International Journal of Development, Vol.5, No.(1) (2016): 109-128
ISSN: 2314-5536 **e-ISSN: 2314-5544 (Online)**
www. ijd.byethost13.com **e-mail: fas_ijd@yahoo.com**

Effect of some food additives on color enhancement of koi fish, *Cyprinus carpio* (Linnaeus, 1758)

Ahmad M. Azab, Hassan M.M. Khalaf-Allah and Hany Maher
Marine Biology branch, Zoology Department, Faculty of Science,
Al-Azhar University, Cairo, Egypt

ABSTRACT

The present work was carried out to investigate the effect of some food additives (turnip leafs and roots, grape leafs and carrot roots) on enhancement colour of koi fish, *Cyprinus carpio.* Fish were divided into 8 groups in 16 glass aquaria (100 x 30 x 40 cm) with stocking density of 10 fish / aquarium (2 replicate aquaria were assigned for each experimental group). Fish were fed on plain food as a control (C), plain diet+ 5% turnip (T_1), plain diet+ 5% grape (T_2), plain diet+ 5% carrot (T_3), plain diet+ 5% grape + carrot (T_4), plain diet+ 5% grape + turnip (T_5), plain diet+ 5% carrot + turnip (T_6) and plain diet+ 5% turnip + grape + carrot (T_7) over 4 months.

Results showed that, the highest amount of the red color pixels was recorded in the treated fish group T_7 (fed on the 3 mixed food additives) at 30, 60, 90 and 120 days; followed by that of T_6 (carrot + turnip) at 60 and 90 days and in T_4 (grape +carrot) at 90 and 120 days.

Results also revealed that, the highest amount of the yellow color pixels was recorded at 90 and 120 days in the treated fish group T_3 (carrot). But, the highest amount of the yellow color pixels was recorded in T_4 (grape +carrot) at 30 and 60 days; followed by that of T_6 (carrot + turnip) at 60, 90 and 120 days.

Results showed that, the highest amount of total color pixels was recorded in T_3 (carrot) at 60, 90 and 120 days; followed by that of T_6 (carrot + turnip) and in T_4 (grape +carrot) at 60, 90 and 120 days.

In conclusion the best enhancement of red color in koi fish, *C. carpio* was recorded in the fish fed on the diet containing 5% of the mixture of all experimental additives (carrot roots+ grape leafs+ turnip leafs and roots). The enhancement of yellow color was recorded in fish fed on the diet containing 5% "carrot roots" for more than 30 days. The enhancement of total color of koi fish, *C. carpio* was recorded in fish fed on the diet containing 5% "carrot roots, or carrot roots with turnip leafs and roots".

Key words: food additives; enhancement; color; koi fish, *Cyprinus carpio*

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. The growing interest in aquarium fish had resulted in a steady increase in aquarium fish trade globally. This trade has a turnover rate of 5 billion $USD and an annual growth rate of 8% and offer a wide of scope for development. The top exporting country is Singapore followed by Hong Kong, Malaysia, Thailand, Philippines, Sri Lanka, Taiwan, Indonesia and India. The largest importer of ornamental fish is the USA followed by Europe and Japan. The emerging markets are China and South Africa. Over US $ 500 million worth of ornamental fish are imported into the USA each year[(1)].

The common carp, *Cyprinus carpio* (Linnaeus 1758), are members of the family Cyprinidae, or minnow family. Koi fish is an ornamental variety of the common carp. The Japanese ornamental koi carp, *C. carpio* is a popular decorative fish in many countries all over the world. Koi were developed approximately two centuries ago in Japan and are characterized by a wide diversity of colors and color patterns [2 - 3]. The colour and scale pattern (squamation) of the species is highly variable. A variety of colours and colour patterns have since been developed; common colours include white, black, red, yellow, blue, and cream. They grow up to 100 cm total length with an elongate body measuring 3 to 4 times less in height than total length. In their natural habitat, koi carp live up to 15-24 years. Males are known to live longer than females[3].

Color is one of the major factors, which determines the price of aquarium fish in the world market. Ornamental fish are acceptable to consumers if they have striking and vibrant colors. Fish skin color is mainly attributed to the presence of chromatophores that contain pigments including melanin, pteridines, purines and carotenoids. Fish are capable of producing some pigments. Since fish, like other animals are unable to synthesize carotenoids[4 - 6].

However, dietary sources of pigments also play a role in determining some pigments. The carotenoid pigmentation of fish results from the pigment present in the diet. So, skin color is highly dependent on the carotenoids presents in the diets of most fish. Hence, a direct relationship between dietary carotenoids and pigmentation exists in them[7 - 8].

Color enhancing diets may contain additional natural pigments to enhance colors of ornamental fish. People involved in the trade of ornamental fish are constantly exploring methods of enhancing skin coloration. Many studies have proved that, the fish can be pigmented by supplementation of plants sources[6, 9 – 13].

Thus, this work aimed to study the effect of some plant additives (leaf and root of turnip, leaf of grape and carrot roots) to fish diet on the color enhancement of koi fish, *Cyprinus carpio* (Linnaeus, 1758).

MATERIAL AND METHODS

1. Experimental fish:

A total of 160 specimens of Koi fish, *Cyprinus carpio* (Fig. 1), with a good condition were obtained from a private farm at Kafr El-Shaikh Governorate. All fish samples were pre-adults, and they greatly similar in weight (5.07±1 g). Fish were transported to the fish laboratory at Animal House of Zoology Department, Faculty of Science, Al-Azhar University; in large plastic bags, each containing approximately 20 L of water and a lot of oxygen. In the laboratory, fish were acclimatized for one week in well aerated large glass tanks (100x50x 50 cm). Fish were fed daily on a commercial fish diet. All fish specimens were kept in the laboratory conditions (temperature: 20±2ºC); and fed on the main experimental diet for 2 months, before the beginning of the experiment.

2. Experimental diets:

The main experimental fish diet 30% protein was bought from fish diet factory at October City. This fish diet was sinking pellets. It used for all fish during the preparatory period (the first 2 months). Also, it used as a control diet for control fish group. Then, it used as a plain for all the treatment diets. Seven treatment diets were prepared by adding 50 grams (5%) of testing additive to 950 grams (95%) of basic diet. The basic diet was grinded and milled.

The experimental additives are root of carrot, grape leaf and leaf and root of turnip. These additives were hot air dried in electric oven at 40-50ºC for 3 hours; then they were grinded and milled. The ingredients of basic diet and the proper additives were weighed and mixed. Then, they were pelleted with meat mincer through a 0.8 mm diameter. After cold pelleting, the feeds were hot air dried in electric oven at 40ºC for 4 hours. All prepared diets were put in air tight containers, and stored in dry-weathered place until it used in fish feeding. Feed preparation was carried out monthly to prevent long storage. The experimental eight diets were prepared as diagrammatically shown in Figure (2).

