of marine shrimp, *Aristeus alcocki* is a cheap source of astaxanthin. An attempt has been made in the present study to increase the coloration of koi carp (*Cyprinus rubrofuscus*) by feeding diets with different levels of shrimp shell waste meal (SSWM) prepared from *A. alcocki*. Four different dietary treatments with varying percentage of SSWM: 0% (F1, control), 5% (F2), 10% (F3) and 20% (F4) were used for the study. The feeding was done for 90 days at the end of which fish were evaluated for growth and colouration. The results indicated that the weight gains in fish fed different levels of SSWM were not significantly different from weight gain in fish fed control diet. The results of the feeding study also demonstrated that feeding diets containing shell waste from *Aristeus alcocki* enhanced colour of koi carp and increased the carotenoid content in the skin of the fish. It may be concluded that shell waste of *A. alcocki* could be used as an efficient protein source to replace costly fish meal in the diet of *C. rubrofuscus*. Moreover, inclusion of shrimp shell waste as a natural carotenoid source in the diet of *C. rubrofuscus* can ensure appealing skin colour and hence can increase consumer acceptability and market value of the fish.

Keywords

Koi Carp, Shrimp Shell Waste Meal, Astaxanthin, Carotenoid Content, Skin Colour, Weight Gain and Pigmentation

1. Introduction

Ornamental fishes attract and fascinate people of all ages, making fish keeping one of the most popular hobbies worldwide by hobbyist, aquarists, and fish enthusiasts. It is easy, pleasing to the eyes and a stress relieving hobby. This hobby of ornamental fish keeping has shown a gradual increase in the ornamental fish trade globally and is increasing day by day. Asia is the biggest exporter of ornamental fish. India's share in ornamental fish trade is estimated to be Rs.158.23 lakhs

which is only 0.008% of the global trade [1].

The Koi carp, *Cyprinus rubrofuscus*, are members of the family Cyprinidae, or minnow family. They are colorful, ornamental versions of common carp (*Cyprinus carpio*). They were developed approximately two hundred ago in Japan and are characterized by a wide diversity of colours and colour patterns [2, 3]. Majority of fishes are vividly and brightly coloured. Color is a major factor that determines the value of

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ornamental fish in the global market. The fish tend to lose their color by fading when maintained in captivity, and this decreases their market value. Fish skin colouration is mainly due to the presence of chromatophores that contain pigments including melanin, pteridines, purines and carotenoids. Fish are capable of producing some pigments. But fish, like other animals, are unable to synthesize carotenoids [4-6]. The carotenoid pigmentation of fish results from the pigment present in the diet. So, skin color is highly dependent on the carotenoids present in the diets of most fish. Hence, a direct relationship between dietary carotenoids and pigmentation exists in them [7, 8]. Exploration of various methods to enhance the vibrant colouration in ornamental fishes are being sought by people involved in the ornamental fish trade. Many studies have proved that the fish can be pigmented by dietary supplementation of carotenoid sources. Astaxanthin is a xanthophyll carotenoid which is abundant in marine animals, such as salmon and shrimp. The shell of Arabian red shrimp *Aristeus alcocki* is a rich source of astaxanthin [9]. The present work is on the effects of feeds containing different levels of shrimp shell waste meal (SSWM) prepared from the shell of *Aristeus alcocki* on growth and colouration of koi carp, *Cyprinus rubrofuscus*.

2. Materials and Methods

2.1. Preparation of Shrimp Shell Waste Meal (SSWM) from *Aristeus alcocki*

Shell waste from the deep sea shrimp *Aristeus alcocki* was collected from a processing plant at Cochin, cleaned and dried under shade. The dried shell was finely powdered and kept in an airtight container in a refrigerator till used.

2.2. Preparation of Feeds

The ingredient composition of the feeds containing different levels of shrimp shell waste meal is given in Table 1. Fish meal was prepared by drying and powdering a cheap quality fish (*Chanda nama*). Groundnut oil cake (GOC) was purchased from the market and powdered. Rice bran was purchased from the market. Tapioca flour was prepared from raw tapioca by drying and powdering it.

Table 1. Ingredient composition of feeds.

Ingredients	F1 (Control)	F2	F3	F4
Shrimp shell waste meal (SSWM)	0 g	5 g	10 g	20 g
Fish meal	40 g	35 g	30 g	20 g
GOC	25 g	25 g	25 g	25 g
Rice bran	25 g	25 g	25 g	25 g

Ingredients	F1 (Control)	F2	F3	F4
Tapioca flour	10 g	10 g	10 g	10 g

The ingredients except shrimp shell waste meal were weighed and mixed in a suitable container with 100 ml of water to get a dough of good consistency. It was then autoclaved at ambient pressure for 30 minutes. The dough was cooled and mixed with shrimp shell waste meal. Each feed was then extruded through a noodle making machine and dried at 60 °C in a hot air oven for 12 hours. Each feed was stored in labeled airtight containers.

