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Research Article

HIBISCUS PETALS AND SPIRULINA SUPPLEMENTED DIET INDUCED CAROTENOID CHANGES IN FRESHWATER GOLD FISH *CARASSIUS AURATUS*

Beena Somanath* and K. Jayala Jasmin
Department of Zoology, Rani Anna Government College for Women,
Tirunelveli-627 008, Tamil Nadu, India

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Abstract

An attempt has been made to assess the efficiency of Hibiscus petals and Spirulina added diets on variation in biochemical constituents and total carotenoids in skin and muscle tissue of gold fish *Carassius auratus*. For this isonitrous (37% protein) diets (D1 and D2) supplemented with Hibiscus petals (5%) and Spirulina (5%) were prepared individually and offered to the candidate fish in 5% body weight at *ad libitum* for a period of 40 days. Simultaneously a control diet (C) with 37% protein but devoid of neither Hibiscus petals nor Spirulina was also prepared and used for experimentation. The results indicated that in the skin and muscle tissues of candidate fish, the protein, carbohydrate and lipid contents were found to vary between diets and also during various time intervals of experimental period. In Hibiscus petals supplemented diet fed fishes, the skin and muscle protein content varied from 4.65 ± 0.110 to 6.46 ± 0.240 mg/100 mg wet tissue and from 7.41 ± 0.280 to 9.26 ± 0.610 mg/100 mg wet tissue, respectively. Likewise, in Spirulina fed fishes, the skin and muscle protein content varied from 4.65 ± 0.160 to 6.98 ± 0.380 mg/100 mg wet tissue and from 7.41 ± 0.280 to 9.92 ± 0.520 mg/100 mg wet tissue, respectively. But in control diet fed fishes, the skin and muscle protein content recorded was less when compared to experimental diets (D1 and D2) fed groups and here the values registered were: 4.65 ± 0.146 to 5.48 ± 0.168 mg/100 mg wet tissues and 7.41 ± 0.280 to 8.14 ± 0.320 mg/100 mg wet tissues. More or less, a similar trend was noticed for the skin and muscle carbohydrate and lipid contents of experimental fishes, which received D1 and D2 diets. The dietary addition of Hibiscus petals and Spirulina has also influenced the total carotenoid content in the skin and muscle tissues of *C. auratus*. Two-way analysis of variance indicated that both the feed supplement and experimental duration significantly ($P < 0.001$ to < 0.05) influenced the total carotenoid content of skin and muscle tissue of *C. auratus*.

Key words: Hibiscus, Spirulina, Carotenoid, *Carassius auratus.*

INTRODUCTION

Ornamental fishes are acceptable to consumers if they have striking and vibrant colours. Colouration is one of the factors deciding the market value of the ornamental fishes. Carotenoid pigments are responsible for many examples of sexually attractive red, orange and yellow colouration in animals including fishes and play an important role in antioxidant and immune defenses. Colour enhancement through the use of carotenoids in feed has been confirmed by a number of authors (Fey and Meyers, 1980; Ako *et al.*, 2000; Buttle *et al.*, 2001; Kiessling *et al.*, 2003; Alagappan *et al.*, 2004). Ako *et al.* (2000) reported an intense colouration of freshwater red velvet sworal tails (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasana myrnae*) when fed diet containing carotenoid rich strain of *Spirulina plakensis* and *Haematococcus pluvialis*. Wallat *et al.* (2005) used six different commercial diets

*****Corresponding author e-mail: beenapalavesam@gmail.com

and reported different resultant colouration of red oranda variety of gold fish (*Carassius auratus*). Sinha and Asimi (2007) reported that the China rose (*Hibiscus rosasinensis*) petal is a potent natural carotenoid source for gold fish (*Carassius auratus*) to enhance its colour. Ezhil *et al.* (2008) reported an encouraging results on colouration of red sword tails (*Xiphophorous helleri*) fed diet containing marigold petal meal. Numerous studies on the addition of carotenoids to fish diets have been conducted on salmonids. In contrast, a limited number of studies have been conducted on carotenoid additions to diet of ornamental fishes (Hancz *et al.*, 2003; Gouveia and Rama, 2005). Viewing the information provided above, this study was designed to assess the effect of Hibiscus petals and Spirulina supplemented diet on biochemical changes and carotenoid level in the skin and muscle tissues of gold fish, *Carassius auratus*.