3. Fish grouping and experiment:

After acclimatization and preparatory periods, fish divided into 8 groups in 16 glass aquaria (100 x 30 x 40 cm) with stocking density of 10 fish / aquarium (2 replicate aquaria were assigned for each experimental group). Fish groups were fed with different fed rations (C, T1, T2, T3, T4, T5, T6 and T7) respectively. Fish were feeding by plain food as a control (C), diet contains 5% turnip (leaf and root) (T1), diet contains 5% grape (leaf) (T2), diet contains 5% carrot (root) (T3), diet contains 5% grape(leaf) + carrot (root) (T4), diet contains 5% grape (leaf) + turnip (leaf and root) (T5), diet contains 5% carrot (root) + turnip (leaf and root) (T6) and diet contains 5% turnip (leaf and root) + grape (leaf) + carrot (root) (T7). Fish were fed twice daily, six days a week at a fixed feeding rate of 2% of body fish weight (dry feed/ day).

The feeding rate adjusted at monthly intervals- where 32 fish were randomly selected from all group (2 fish from each aquarium), weighted; and the average fish weight was obtained and the monthly feed intake (g feed / fish /month) was calculated for each group. Half of the water volume, for all aquaria, was replaced once every week with de-chlorinated fresh tap water after removing the wastes (diet and excreta).

Fig (1): A photograph of a Koi fish, *Cyprinus carpio* at 0 time of experiment.

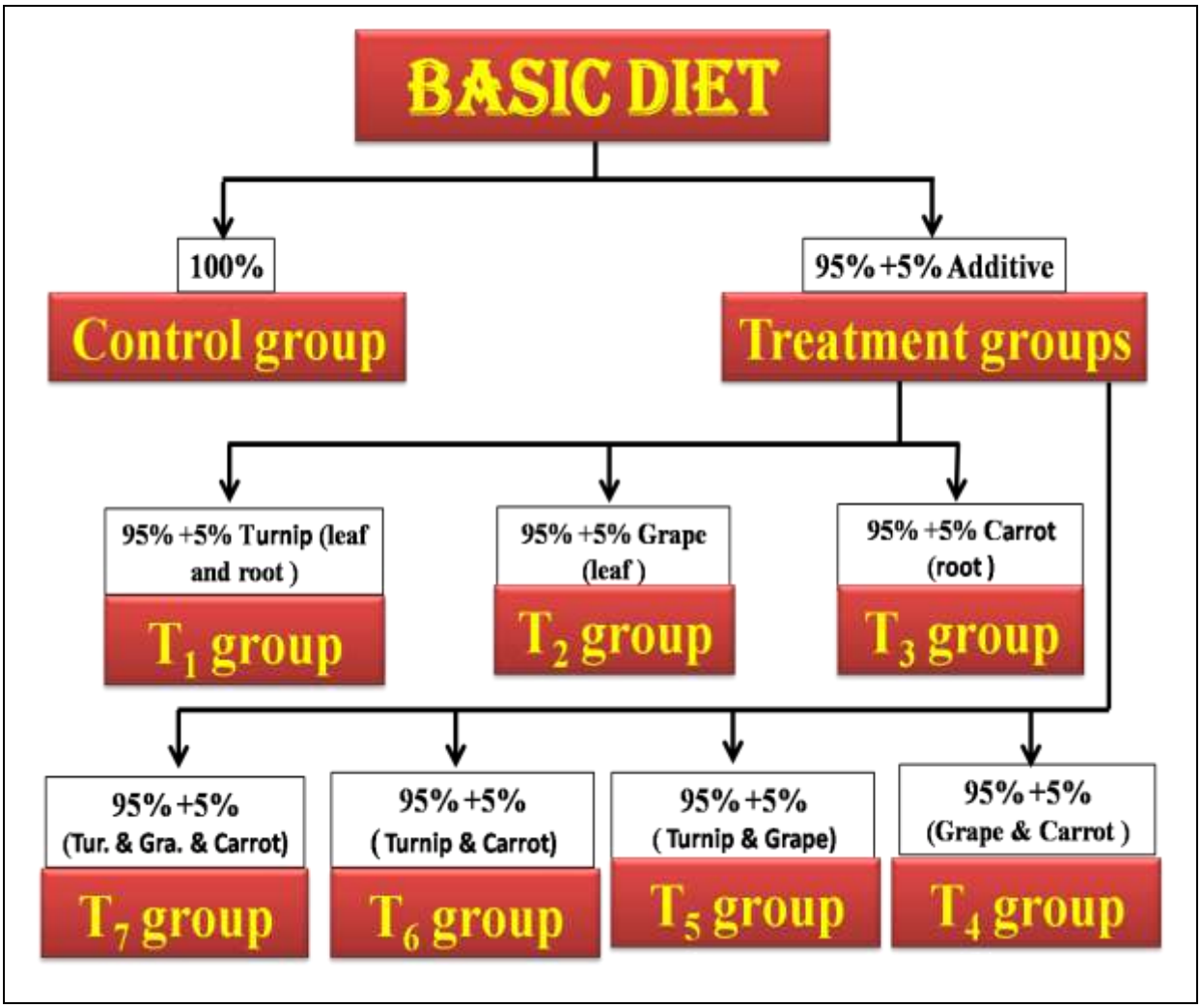

Fig. (2): A diagram showing the preparation of different experimental diets.

4. Coloration studies:

To evaluate the enhancement of fish color, 10 fishes from each group (control and treatment groups) were monthly photographed by a digital camera. These fish photographs were taken with similar dimensions and resolution. The digital photographs were then treated by the Adobe Photoshop 7 computerized program. These photographs were re-formatted as follows: Pixel dimensions: 400 width x 300 height pixels. Document size: 5.556 x 4.167 inches (width x height). Resolution: 72 pixels / inch.

In Adobe Photoshop program, the amount of pixels for the very important colors of koi **fish (Red and Yellow) was** detected and recorded.

4.1. Amount of color pixels:

The method of detecting the amount of red color pixels can be summarized in the following steps:

1) Press on File button in the tools bar; then press on Open in its list to select the fish picture.
2) Press on Select button in the tools bar; then press on Color Range in its list.

3) In appeared window, select the Red color and press OK. The red color pixels in the picture will be flashed on.
4) Press on Image button in the tools bar; then press on Histogram in its list.
5) In the appeared window, we find the detected Pixels amount of the red color.

For detecting the amount of yellow color pixels, we repeat the steps (2 - 5).

RESULTS

1. Effect of food additives on color pixels amount:

The effect of different feed rations (C, T1, T2, T3, T4, T5, T6 and T7) on the color pixels amount in the digital photographs of koi fish, *C. carpio,* reared for 120 days are given in Tables (1- 3), Figures (3 - 5) and Plates (I – II).

1.1. Red color:

The amount of red color pixels in the digital photographs of koi fish, *C. carpio*, at zero time and after feeding on the different feed rations for 30, 60, 90 and 120 days are shown in Table (1) and graphically presented in Figure (3).

At zero time (T_0), the amount of red color pixels in the digital photographs of koi fish, *C. carpio*, was very low, ranged from 0 to 77 pixels with an average of 16.2±24.92 pixels (Table, 1).