2.3. Feeding Study

The feeding study was conducted in aquarium tanks located indoors having uniform day light. Uniform sized koi carp fingerlings obtained from the larval rearing facility of the department of aquaculture at S H College were used for the feeding study. The fish were distributed randomly among 12 aquarium tanks (60 x 30 x 30 cm) at a stocking density of 10/tank, with treatments in triplicates, also arranged at random. Each tank contained 30 litres of aerated freshwater. They were acclimatized in the tanks for one week while being fed with control diet. Fish from each tank was weighed collectively and mean initial weight was calculated at the beginning of the feeding experiment. Fish were fed ad libitum in the morning and evening. Faeces and feed remnants were removed by siphoning before fresh feed was given. One third of water in each tank was replaced daily and it was fully replaced once in a week. Weekly measurements showed that dissolved oxygen, pH, and temperature ranged from 6.3 to 7.5 mg/l, 7.8 to 8.3 and 28 to 30 °C, respectively. The fish were fed for 90 days, at the end of which the fish from each tank was weighed collectively to determine the final mean weight. The data was used to calculate the mean gain in weight of fish of each tank.

2.4. Evaluation of Skin Colour and Carotenoid Content in Skin

The colour of the fish from each tank was visually assessed. Carotenoid pigments from the skin (including scales) of fish from each treatment was extracted using acetone and determined by measuring the absorbance at 440 nm [10]. For this kept one gram skin including scales into 10 ml acetone in a stoppered tube. The tube was kept in a refrigerator for overnight. Filtered the extract through filter paper, made upto 10 ml and read its absorbance at 440 nm using acetone as blank.

2.5. Statistical Analysis

The data were subjected to analysis of variance and differences determined at 1% level of probability [11].

3. Results and Discussion

3.1. Effects of Different Dietary Levels of SSWM on Growth of *C. rubrofasciatus*

The growth response of koi carp fed different levels SSWM are given in Table 2.

Table 2. Effect of different dietary levels of SSWM on growth of *C. rubrofasciatus* after 90 days of feeding.

Feed No.	%SSWM	Mean initial weight \pm S.D (g.)	Mean final weight \pm S.D (g.)	Mean weight gain \pm S.D (g.)
F1(Control)	0	15.58 \pm 1.17	26.46 \pm 0.99	10.88 \pm 0.20
F2	5	15.70 \pm 0.69	26.78 \pm 1.72	11.08 \pm 1.09
F3	10	17.39 \pm 1.08	29.30 \pm 1.97	11.91 \pm 0.94
F4	20	17.04 \pm 1.86	29.17 \pm 4.33	12.13 \pm 2.46

Values are mean of 3 replicates.

Analysis of variance (Table 3) for different weight gains obtained for different feeds demonstrated that the weight gains were not significant for different levels of SSWM as the calculated F value (0.5526) was much less than the table F value (4.07) at 5% level.

Table 3. ANOVA for weight gains for different levels of SSWM.

Source of variation(SV)	Degrees of freedom(df)	Sum of squares(SS)	Mean sum of squares(MS)	F value	Table F
Between feeds	3	3.3858	1.12860	0.5526	4.07(5%)
Error	8	16.3396	2.04245		
Total	11	19.7254			

The results thus, clearly indicated that the weight gains in fish fed different levels of SSWM were not significantly different from weight gain in fish fed control diet. However, the weight gains were comparable with that obtained for control and showed a slight increase as the level of SSWM increased, though the increase was not statistically significant.

Shrimp shell waste of *Aristeus alcocki* is high in protein [9]. The present study indicated that shell waste from *Aristeus alcocki* could be used as an efficient protein source to replace the costly fish meal in fish feed. This is in agreement with several other studies conducted in other fish species [12].

Utilization of shrimp shell waste as a protein ingredient in aquafeeds can reduce cost of production and minimize environmental pollution caused by its unlawful disposal.

3.2. Effects of Different Dietary Levels of SSWM on Colour and Carotenoid Content in the Skin of *C. rubrofasciatus*

The effects of different feeds on skin color and carotenoid content in the skin of koi carp are given in Table 4.

Table 4. Effects of different dietary levels of SSWM on colour and carotenoid content in the skin of koi carp.