MATERIALS AND METHODS

Collection and maintenance of experimental fish

For the present experiment, gold fish *Carassius auratus*, a red variety obtained from a local commercial aquarium centre was kept under quarantine conditions for two weeks and then acclimatized to the experimental conditions. During this period, the fish were fed with control diet (Basal diet) at 5% of their body weight. The fishes with same colour and initial weight of 3.0 ± 0.5g were selected. The fishes were starved for two days before taking the initial weight in order to evacuate their gut contents.

Feed preparation

In the present study, three diets (C1, D1 and D2) were prepared. The ingredients such as fish meal (30.28%), chicken intestine (30.28%), rice bran (19.72%), maida (18.72%) and vitamin and mineral mix (1%) were used for the basal diets. The control diet (C1) was prepared with the above mentioned basal feed ingredients and it was devoid of carotenoid supplement. The experimental diets (D1 and D2) were prepared by the addition of carotenoid feed supplement such as powdered Hibiscus petals and Spirulina at 5% level and were added separately in the basal diet. The dried dietary ingredients were weighed according to the formulation and mixed well by adding sufficient quantity of water and made into dough. The dough was steamed in a pressure cooker for 20 minutes. After steaming, the dough was taken out and then vitamin and mineral mixture and the carotenoid supplementation were added (Hibiscus petals and Spirulina powder) and remixed. Then it was extruded through the pelletizer having a diameter of 1.5 mm die. The controls as well as the experimental diets were dried in room temperature to avoid the carotenoid loss. The dried pellets were stored individually in an air-tight plastic container for further use.

Experimentation

To test the efficiency of Hibiscus petals and Spirulina supplemented diets on *C. auratus* indoor culture experiment was carried out for a period of 40 days. During this experimentation, fishes were cultured in 200l plastic trough at the rate of 10 fish per trough and were fed with respective control and experimental diets twice daily at 5% body weight. Everyday morning unfed remains was collected and 50% water exchange was made. The water quality parameters such as dissolved oxygen (> 5.8 mg/l), pH (7.6), temperature (28.7^{o}C), ammonia (0.2 mg/l) were maintained at the optimum level and continuous aeration was also provided. During experimentation, fishes were withdrawn at frequent intervals (10, 20, 30 and 40 days), sacrificed and the muscle and skin tissues were dissected out in aseptic condition and were used for further analysis.

Chemical analysis

Biochemical constituents such as protein (Lowry *et al.*, 1951), carbohydrate (Seifter *et al.*, 1950) and lipid (Folch *et al.*, 1957) contents of the muscle and skin tissues of control and experimental diets fed fishes were measured individually by following the standard methods. Quantitative estimation of carotenoid was also made in the respective tissue samples using spectrophotometer.

Carotenoid analysis

The carotenoid content of skin and muscle were extracted following the method of Torrissen and Naevdal (1984). Muscle and skin (500 mg) samples were ground in acetone and methanol solvent system separately with a homogenizer. In each solvent system, the extraction was done repeatedly to get all the carotenoids. The total extracts were pooled individually for acetone and methanol, centrifuged at 5000 rpm for 15 minutes and then optical density was measured in a Spectrophotometer at 444 nm.

Statistical analysis

The results obtained in the present study were subjected to statistical analysis (Mean ± SD, ANOVA and Regression) following the standard methods described in Zar (1986).