After 30 days, the highest amount of red color pixels was recorded in T_7 (diet contained the 3 mixed food additives), ranged from 26 - 2721 pixels, with an average of 1127.3±1069.82 pixels. Followed by T_3 (diet contained carrot), ranged from 0- 2381 pixels, with an average of 974.8±1133.43 pixels. While the lowest red pixels amount was occurred in T_4 (fed on carrot + grape), ranged from 5 - 755 pixels, with an average of 253.2± 235.74 pixels; compared with control, ranged from 0 – 1186 pixels, with an average of 296.4±413.05 pixels (Table 1 and Fig. 3).

After 60 days, the red color was slightly improved in all treated groups, except T_3, in which the red color pixels amount greatly decreased, recording the lowest value (541.3±1472.22 pixels) after the control group (340.3±723.14 pixels). Also, the highest red color pixels amount was recorded in T_7, in which it ranged from 0 - 8251 pixels, with an average of 1489.1 ±2626.94 pixels. It followed by T_6, with an average of 1158.8 ± 1092.39 pixels (Table 1 and Figure, 3).

After 90 days, the red color pixels amounts were moderately increased in all experimental groups, except T_3, in which it was slightly decreased, recording the lowest value (420.1±703.61 pixels), and it followed by the control group (458.0±402.1 pixels). In the same manner, the highest average of red color pixels amount (2184.6±1203.53 pixels) was recorded in T_7. It followed by T_6, with an average of 1672.5±1147.87 pixels (Table 1 and Fig. 3).

After 120 days, the red color pixels amounts reached the maximum values in all experimental groups, except T_3, in which it was greatly decreased, recording its minimum value (298.8±396.41 pixels). The highest average of red color pixels amount was recorded in T_7, ranged from 2069 - 3783 pixels, with an average of 2880.2±528.44 pixels. Followed by T_4 and T_6 (diets contained carrot), with averages of 2487.5±3579.06 and 2186.2±2121.94 pixels, respectively (Table 1, Fig. 3 and Plates, I&II).

The results showed that, the amount of red color pixels was significantly increased, with increase of feeding period in T_7, T_6 and T_4 (in which carrot was mixed with another additive).

While, it was significantly decreased with increase of feeding period in T_3, in which feed ration contained carrot only (Table 1, Fig. 3 and Plates, I&II).

Table (1): Amount of red color (pixels) in the pictures of koi fish, *C. carpio*, fed on different food additives, during different periods.

Treatments		30 days	60 days	90 days	120 days
T_0	Range	0 - 77			
	Mean ± SD	16.2 ± 24.92			
C	Range	0 - 1186	0 - 2298	52 -1288	17 - 1735
	Mean ± SD	296.4±413.05	340.3 ±723.14	458.0 ±402.10	669.3±500.04
T1	Range	0 - 2124	0 - 2208	0 - 2262	0 - 3478
	Mean ± SD	452.1±672.6	785.7±925.44	942.1±732.78	1098.5±1206.99
T2	Range	0 - 1356	0 - 3201	0 - 2700	0 - 4844
	Mean ± SD	320.9±490.89	1096.3±1393.57	1215.1±1198.61	1334.0±1503.11
T3	Range	0 - 2381	0 - 4708	0 - 2354	0 - 921
	Mean ± SD	974.8±1133.43	541.3±1472.22	420.1±703.61	298.8±396.41
T4	Range	5 - 755	0 - 1341	222 - 6541	405- 12000
	Mean ± SD	253.2±235.74	617.1±533.56	1552.3±1905.8	2487.5±3579.0
T5	Range	0 - 1106	0 - 1567	1 - 1978	0 - 3009
	Mean ± SD	308.3±438.71	460.8±630.19	496.4±650.71	532.1±1144.65
T6	Range	0 - 2616	0 - 2954	737 - 5078	224 -8540
	Mean ± SD	556.1±682.83	1158.8±1092.3	1672.5±1147.8	2186.2±2121.9
T7	Range	26 - 2721	0 - 8251	1073 - 5160	2069 -3783
	Mean ± SD	1127.3±1069.82	1489.1±2626.94	2184.6±1203.53	2880.2±528.44