Feed No.	%SSWM	Visual color intensity	Mean carotenoid content in terms of absorbance at 440 nm \pm S.D
F1 (Control)	0	+	0.060 \pm 0.005a
F2	5	++	0.140 \pm 0.005b

Feed No.	%SSWM	Visual color intensity	Mean carotenoid content in terms of absorbance at 440 nm \pm S.D
F3	10	+++	0.210 \pm 0.005c
F4	20	++++	0.2883 \pm 0.005d

Values with different superscripts are significantly different at 1% level.

After feeding for 90 days the fish were visually assessed for their skin color and found that the skin of fish was yellow in color. The extent and intensity of color increased as the level of SSWM in the feeds increased (Figures 1, 2, 3 & 4).

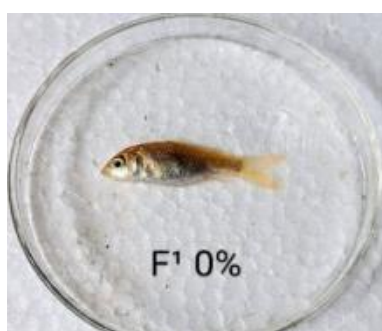


Figure 1. F1 control 0% SSWM.

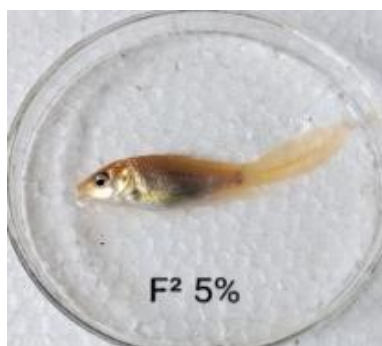


Figure 2. F2 5%SSWM.



Figure 3. F3 10% SSWM.

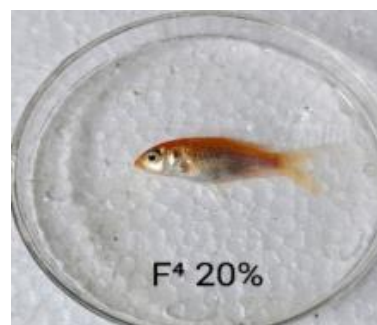


Figure 4. F4 20% SSWM.

The carotenoid content per gram of skin also increased as the SSWM level increased (Table 4). Analysis of variance (Table 5) of the data demonstrated that the carotenoid contents in fish fed different levels of SSWM were significantly different at 1% level. The carotenoid content was the lowest in the fish fed control diet and the highest in fish fed diet containing 20% SSWM. Thus, there was a proportionate increase in skin color and carotenoid content of the skin as the level of SSWM increased in the diet.

Table 5. ANOVA for carotenoid contents for different levels of SSWM.

Source of variation (SV)	Degrees of freedom (df)	Sum of squares (SS)	Mean sum of squares (MS)	F - value	Table F
Between feeds	3	0.085550	0.0285166667	1303.62**	7.59
Error	8	0.000175	0.000021875		
Total	11	0.085725			

**Significant at 1% level. Critical difference 0.0128.

The current study, thus demonstrated that feeding diets containing shell waste from *Aristeus alcocki* enhances color of koi carp and increases the carotenoid content in the skin of the fish. Shell waste of Arabian red shrimp *Aristeus alcocki* is a good source of the carotenoid pigment astaxanthin [9]. Carotenoids are the primary source of pigmentation in the skin of ornamental fishes. But they are unable to synthesize them *de novo* and the fish must get the pigments from their diet. The current study revealed that astaxanthin present in shell waste of *A. alcocki* could enhance colour of the ornamental fish *C. rubrofasciatus* under laboratory conditions. Inclusion of astaxanthin from the microalga *Haematococcus pluvialis* could enhance the colour and growth of red tilapia [13]. The yeast *Phaffia rhodozyma* contains about 85% astaxanthin and is used as pigmentation source in commercial aqua feeds [14]. The present work also shows that astaxanthin from shell waste of *A. alcocki* can serve as a natural carotenoid source in *C. rubrofasciatus* diet to impart appealing color in the fish which in turn can enhance consumer acceptability and hence market value.

4. Conclusion

It may be concluded that shell waste of *Aristeus alcocki* could be used as an efficient protein source to replace costly fish meal in the diet of *Cyprinus rubrofasciatus*. This can not only reduce the cost of production of ornamental fish feed but also minimize hazard to environment caused by unlawful disposal of shrimp shell waste. Moreover, inclusion of shrimp shell waste as a natural carotenoid source in the diet of *Cyprinus rubrofasciatus* can ensure appealing skin color and hence can increase consumer acceptability and market value of the fish.

Abbreviations

SSWM Shrimp Shell Waste Meal
GOC Groundnut Oil Cake

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Conflicts of Interest

The authors declare no conflicts of interest.

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