RESULTS

Table 1 provides the data on biochemical constituents in the skin and muscle tissues of *C. auratus* fed with Hibiscus petals and Spirulina supplemented diets for different duration i.e., 10, 20, 30 and 40 days. The tested biochemical constituents (protein, carbohydrate and lipid) showed much variation between control, Hibiscus petals and Spirulina supplemented groups. In Hibiscus petals supplemented diets fed groups, the initial (0 day) protein content in the skin tissue was 4.65 ± 0.160 mg/100 mg wet weight. In the experimental period, it was high and varied much from 5.06 ± 0.240 mg/100 mg wet weight to 6.46 ± 0.240 mg/100 mg wet weight respectively in 10 to 40 days fed fishes. The skin carbohydrate content of *C. auratus* was 2.65 ± 0.080 mg/100 mg wet weight on 0 day and it ranged from 2.98 ± 0.095 to 3.92 ± 0.196 mg/100 mg wet weight for 10 to 40 days fed experimental fishes. The initial (0 day) skin lipid content was 2.03 ± 0.056 mg/100 mg wet weight and during experimental period it ranged from 2.54 ± 0.061 to 3.53 ± 0.140 mg/100 mg wet weight (Table 1).

Likewise, the muscle protein, carbohydrate and lipid contents of Hibiscus petals supplemented diets fed fishes also showed much variation. The initial (0 day) protein content of muscle tissue was 7.41 ± 0.280 and during experimental period it ranged from 7.94 ± 0.240 mg/100 mg wet weight (10 days) to 9.26 ± 0.610 mg/100 mg wet weight (40 days). More or less, a similar variation was also noticed for skin carbohydrate and lipid contents (Table 1).

In Spirulina supplemented diet, the protein content registered in the skin tissue of experimental fish were: 5.45 ± 0.260 (10 days), 6.04 ± 0.360 (20 days), 6.62 ± 0.450 (30 days) and 6.98 ± 0.380 mg/100 mg wet weight (40 days); whereas at the initial period, it was 4.65 ± 0.160 mg/100 mg wet weight. The skin carbohydrate content ranged from 2.65 ± 0.080 during 0 day to 4.02 ± 0.220 mg/100 mg wet weight in 40 days experimental group. Likewise, the skin lipid content of initial and experimental fishes was ranged from 2.03 ± 0.056 mg/100 mg wet weight to 3.87 ± 0.240 mg/100 mg wet weight respectively for initial and 40 days fed fishes (Table 2).

The protein, carbohydrate and lipid content of muscle tissue of *C. auratus* fed with Spirulina supplemented diet also showed much variation. The initial (0 day) muscle protein content recorded was 7.41 ± 0.280 mg/100 mg wet weight and in experimental group it ranged from 8.16 ± 0.360 to 9.92 ± 0.520 mg/100 mg wet weight. The carbohydrate content also varied from 4.97 ± 0. 176 mg/100 mg wet weight (0 day) to 6.96 ± 0.440 mg/100 mg wet weight (40 days). Likewise the lipid content during initial period (0 day) was 1.26 ± 0.045 mg/100mg wet weight and in experimental group, it ranged from 1.94 ± 0.060 (10 days) to 2.90 ± 0.080 mg/100mg wet weight (40 days) (Table 2).

Compared to that of those fishes fed on experimental diets, the changes in tissue biochemical constituents was not much obvious in control diet fed groups (Table 3). For instance, the skin protein content was fluctuated between 4.65 ± 0.146 to 5.48 ± 0.168 mg/100g wet tissue during initial and at 40th day of the experiment. Similarly, the muscle protein content was ranged between 7.41 ± 0.280 and 8.14 ± 0.329 mg/100

mg wet tissue, respectively during 0 and 40th day of the experiment. The trend noticed for the changes in skin and muscle carbohydrate and lipid contents was similar to that of noticed for protein content.

Total carotenoid content in the skin tissue

The total carotenoid content in the acetone extract of skin tissue of *C. auratus* fed with Hibiscus petals supplemented diet was high when compared to methanol extracted skin tissues. Further among the tested diets, the total carotenoid content was more in Spirulina supplemented diets fed fishes, and it was less in Hibiscus petals supplemented and control diets fed groups. For instance, the total carotenoid content in the acetone extracted skin of *C. auratus* fed with Hibiscus petals supplemented diets varied from 0.262 ± 0.013 (0 day) to 0.626 ± 0.036 µg/g wet tissue (40th days of experiment). In the skin of same diets fed fishes, but extracted with methanol, the total carotenoid content varied from 0.230 ± 0.010 (0 day) to 0.420 ± 0.031 µg/g wet tissue. On the other hand, in control diet fed group, the initial and final skin carotenoid content varied from 0.262 ± 0.014 to 0.380 ± 0.036 and from 0.230 ± 0.010 to 0.330 ± 0.041 µg/g wet tissues respectively in acetone and methanol extracts (Table 4).