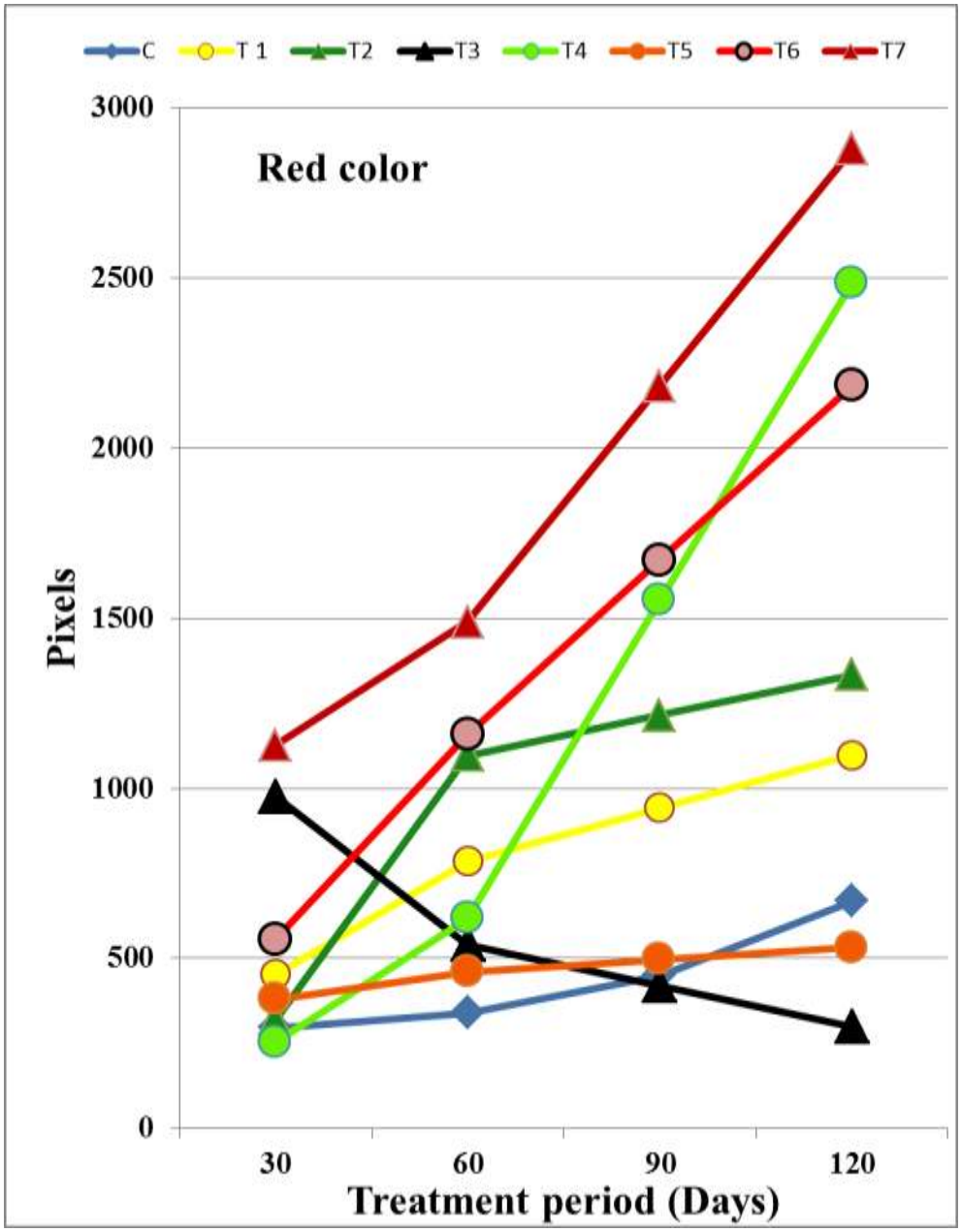

Fig. (3): Relationship between the red color pixels amounts (pixels) and the treatment period (days) in the pictures of koi fish, *C. carpio*, fed on different food additives.

1.2. Yellow color:

The amount of yellow color pixels in the digital photographs of koi fish, *C. carpio*, at zero time and after feeding on the different feed rations for 30, 60, 90 and 120 days are shown in Table (2) and graphically presented in Figure (4).

At zero time (T_0), the amount of yellow color pixels in the digital photographs of koi fish, *C. carpio*, was very low, ranged from 0 to 964 pixels with an average of 202.3±255.79 pixels (Table 2).

After 30 days, the highest yellow color pixels amount was recorded in T_4 (diet contained carrot and grape). It ranged from 108 - 3625 pixels, with an average of 1415.0±1256.96 pixels. Followed by T_3 (diet contained carrot), ranged from 45 – 2761 pixels, with an average of 1076.1±1239.66 pixels. While the lowest yellow pixels amount was occurred in T_6 (fed on carrot + turnip), ranged from 0 - 98 pixels, with an average of 8.2± 28.29 pixels; compared with the control group, ranged from 0 – 301 pixels, with an average of 37.6±90.76 pixels (Table, 2 and Fig. 4).

After 60 days, the yellow color was greatly improved in all treated groups, except T_1, (feed contained turnip), in which the yellow color pixels amount greatly decreased, recording the lowest value (63.8±84.13 pixels) comparing to the control group (448.4±256.16 pixels). The highest yellow color pixels amount was recorded in T_4 (diet contained carrot and grape). It ranged from 63 - 4547 pixels, with an average of 2104.3±1732.95 pixels. Followed by T_3 (diet contained carrot), ranged from 8 – 2197 pixels, with an average of 1767.8±3301.11 pixels. Then, it followed by T_6, with an average of 1561.9 ± 2322.57 pixels (Table 2 and Fig. 4).

After 90 days, the yellow color pixels amounts were greatly varied in all experimental groups. It was greatly increased in T_3 and reached to the highest yellow color pixels amount at 90 days of treatment; ranging from 314 – 9267 pixels, with an average of 4875.5±3755.36 pixels. It followed by T_6, with an average of 2428.4 ± 1364.6 pixels. The lowest yellow color pixels amount was recorded in the control group and T_1; with averages of 57.4±91.24 pixels and 66.1±95.64 pixels, respectively (Table 2 and Fig. 4).

After 120 days, the yellow color pixels amount was greatly increased and reached the maximum values in T_3, ranged from 27–18522 pixels, with an average of 7983.1±8458.72 pixels. It followed by T_6, which ranged from 0-8577 pixels with an average of 3294.8±3644.5 pixels. The lowest yellow color pixels amount was recorded in T_7, which ranged from 0 - 98 pixels, with an average of 49.5±28.29 pixels (Table 2, Fig. 4 and Plates, I&II).

The results showed that, the yellow color pixels amount was significantly increased with increase of feeding period in T_3 and T_6 (in which carrot was alone or mixed with turnip). But, it was very low during the whole period of study in T_7 (Table 2, Fig. 4 and Plates, I&II).

Table (2): Amount of yellow color pixels in the pictures of koi fish, *C. carpio* fed on different food additives, during different periods.

Treatments		30 days	60 days	90 days	120 days
T_0	Range	0 - 964			
	Mean ± SD	202.3 ± 255.79			
C	Range	0 – 301	14 – 791	0 - 295	2 – 598
	Mean ± SD	37.6 ±90.76	448.4 ±256.16	57.4 ±91.24	169.0 ±215.57
T1	Range	0 - 789	0 - 179	0 - 316	0 - 630
	Mean ± SD	248.3 ±211.27	63.8 ±84.13	66.1± 95.64	68.4 ±197.65
T2	Range	0 - 283	0 - 850	0 - 826	0 - 1077
	Mean ± SD	99.9 ±103.64	350.6 ±370.43	257.5 ± 268.76	164.3 ±379.72
T3	Range	45- 2761	8 - 2197	314 - 9267	27- 18522
	Mean ± SD	1076.1±1239.66	1767.8 ±3301.11	4875.5±3755.36	7983.1±8458.72
T4	Range	108-3625	63 -4547	83 - 3823	8 -7524
	Mean ± SD	1415.0±1256.96	2104.3±1732.95	1566.2±1134.09	1028.1±2096.28
T5	Range	0 - 5039	0 - 9739	3 - 4873	5 - 2639
	Mean ± SD	827.4±1802.44	1103.5±2780.69	1112.2±1338.01	1120.9±1039.27
T6	Range	0 - 98	0 - 5319	2 - 4289	0 - 8577
	Mean ± SD	8.2 ±28.29	1561.9±2322.57	2428.4±1364.6	3294.8±3644.5
T7	Range	17 - 574	0 - 2915	0 - 1469	0 - 98
	Mean ± SD	243.4±171.17	412.8 ±859.94	231.1±427.41	49.5±28.29

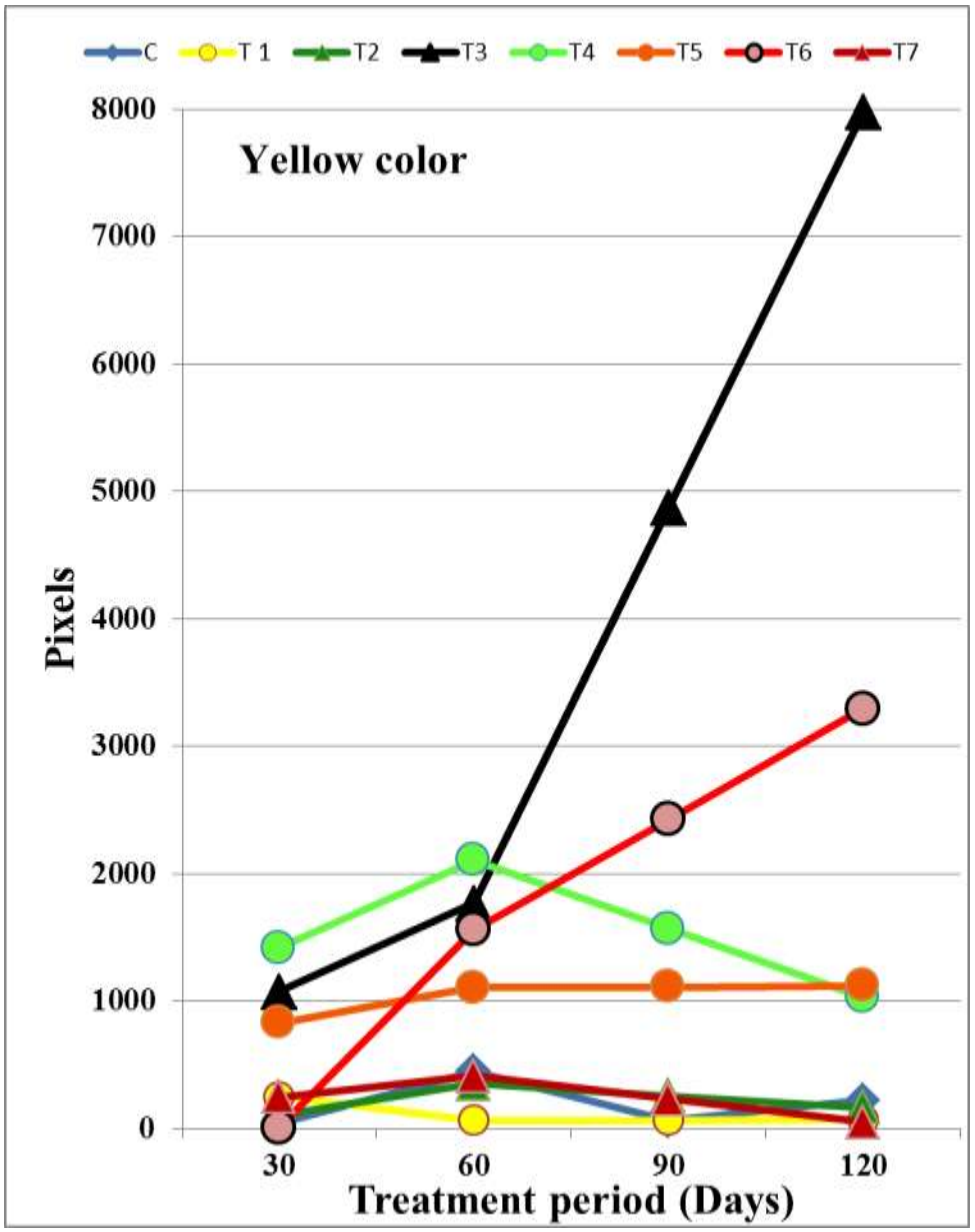

Fig. (4): Relationship between the yellow color pixels amounts (pixels) and the treatment period (days) in the pictures of koi fish, *C. carpio*, fed on different food additives.

1.3. Total color:

The amount of total (red and yellow) color pixels in the digital photographs of koi fish, *C. carpio*, after feeding on the different feed rations for 30, 60, 90 and 120 days are shown in Table (3) and graphically presented in Figure (5).

At zero time (T_0), the amount of total color pixels in the digital photographs of koi fish, *Cyprinus carpio*, was very low, ranged from 0 to 979 pixels with an average of 218.5±250.55 pixels (Table 3).

After 30 days, the highest total color pixels amount was recorded in T_3 (diet contained carrot), it ranged from 155 - 2761 pixels, with an average of 2050.9±865.23 pixels. Followed by T_4 (diet contained carrot and grape), it ranged from 390 – 3630 pixels, with an average of 1668.2±1157.76 pixels. While the lowest total color pixels amount was occurred in control group, it ranged from 4 - 1194 pixels, with an average of 333.9± 411.06 pixels (Table 3 and Fig. 5).

After 60 days, the total color was slightly improved in all treated groups. The highest total color pixels amount was recorded in T_4, in which it ranged from 122 - 4547 pixels, with an average of 2721.4±1481.33 pixels. Also, T_6 recorded the highest value of total color pixels, ranged from 1004 – 5325 pixels, with an average of 2720.7±1626.24 pixels. While the lowest total color pixels amount was recorded in the control group and T1, with averages of 788.6±546.16 and 849.5±867.31 pixels, respectively (Table 3 and Fig. 5).

After 90 days, the total color pixels amounts were greatly increased in all experimental groups, except the control group, in which it was slightly decreased, recording the lowest value (506.1±420.7 pixels). It also followed by T_1 with an average of 1008.2±708.88 pixels. The highest total color pixels amount was recorded in T_3, ranged from 710 – 11202 pixels, with an average of 5259.5±3951.91 pixels. It followed by T_6, it ranged from 1574 – 9366 pixels, with an average of 4100±2112.5 pixels (Table 3 and Fig. 5).

After 120 days, the total color pixels amounts reached the maximum values in all experimental groups. The lowest average of total color pixels (1166.9±1198.27 pixels) was recorded in T1 (diet contained turnip). The highest average of total color pixels amount was recorded in T_3, ranged from 45 - 18522 pixels, with an average of 8281.9±8151.67 pixels. It followed by T_6, ranged from 236 – 17117 pixels, with averages of 5481±5137.92 pixels (Table 3, Fig. 5 and Plates I&II).

These results showed that, the total color pixels amount was significantly increased, with increase of feeding period, in T_3, T_6 and T_4 (in which carrot was mixed with another additive). While, it was slightly decreased with increase of feeding period in T_7 and T_5, in which feed ration contained grape and turnip (Table 3, Fig. 5 and Plates I&II).

Table (3): Amount of total color pixels in the pictures of koi fish, *C. carpio* fed on different food additives, during different periods.

Treatments		30 days	60 days	90 days	120 days
T0	Range	0 - 979			
	Mean ± SD	218.5 ± 250.55			
C	Range	4 - 1194	367 - 2312	52 - 1194	543 - 2797
	Mean ± SD	333.9 ±411.06	788.6±546.16	506.1±420.70	1540.4±887.26
T1	Range	11 - 2274	114 - 2208	57 - 2274	0 - 3478
	Mean ± SD	700.4 ±665.64	849.5±867.31	1008.2±708.88	1166.9±1198.27
T2	Range	34 - 1356	254 - 3201	226 - 2813	0 - 4844
	Mean ± SD	420.8 ±427.19	1446.8 ±1142.43	1472.6±1054.07	1498.3±1474.35
T3	Range	155 - 2761	11 - 10928	710 - 11202	45 - 18522
	Mean ± SD	2050.9±865.23	2309.1±3498.05	5295.5±3951.91	8281.9±8151.67
T4	Range	390 - 3630	122 - 4547	1016 - 6626	471 - 12026
	Mean ± SD	1668.2±1157.76	2721.4±1481.33	3118.5±1555.08	3515.6±3838.81
T5	Range	0 - 5039	0 - 10685	62 - 6850	124 - 3015
	Mean ± SD	1207.7±1663.28	1564.3±2953.95	1608.6±1790.7	1653.0±1054.92
T6	Range	56 - 2616	1004- 5325	1574 - 9366	236 - 17117
	Mean ± SD	564.3±676.13	2720.7±1626.24	4100±2112.5	5481±5137.92
T7	Range	266 - 2818	73 - 11166	1073 - 6629	2072 - 881
	Mean ± SD	1370.6±941.99	1901.9±3355.63	2415.8±1561.93	2929.6±550.4