Similarly in the acetone extracted skin of *C. auratus* fed with Spirulina supplemented diet, the total carotenoid content varied from 0.262 ± 0.013 µg/g wet tissue (0 day) to 0.747 ± 0.036 µg/g wet tissue (40 days of experiment). In the methanol extracted skin of *C. auratus* fed on same group of experimental diets, the total carotenoid content varied from 0.230 ± 0.010 µg/g wet tissue (0 day) to 0.510 ± 0.032 µg/g wet tissue (Table 4).

Total carotenoid content in the muscle tissue

The total carotenoid content in the muscle tissue of *C. auratus* extracted with acetone and methanol but fed with Hibiscus petals and Spirulina supplemented diets are shown in Table 5. The results indicated that the total carotenoid content in the muscle tissue of *C. auratus* extracted with acetone was obviously more when compared to methanol extract. Also, the total carotenoid content was more in fishes received Spirulina supplemented diets and it was less in Hibiscus petals supplemented and control diets fed fishes. For example, in the muscle tissue of Hibisucs supplemented diets fed *C. auratus* extracted with acetone, the total carotenoid content varied from 0.086 ± 0.004 µg/g wet tissue (0 day) to 0.440 ± 0.036 µg/g wet tissue (40th days of experiment). In the methanol extracted muscle tissue of *C. auratus* fed with same experimental diets, it ranged from 0.065 ± 0.003 µg/g wet tissue (0 day) to 0.280 ± 0.012 µg/g wet tissue (40th days of experiment).

Likewise, the total carotenoid content in the muscle tissue of *C. auratus* fed with Spirulina added diets but extracted with acetone varied between 0.086 ± 0.004 µg/g wet tissue on 0 day to 0.502 ± 0.036 µg/g wet tissue on 40th day of experiment. But in the muscle tissue *C. auratus* extracted with methanol, the total carotenoid content ranged from 0.065 ± 0.003 mg/100 mg wet weight tissue on 0 day to 0.410 ± 0.020 mg/100 mg wet weight on 40th day of experiment. In control diet fed fishes, the initial (0 day) and final muscle carotenoid content were less when compared to experimental fishes (Table 5).

Table 6 provides the summary of two-way ANOVA for the data on total carotenoid content in the tested tissues of *C. auratus* extracted in two different solvent systems as a function of source of diet and experimental duration. The results inferred that, among the variables tested, experimental duration had more significant influence ($P < 0.001$ to < 0.05) when compared to the influence exerted by the dietary source ($P < 0.05$). Further, it revealed that among the solvent systems used, acetone supported the extraction of total carotenoids at a higher level than that of methanol.

Table 1. Changes in biochemical constituents in the skin and muscle tissues of gold fish, *Carassius auratus* fed with Hibiscus petals supplemented diet. Each value mean ± SD is the mean of three estimates.

Tissues	Experimental duration (days)	Biochemical constituents (mg/100 mg wet weight)		
		Protein	Carbohydrate	Lipids
Skin	Initial (0)	4.65 ± 0.160	2.65 ± 0.080	2.03 ± 0.056
	10	5.06 ± 0.240	2.98 ± 0.095	2.54 ± 0.061
	20	5.46 ± 0.202	3.42 ± 0.110	2.94 ± 0.066
	30	5.92 ± 0.210	3.68 ± 0.164	3.32 ± 0.080
	40	6.46 ± 0.240	3.92 ± 0.196	3.53 ± 0.140
Muscle	Initial (0)	7.41 ± 0.280	4.97 ± 0.176	1.26 ± 0.045
	10	7.94 ± 0.240	5.36 ± 0.180	1.68 ± 0.056
	20	8.64 ± 0.340	5.92 ± 0.210	2.08 ± 0.065
	30	9.04 ± 0.416	6.42 ± 0.360	2.42 ± 0.074
	40	9.26 ± 0.610	6.70 ± 0.410	2.62 ± 0.070

Table 2. Changes in biochemical constituents in the skin and muscle tissues of gold fish, *Carassius auratus* fed with Spirulina supplemented diet. Each value mean ± SD is the mean of three estimates.