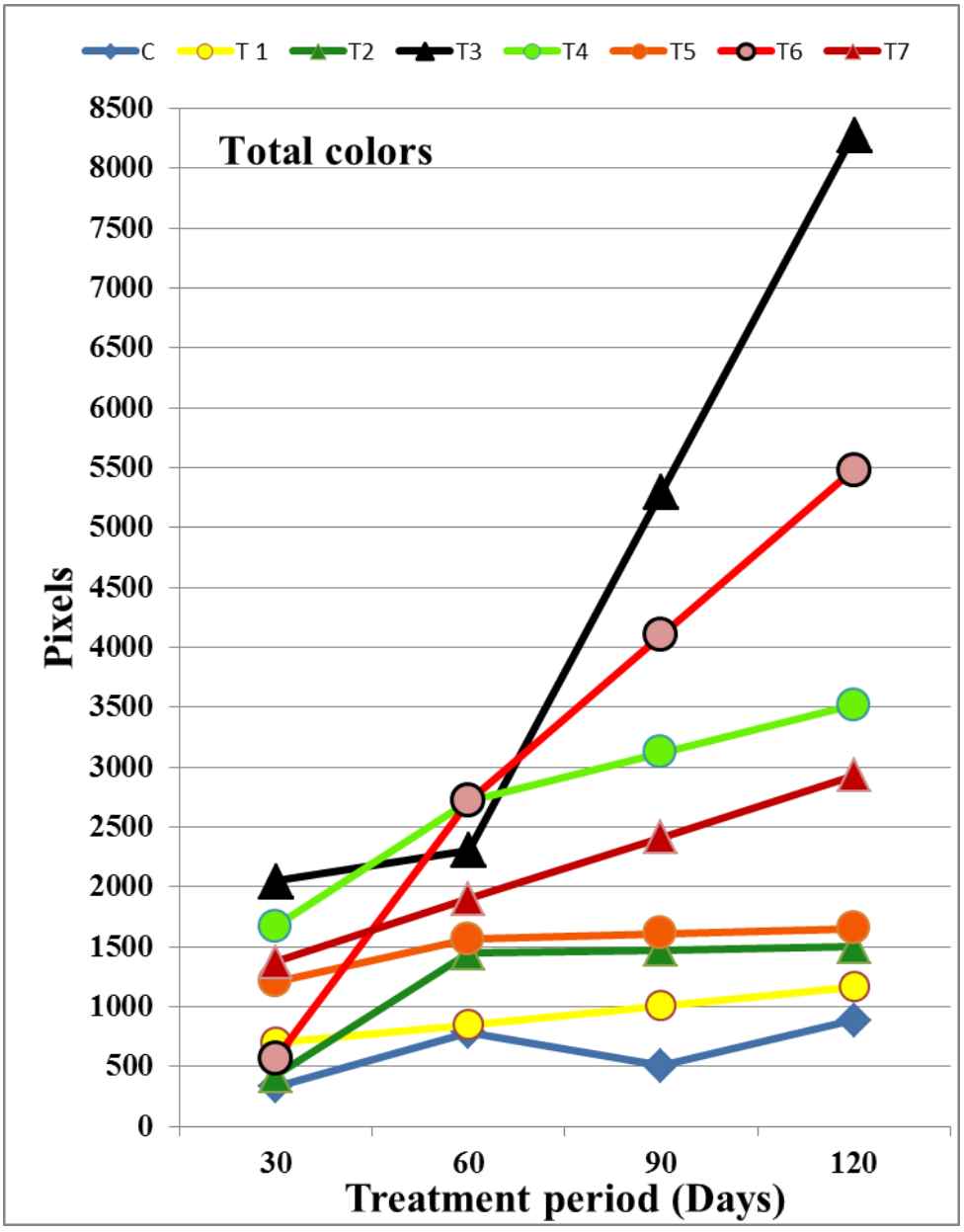

Fig. (5): Relationship between the total color pixels amounts (pixels) and the treatment period (days) in the pictures of koi fish, *C. carpio*, fed on different food additives.

C	
T1	
T2	
T3	

PLATE I: Photographs of some Koi fish, *C. carpio*, fed on plain food as a control (C), diet contains 5% turnip (T1), diet contains 5% grape (T2) and diet contains 5% carrot (T3) for 120 days.

T4	
T5	
T6	
T7	

PLATE II: Photographs of some Koi fish, *C. carpio*, fed on diet contains 5% grape + carrot (T4), diet contains 5% grape + turnip (T5), diet contains 5% carrot + turnip (T6) and diet contains 5% turnip + grape + carrot (T7) for 120 days.

DISCUSSION

Ornamental fish are characterized by a wide diversity of colours, colour patterns and success in the ornamental fish trade is very much dependent on the bouncy colour of the fish. Color is one of the major factors, which determines the price of aquarium fish in the world market [5].

Color enhancing diets may contain additional natural pigments to enhance colors of ornamental fish. People involved in the trade of ornamental fish are constantly exploring methods of enhancing skin coloration. A direct relationship between dietary carotenoid and pigmentation exists in fishes. Unlike other animals, fish lack the ability to synthesis carotenoids and entirely depend on their dietary sources. If enhancement of colouration can be done by administering pigment enriched feed, it will definitely improve the quality and cost of the fish[14]

In the present study, the highest amount of the red color pixels was recorded in T_7 (3 mixed food additives: carrot + turnip + grape) at 30, 60, 90 and 120 days; in T6 (carrot + turnip) at 60, 90 and 120 days; and in T_4 (carrot + grape) at 30, 90 and 120 days. It may be due to the source of red colour, which found in different additives of carotenoids (carrot, turnip and grape). But, the lowest amount of the red color pixels occurred in T_5 (grape + turnip) at 60, 90 and 120 days; in T3 (carrot) at 60, 90 and 120 days and as well as the control group at the whole period of experiment.

In the present study, the average in the amount of red color pixels reached its maximum value (2880.2±528.44) in T_7 (turnip + grape + carrot) at the end of experiment (120 days). The enhancement of the amount red color pixels may be due to the combination between the carotenoids in the three experimental additives.