Tissues	Experimental duration (days)	Biochemical constituents (mg/100 mg wet weight)		
		Protein	Carbohydrate	Lipids
Skin	Initial (0)	4.65 ± 0.160	2.65 ± 0.080	2.03 ± 0.056
	10	5.45 ± 0.260	3.04 ± 0.140	2.68 ± 0.130
	20	6.04 ± 0.360	3.66 ± 0.176	3.21 ± 0.160
	30	6.62 ± 0.450	3.94 ± 0.240	3.66 ± 0.185
	40	6.98 ± 0.380	4.02 ± 0.220	3.87 ± 0.240
Muscle	Initial (0)	7.41 ± 0.280	4.97 ± 0.176	1.26 ± 0.045
	10	8.16 ± 0.360	5.60 ± 0.260	1.94 ± 0.060
	20	8.98 ± 0.410	6.40 ± 0.330	2.20 ± 0.072
	30	9.61 ± 0.382	6.76 ± 0.410	2.64 ± 0.076
	40	9.92 ± 0.520	6.96 ± 0.440	2.90 ± 0.080

Table 3. Changes in biochemical constituents in the skin and muscle tissues of gold fish Carassius auratus fed with control diet. Each value mean ± SD is the mean of three estimates.

Tissues	Experimental duration (days)	Biochemical constituents (mg/100 mg wet tissue)		
		Protein	Carbohydrate	Lipid
Skin	Initial (0)	4.65 ± 0.146	2.65 ± 0.084	2.03 ± 0.055
	10	4.74 ± 0.152	2.74 ± 0.082	2.14 ± 0.052
	20	4.96 ± 0.164	2.80 ± 0.091	2.36 ± 0.063
	30	5.24 ± 0.172	2.86 ± 0.084	2.58 ± 0.071
	40	5.48 ± 0.168	3.04 ± 0.094	2.62 ± 0.074
Muscle	Initial (0)	7.41 ± 0.280	4.97 ± 0.170	1.26 ± 0.045
	10	7.58 ± 0.310	5.04 ± 0.162	1.34 ± 0.052
	20	7.82 ± 0.274	5.18 ± 0.174	1.56 ± 0.061
	30	8.04 ± 0.286	5.26 ± 0.182	1.78 ± 0.072
	40	8.14 ± 0.320	5.46 ± 0.140	2.06 ± 0.068

Table 4. Total carotenoid content in the skin tissue of gold fish, *Carassius auratus* fed with control and experimental diets. Each value mean ± SD is the mean of three estimates.

Solvent systems	Experimental duration (days)	Total carotenoid content (µg/g wet tissue)		
		Control (C)	Hibiscus petals (D1)	Spirulina (D2)
Acetone	Initial (0)	0.262 ± 0.014	0.262 ± 0.013	0.262 ± 0.013
	10	0.282 ± 0.016	0.314 ± 0.016	0.361 ± 0.016
	20	0.296 ± 0.010	0.426 ± 0.024	0.492 ± 0.020
	30	0.324 ± 0.024	0.494 ± 0.032	0.616 ± 0.034
	40	0.380 ± 0.036	0.626 ± 0.036	0.747 ± 0.036
Methanol	Initial (0)	0.230 ± 0.010	0.230 ± 0.010	0.230 ± 0.010
	10	0.276 ± 0.020	0.280 ± 0.016	0.312 ± 0.030
	20	0.284 ± 0.030	0.342 ± 0.020	0.392 ± 0.021
	30	0.310 ± 0.034	0.380 ± 0.026	0.460 ± 0.036
	40	0.330 ± 0.041	0.420 ± 0.031	0.510 ± 0.032

Table 5. Total carotenoid content in the muscle tissue of gold fish, *Carassius auratus* fed with control and experimental diets. Each value mean ± SD is the mean of three estimates.

Solvent systems	Experimental duration (days)	Total carotenoid content (µg/g wet tissue)		
		Control (C)	Hibiscus petals (D1)	Spirulina (D2)
Acetone	Initial (0)	0.086 ± 0.003	0.086 ± 0.004	0.086 ± 0.004
	10	0.092 ± 0.010	0.160 ± 0.006	0.176 ± 0.010
	20	0.098 ± 0.020	0.248 ± 0.008	0.312 ± 0.020
	30	0.104 ± 0.024	0.330 ± 0.018	0.380 ± 0.040
	40	0.124 ± 0.030	0.440 ± 0.036	0.502 ± 0.036
Methanol	Initial (0)	0.065 ± 0.002	0.065 ± 0.003	0.065 ± 0.003
	10	0.068 ± 0.020	0.078 ± 0.004	0.090 ± 0.007
	20	0.086 ± 0.030	0.124 ± 0.006	0.240 ± 0.012
	30	0.099 ± 0.036	0.180 ± 0.009	0.360 ± 0.030
	40	0.114 ± 0.042	0.280 ± 0.012	0.410 ± 0.020

Table 5. Summary of two-way analysis of variance for the data on total carotenoid content in the skin and muscle tissue of *C. auratus* extracted with acetone and (or) methanol as a function of source of diet and experimental duration.

Tissue	Source of Variation	df	MS	F	P-value
Skin	Variation due to diet (Acetone)	2	0.0444	7.54	< 0.05
	Variation due to experimental duration (Acetone)	4	0.0489	8.30	< 0.05
	Variation due to diet (Methanol)	2	0.0506	7.77	< 0.05
	Variation due to experimental duration (Methanol)	4	0.0335	5.14	< 0.05
Muscle	Variation due to diet (Acetone)	2	0.0169	7.76	< 0.05
	Variation due to experimental duration (Acetone)	4	0.0169	11.72	< 0.001
	Variation due to diet (Methanol)	2	0.0272	5.66	< 0.05
	Variation due to experimental duration (Methanol)	4	0.0225	4.69	< 0.05