Generally, the present study showed that, the amount of red color pixels was significantly increased, with increase of feeding period in T_7, T_6 and T_4 (in which carrot was mixed with another additive). While, it was significantly decreased with the increase of feeding period in T_3, in which feed ration contained carrot only. Many reports have demonstrated that, skin color change over time depended on the level of carotenoid in the diet and differed among species[6, 15 - 17].

Carotenoids form the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables. Carotenoids are of great interest due to their essential biological functions in both plants and animals[18]. Carotenoids are the primary source of pigmentation in ornamental tropical fish, responsible for various colours like yellow, red and other related colours. Normally these are obtained through substances rich in carotenoid content in the food chain of the aquatic organisms. But commercial feed ingredients such as yellow corn, corn gluten meal and *alfa alfa* are used as sources of carotenoids such as zeaxanthin and lutein[19 - 20].

Some authors used another sources of carotenoids for enhansment of color. [21] used *Haematococcus pluvialis* as a safe natural source of astaxanthin derived from micro algae which resulted in extensive pigmentation in koi and tropical fishes. [22] observed a positive effect of dietary carotenoid on the growth of red tilapia. [23] reported that, the enhancement of colour in male gourami when fed with carotenoid pigment source. [24] reported that, *Spirulina* induced the growth and body colour of crucian carp and [20] mentioned that, *Rosa rubiginosa* petals enhanced the coloration of soardtail fish, *Xiphophorus helleri.*

In the present study, the highest amount of yellow color pixels was recorded in T_4 (grape+carrot) at 30 and 60 days only. But, this amount of yellow color pixels started to increase in T_3 (carrot) after 60 days; reached the maximum value (7983.1± 8458.7) after 120 days. This

means that, the sources of red color in carotinoids of carrot, which gave high amount of red color pixels after 30 days only, they shared sources of yellow color in carotinoids of carrot giving more yellow color pixels after a long period.

These findings were in agreement with some other studies[25 - 27]. It was found that, carrot (*Daucus arota*) is a good source of β carotene (10800 μg g^{-1} wet weight) and α carotene (3610 μg g^{-1} wet weight) which is higher than many fruits, uncooked vegetables and seeds. In addition due to its low price and easy access, carrot is a good source of food additive in fish culture.

The enhancement of the yellow color pixels may be due to carotenoids in carrot; where, carotenoid pigments play a decisive role in intermediary metabolism and that could also enhance nutrient utilization and various sources of carotenoid pigments were tried for enhancing pigmentation[28 - 29].

The lowest amounts of yellow color pixels occurred in T_6 (carrot + turnip) after 30 days and in T_1 (turnip) and in T_7 (grape+ carrot+ turnip), after 60, 90 and 120 days. This means that, the sources of yellow color in carotinoids of turnip were very low.

In the present study, the total amount of color pixels over the rearing period of 120 days exhibited greater in all treatments compared with the control. It may be due to the carotenoids in turnip, grape and carrot.

Although some authors claim that, the biological functions of carotenoids in fish are still speculative [30], other consider these compounds as important micronutrients that fish are not able to synthesize, therefore, must be included in the diet[31].

In the present study, the highest amounts of total color pixels were recorded in T_3 (carrot) after 30, 60, 90 and 120 days. But, the lowest amounts of total color pixels occurred in T_2 (grape) at 30 days and in control group and in T_1 (turnip) during the whole period of the experiment. This means that, the sources of total colour in carotinoids of carrot were more than grape and turnip and the total colour in carotinoids of turnip and grape were very small.

REFERENCES

1. FAO (Food and Agricultural Organisation) (2000). World status of ornamental fish, Pp: 225.
2. Kuroki, T. (1981): The latest manual to nishikigoi.Shin-Nippon Kyoiku-Tosho Co. Ltd. Japan, Pp: 272.
3. Kailola, P.J; Williams, M.J.; Stewart, P.C.; Reichelt, R.E.; McNee, A. and Grieve, C. (1993). Australian fisheries resources. Bureau of Resource Sciences,Canberra, Australia, Pp: 422.
4. Goodwin, T.W. (1984). The Biochemistry of the Carotenoids.Volume II Animals. Chapman and Hall, New York, U.S.A., Pp: 224.
5. Saxena, A. (1994). Health; coloration of fish. International Symposium on Aquatic Animal Health: Program and Abstracts. Univ. of California, School of Veterinary Medicine, Davis, CA, U.S.A., Pp: 94.
6. Chatzifotis, S.; Pavlidis, M.; Jimeno, C.D.; Vardanis, G.; Sterioti, A. and Divanach, P. (2005). The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). Aquacult Res; 36 (15):1517-1525.
7. Hata, M. and Hata, M. (1973). Studies on astaxanthin formation in some freshwater fishes. Tohoku. J. of Agricultural Research, 24(4): 192-196.

8. Halten, B.; Arnmesan, A.; Jobling, M. and Bjerkeng, B. (1997). Carotenoid pigmentation in relation to feed intake, growth and social integration in Arctic char, *Salvelinus aipinus* (L.), from two anadromous strains, Aquaculture. Nutr., 3: 189-199.
9. Lovell, R.T. (2000). Nutrition of Ornamental Fish. In: Kirk's Current Veterinary Therapy XIII-Small Animal Practice. Bonagura J. (Ed.), Saunders, W.B., Philadelphia, USA; p. 1191-1196.
10. Kowsalya, S.; Chandrasekhar, U. and Balasasirekha, R. (2001). Beta carotene retention in selected green leafy vegetables subjected to dehydration. Ind. J. Nutr. dietet., 38: 374-383.
11. Sales, J. and Janssens, G.P.J. (2003). Nutrient requirements of ornamental fish. Aquat Living Resour, 16(6): 533-540.
12. Sinha, A. and Asimi, O.A. (2007). China rose (*Hibiscus rosasinensis*) petals: a potent natural carotenoid source for goldfish (*Carassius auratus* L). Aquaculture Res., 38: 1123-1128.
13. Ahilan, B.; Jegan, K.; Felix, N. and Ravaneswaran, K. (2008). Influence of botanical additives on the growth and coloration of adult, *Carassius aurates* (Linnaeus), Tamil Nadu J. Veterinary & Animal Sciences, 4 (4): 129-134.
14. Golandaj, A.; Shyama, S.; Dinesh, K.; Sreenath V.R. and Swain, S. (2015). Colour enhancement potential of selected local flowers in Sword tail, *Xiphophorus helleri* through dietary incorporation. Research Journal of Recent Sciences, 4 (ISC): 37- 43.
15. Dharmaraj S. and Dhevendaran K. (2011). Application of microbial carotenoids as a source of colouration and growth of ornamental fish, *Xiphophorus helleri*. World Journal of Fish and Marine Sciences, 3(2):137-144.
16. Ho, A.L.F.C.; Zong, S. and Lin, J. (2014). Skin color retention after dietary carotenoid deprivation and dominance mediated skin coloration in clown anemonefish, *Amphiprion ocellaris*. AACL Bioflux, 7(2):103-115.
17. Yedier, S.; Gümüs, E.; Livengood, E.J. and Chapman F.A. (2014). The relationship between carotenoid type and skin color in the ornamental red zebra cichlid, *Maylandia estherae*. AACL Bioflux, 7(3):207-216.
18. Eldahshan, O.A. and Singab, A.B. (2013). Carotenoids. Journal of Pharmacognosy and Phytochemistry, 2 (1): 225 – 234.
19. Lovell, R.T. (1992). Dietary enhancement of colour in ornamental fish. Aquaculture Magazine, 18: 77-79.
20. Arulvasu, C.; Ramya, S.; Meena, I.; Chandhirasekar, D. and Sivaganam, S. (2013). Evaluation of natural sources of carotenoid pigments from *Rosa rubiginosa* on growth, survival and coloration of *Xiphophorus helleri* fish fry. European Journal of Biological Sciences, 5 (2): 44-49.
21. Ronneberg, H.; Borch, G.; Fox, D. and Jensen, S.L. (1979). Animal carotenoid 19, Natu. Rose. (Technical bulletin 054 Revision Date: 3/27/98).
22. Boonyaratpalin, M. and Unprasert, N. (1989). Effects of pigments from different sources on color changes and growth of red *Oreochromis niloticus*. Aquaculture, 79: 375-380.
23. Fay, M.F.; Lledó, M.D.; Kornblum, M.M. and Crespo, M.B. (1999). From the waters of Babylon? *Populus euphratica* in Spain is clonal and probably introduced. Biodiversity and Conservation, 8: 769-778.
24. Peimin, H.; Yinjiong, Z. and Wenhui, H. (1999). Effect of the *spirulina* feed on the growth and body colour of crucian carp. J. Fish. China/Shuichan Xuebao., 23: 162-168.