Note : $P < 0.05$ is statistically significant

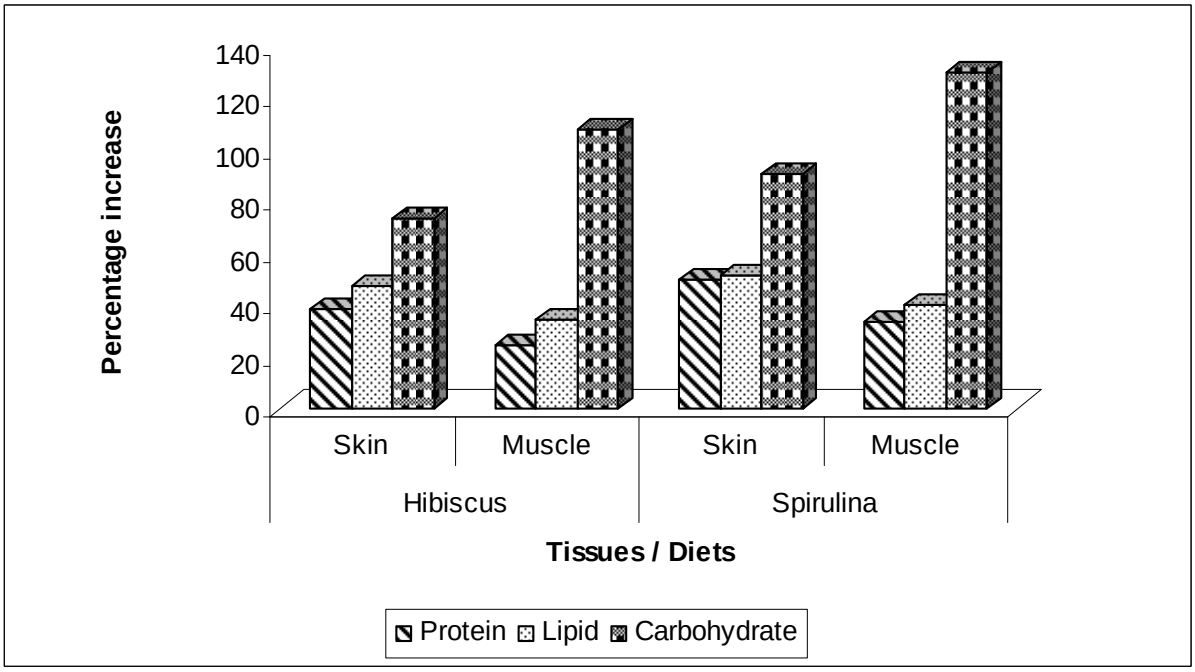

Figure 1. Percentage increase in biochemical constituents in the skin and muscle tissue of *C. auratus* fed with Hibiscus petals and Spirulina supplemented diets.

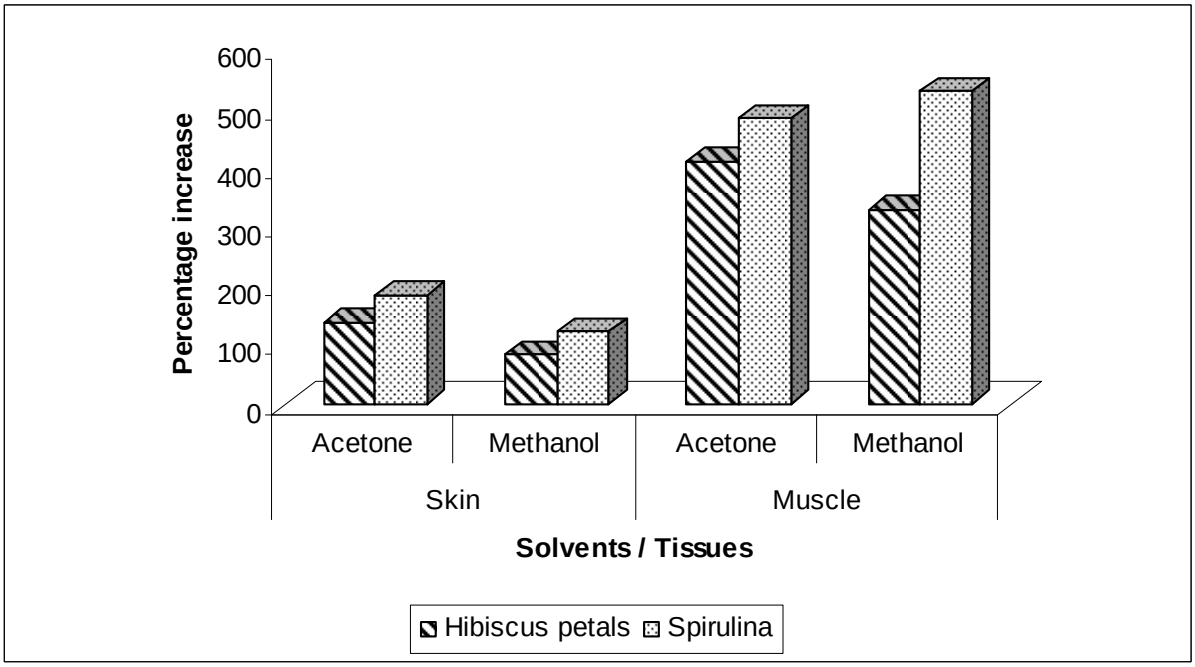

Figure 2. Percentage increase in total carotenoid content in the skin and muscle tissue of *C. auratus* fed with Hibiscus petals and Spirulina supplemented diets extracted in acetone and methanol.

DISCUSSION

One of the major problems in home and public aquaria is how to provide them with adequate diets (Pannevis and Earle, 1995; Macartney, 1996). Because dietary sources of pigment play a critical role in determining the fish colour. Carotenoids are also vital nutrients for healthy growth, metabolism and reproduction as well as colour (Miki, 1991). Since fish, like other animals not able to perform denovo synthesis of carotenoids (Goodwin, 1984), they have to be obtain them from dietary sources.

In the present study, irrespective of the dietary supplementation, the biochemical constituents (protein, carbohydrate and lipid) of skin and muscle tissues of *C. auratus* showed an enhancing trend with the advancement of experimentation. This trend was much more obvious for both Hibiscus petals and Spirulina diets fed fishes, when compared to those fishes received control diet. In the skin tissue of *C. auratus* fed with Hibiscus petals supplemented diets, the increase of 38.92%, 47.92% and 73.89% were observed respectively for protein, carbohydrate and lipid content. In the muscle tissue, the increase was 24.97%, 34.81% and 107.94% over the initial value (0 day). More or less, a similar variation was noticed in Spirulina diets fed fishes. In these groups, the increase in skin protein, carbohydrate and lipid content was 50.11%, 51.70% and 90.64% over the initial value (0 day). Likewise in the muscle tissue, the enhancement noticed was 33.87% (protein), 40.04 % (carbohydrate), 130.16% (lipid) over the initial value (Fig. 1). These results inferred that the test diets not only alter the survival and growth of candidate species but also supported the synthesis of essential macro and micro nutrients. It was reported that carotenoid deposition can vary according to fish size or age (Halten *et al.*, 1995). Khatoon *et al.* (2010) evaluated the algae based on feed in gold fish (*Carassius auratus*) nutrition and inferred that it improved the overall biochemical composition including carotenoid.

It is generally accepted that market value of gold fish increases with increasing degree of colouration in the skin. Therefore, the higher level of carotenoid in the skin of gold fish means the better acceptance by consumers (Yanar *et al.*, 2008). In the present study, the total carotenoid content of skin and muscle tissues of *C. auratus* showed dietary dependant variation. It was also noticed that, the total carotenoid in the tested tissues of *C. auratus* was more in Spirulina diet fed fishes and it was less in Hibiscus petals and control diet fed groups. The total carotenoid of skin tissue of *C. auratus* fed with Hibiscus petals supplemented diets showed an increase of 138.93% (Acetone), 185.11% (Methanol) over the initial value. On the other hand, in the same tissue of *C. auratus* fed with Spirulina supplemented diets, the increase in total carotenoid was 82.61% (Acetone) and 121.74% (Methanol) when compared to initial value (0 day). More or less, a similar trend was noted in the total carotenoid content of muscle tissue of *C. auratus* fed with Hibiscus petals and *Spirulina* supplemented diet. In this tissue, the increase in total carotenoid content was 411.63% (Acetone) and 483.72% (Methanol) in Hibiscus petals diets fed groups when compared to initial value. By the same way, the increase noticed in the Spirulina diet fed group was 330.77% (Acetone) and 530.77% (Methanol) over the initial value (Fig. 2). In control diet fed fishes, the range of variation in total carotenoid content of skin and muscle tissues during initial (0 day) and at end of the experiment was very less when compared to those fishes fed with experimental diets. This study demonstrated that Spirulina supplemented diet supported the carotenoid accumulation in the skin and muscle tissues of *C. auratus*.

Simpson *et al.* (1981) reported that limited dietary availability may impose a trade-off between maintaining ornamental colouration and health. Carotenoids are also vital nutrients for healthy growth, metabolism and reproduction. However, carotenoid cannot be synthesized by most of the animals, including fishes and must be obtained from dietary sources (Hata and Hata, 1971; Torrissen *et al.*, 1989; Storebakken and No, 1992). Torrissen *et al.* (1989) reported the influence of synthetic astaxanthin and canthaxanthin on carotenoid deposition and metabolism in salmonids. Satio and Regier (1971) reported that, dry crustacean meal from red crustacean enhanced the pigmentation on brook trout *Salvelinus fontalis*. Johnson et al. (1980) have also reported that addition of red yeast *Phaffia rhodozyma* supported the pigmentation in salmonids.

CONCLUSION

Further, it could be inferred that, in the present study, addition of spirulina as a dietary carotenoid source obviously enhanced the total

*****Corresponding author e-mail: beenapalavesam@gmail.com

carotenoid content in *C. auratus*. Though Hibiscus petals supported the pigmentation of skin and muscle of *C. auratus*, it could not be used as a sole carotenoid source in the diet of *C. auratus*. Also it could be added with other carotenoid sources as added additives to enhance pigmentation in freshwater or ornamental fish *C. auratus.*

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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