25. Sommer, T.R.; Potts, W.T. and Morrissy, N.M. (1991). Utilization of microalgal astaxanthin by rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 94: 79-88.
26. Scott, K.J. and Hart, D.J. (1994). The carotenoid composition of vegetables and fruit commonly consumed in the UK. IFR Publishing Limited, Norwich, England.
27. Pirnia O. and Shadi A. (2015). Color enhancement of zebra Malawi cichlid (*Pseudotropheus zebra*) using carrot (*Daucus carota*) as Feed Additive. Journal of Fisheries and Aquatic Science 10 (2): 128-131.
28. Amar, E.C.; Kiron, V.; Satoh, S. and Watanabe, T. (2001). Influence of various dietary synthetic carotenoids on bio-defense mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res., 32 (1): 162-163.
29. Kalinowski, C.T.; Robaina, L.E.; Palacios, H.F.; Schuchardt, D. and Izquierdo, M.S. (2005). Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture, 244: 223-231.
30. Choubert, G.; Cravedi, J.P. and Laurentie, M. (2005). Pharmocokinetics and bioavailabilities of 14C-ketocarotenoids, astaxanthin and canthaxanthin in rainbow trout, *Oncorhynchus mykiss*. Aquaculture Research, 15: 1526-1534.
31. Baker, R.T.M.; Pfeiffer, A.M; Schöner, F.J. and Smith-Lemmon, L. (2002). Pigmentation efficacy of astaxanthin and canthaxanthin in fresh-water reared Atlantic salmon, *Salmo salar*. Animal Feed Science and Technology, 99: 97-106.

تأثير بعض الإضافات الغذائية على تحسين اللون فى أسماك الكوى (سيبرينس كاربيو)

أحمد مسعد عزب، حسن مشحوت محمد خلف الله، هانى ماهر
شعبة علوم البحار والأسماك - قسم علم الحيوان- كلية العلوم (بنين) - جامعة الأزهر- القاهرة

المستخلص

يهدف هذا البحث إلى إجراء تجربة توضح تأثير بعض الإضافات الغذائية مثل أوراق وجذور اللفت، ورق العنب وجذور الجزرعلى تحسين الألوان فى أسماك الكوى (سيبرينس كاربيو) لإنتاج سلاله محسنة من هذة الأسماك.

تم تصميم التجربة بتقسيم الأسماك الى 8 مجموعات موزعة على 16 حوض زجاجى (40x30x100)، بكثافة 10سمكة/حوض بواقع 2 حوض لكل معامله. غذيت مجاميع الأسماك على غذاء أساسى لمدة شهرين قبل بداية التجربة. ثم غذيت كل مجموعة من الأسماك لمدة 6 أشهرعلى عليقة مختلفة. حيث غذيت المجموعة الضابطة على الغذاء الأساسى، المجموعة لأولى غذاء به 5% أوراق وجذور اللفت، المجموعة الثانية غذاء به 5% ورق العنب، المجموعة الثالثة غذاء به 5% جذور الجزر، المجموعة الرابعة غذاء به 5% ورق العنب + جذور الجزر، المجموعة الخامسة غذاء به 5% ورق العنب + أوراق وجذور اللفت، المجموعة السادسة غذاء به 5% جذور الجزر + أوراق وجذور اللفت،، والمجموعة السابعة غذاء به 5% أوراق وجذور اللفت+ ورق العنب+ جذور الجزر. هذا وقد خلصت النتائج إلى ما يلى:

أوضحت النتائج أن أعلى كمية من اللون الأحمر سجلت بعد 30، 60، 90 , 120يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% أوراق وجذور اللفت+ ورق العنب+ جذور الجزر. وأقل كمية سجلت بعد 30يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% ورق العنب+ جذور الجزر وبعد 60، 90, 120 يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% جذور الجزر.

بينت النتائج أن أعلى كمية من اللون الأصفر سجلت بعد 30، 60 يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% ورق العنب+ جذور الجزر، وبعد 90 , 120يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% جذور الجزر، وأقل كمية سجلت بعد 30يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% أوراق وجذور اللفت + جذور الجزر، وبعد 60يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% أوراق وجذور اللفت ، وبعد 90 يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% أوراق وجذور اللفت+ ورق العنب+ جذور الجزر.

أوضحت النتائج أن أعلى كمية من مجموع الألوان سجلت بعد 30، 60، 90, 120 يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% جذور الجزر و لكن أقل كمية سجلت بعد 30يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% ورق العنب وبعد 90 ,120يوم فى مجموعة الأسماك التى تغذت على عليقة تحتوى على 5% أوراق وجذور اللفت.

ونخلص من هذا البحث الي أن اللون الأحمر فى أسماك الكوى (سيبرينس كاربيو) يكون أفضل ما يمكن عندما تتغذي الأسماك على عليقة تحتوى على 5% من خليط كل الإضافات(جذور الجزر + ورق العنب + أوراق وجذور اللفت). واللون الأصفر يكون أفضل ما يمكن عندما تتغذي الأسماك على عليقة تحتوى على 5% من جذور الجزر لمدة تزيد عن 30 يوم. أما مجموع الألوان فى أسماك الكوى يكون أفضل ما يمكن عندما تتغذي الأسماك على عليقة تحتوى على 5% من جذور الجزر أو جذور الجزر + أوراق وجذور اللفت.