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Research Article

Effect of safflower (*Carthamus tinctorius*), beetroot (*Beta vulgaris*), and scarlet firethorn (*Pyracantha coccinea*) fruit on fillet and skin pigmentation of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Using beetroot, safflower, and scarlet firethorn with ten treatments, three replications, and 600 fish weighing roughly 230±10 gr that were established in a CRD experimental design for 45 days, this study evaluated the pigmentation of the fillet and skin of rainbow trout. Three groups underwent treatments in which dietary sources of plant pigment were given, while a control group did not receive any plant-based pigmentation additive. Five fish from each replication were randomly chosen and euthanized on day 45 of the experiment to evaluate the skin and fillet colors. Three criteria -L* (Brightness), a* (Redness), and b* (Yellowing) were used to evaluate the colorimetric indices after cutting a piece of fillet with skin on the lateral of the body and cooling it for 24 h. According to the findings, there was no significant difference between the experimental beetroot groups and skin color groups for fillets ($p>0.05$). However, the beetroot group of 10% had the highest percentage of the fillet and skin redness index, while the group without beets had the highest percentage of the yellowing and brightness index. The findings demonstrated that the addition of safflower improved skin discoloration compared to the control group ($p<0.05$). Scarlet firethorn was not significantly different in the experimental groups from the control ($p>0.05$). In conclusion, the supplementation of the rainbow trout diet with 10% safflower resulted in better fillet coloration.

Keywords: Pigmentation, *Oncorhynchus mykiss, Carthamus tinctorius, Beta vulgaris, Pyracantha coccinea*

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Introduction

Nutrition is essential to produce healthy and high-quality seafood in the aquaculture industry and it accounts for a large share of production costs. The color of rainbow trout fillet indicates the quality and biological health of the product and is very influential on the marketability (Zhonggao *et al.,* 2005; Yeganeh *et al*., 2022). The body color of living organisms is a function of two factors: genetics and nutrition. Body color in fish is mostly due to the presence of chromatophores containing pigments in tissues. Therefore, pigments in feed resources play an important role in determining the color of fish (Fuji, 2000). According to the high price of synthetic pigments and the increasing demand for natural materials like natural pigments, efforts to use natural carotenoids have increased in the animal nutrition, food, and cosmetics industries (Karimifar *et al.*, 2022). Today, plants are widely used as a source of natural pigments and research on how to use these substances is in progress (Mashalchi *et al.*, 2000).

The extraction of natural pigments from beetroot (*Beta vulgaris*) is known as an edible dye in various industries and its intensity is higher than many synthetic dyes. The presence of various flavonoids and carotenoids compounds in beetroot, in addition to the presence of a suitable dye known in the industry as E162, is known as a powerful antioxidant and a high anti-stress agent in animals and humans (Clifford *et al.*, 2015). The pigments in beets are rich in red betacyanins and yellow betasanthin. These two groups of pigments are very soluble in water (Fakhari and Baghipour, 2010). Cartamine dye, found in safflower flowers (*Carthamus tinctorius* L.), is used as a food coloring and dyeing of fabrics and silks (Esendal, 2001). Cartamine with the chemical formula C21H22O11 is lipophilic and insoluble in water and alcohol (Liu *et al.,* 2005). Studies showed that safflower flowers contain many flavonoids such as quercetin and pigments including iso-carthamine, safflamin C and A, safflor yellow A, and hydroxysafflor A (Sato *et al.*, 2005; Zhaoa *et al.*, 2005). It has been reported that scarlet firethorn (*Pyracantha coccinea*) contains various flavonoid compounds in different parts of the plant (roots, stems, leaves, flowers, and fruits) during the reproductive stages. The flavonoid compounds in its fruit include hyperoside, isoorientin, isoquercitrin, pyracanthoside, quercetin, quercetin-3-rut-7-glc, rutin, and vitexin-2A-rha (Fico *et al*., 1999). Also, it has been reported that the diet containing B. vulgaris leaf powder in the common carp led to better growth and survival performance (Amiri *et al*., 2019). The results of another research indicated that the inclusion of 2 and 4% red beet juice was more reliable for suitable coloration and growth with which 4% was found the most reliable amongst studied ratios (Asadi Sharif *et al*., 2014). Various studies have been performed on the use of plant resources to improve the color of fish fillets and skin, some of which are mentioned below:

Talebi *et al.* (2013) examined the effect of red pepper on growth, pigment accumulation, and blood factors of rainbow trout and reported that in terms of pigment accumulation, red pepper produced the most color change among Treatments. The results showed that the use of plant materials in the diet of rainbow trout is effective and cost-effective. Kop and Durmaz (2008) compared the discoloration of cichlid skin under the influence of natural pigments such as red algae and astaxanthin and reported an increase in red skin in both cases. Aysun *et al.* (2010) investigated the effect of carrots and red peppers as sources of natural pigments on cichlid pigmentation and reported that food containing natural pigments had an effect on cichlid pigmentation, but had no effect on conversion ratio and fish growth rate. Buyukcapar *et al.* (2007) investigated the accumulation of rainbow trout pigments with parsley and red pepper carotenoids and reported that the synthetic astaxanthin caused the highest carotenoid accumulation in rainbow trout Fillet.

Materials and methods

Research location and culture conditions

This research was conducted in 2018, in a CRD experimental design for 45 days. The steps of maintenance, preparation of breeding ration, treatments, feeding and breeding of rainbow trout, and measuring the color of fish were performed in Khojir cold-water fish research station located in the east of Tehran. The experimental fish were housed in two sheds measuring 100 meters long and 15 meters wide, which contained 30 fiberglass tanks that were used to breed experimental fish. The height of the tanks was 85 cm, the end of the tank was 50 cm and the opening of the tank was 75 cm and the height of the tanks was 3 meters from the ground. The amount of water in each tank was 170 liters. Each tank had one valve at the top and two valves at the bottom. Water came in from the top valve of the tank and came out of the bottom valve of the tank and was circulated in a circular style day and night. The second valve under the tank was used to drain waste and leftover uneaten food and to create oxygen for the water, which was opened and closed several times during the day. There were 20 pieces of fish in each tank. The water supply system was supplied through a water well. Physicochemical parameters such as water and ambient temperature, feeding and disinfection of tanks and water, were completely the same for all experimental groups and based on standard principles of rainbow trout farming.

Test method

600 pieces of rainbow trout weighing 230±10 g (Pre market size) were purchased and placed in 30 experimental units (10 treatments and 3 replications) in a completely randomized design. Accordingly, the

treatments were: (1) Basic ration (Control); (2) Safflower diet with three levels of 5, 10, 15% safflower+basic diet; (3) beetroot ration with three levels of 5, 10, 15% beetroot+basic ration; (4) firethorn fruit ration with three levels of 5, 10, and 15% scarlet firethorn+basic diet.

The diet used

Dieting was performed according to the NRC Nutrition Catalog (1993) and by UFFDA rationing software (Table 1) after determining the number of nutrients in basic foods and experimental materials based on fish requirements.

Table 1: The dry mater-based Chemical composition of practical diets.

Foodstuffs	Control (0%)	Safflower (%)			Beetroot (%)			Scarlet firethorn (%)		
		5	10	15	5	10	15	5	10	15
Fish powder	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
Soybean meal	14	14	14	14	14	14	14	14	14	14
Wheat flour	7	7	7	7	7	7	7	7	7	7
Oil	12	12	12	12	12	12	12	12	12	12
Corn gluten	10	10	10	10	10	10	10	10	10	10
Mineral supplement [1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin supplement [2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Molasses	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Bran	15	10	5	0	10	5	0	10	5	0
Beetroot	0	0	0	0	5	10	15	0	0	0
Safflower	0	5	10	15	0	0	0	0	0	0
Scarlet firethorn	0	0	0	0	0	0	0	5	10	15
Total food	100	100	100	100	100	100	100	100	100	100
Nutrients (calculated)										
Combustible combustible energy (Kcal/Kg)	3390	3345	3340	3465	3330	3330	3330	3392	3422	3395
Crude protein (%)	37.33	39.07	36.66	37.41	38.96	38.96	39.49	35.9	34.78	33.03
Crude fat (%)	11.20	10.90	11.40	11.10	10.80	10.80	10.75	10.84	10.55	10.14
Crude fibers (%)	3.85	3.90	3.70	3.80	4.10	4.10	4.10	4.65	5.51	6.29
Dry matter (%)	94.93	64.60	94.35	94.17	95.02	95.02	95.26	94.98	95.83	95.06
Ash (%)	11.00	11.50	12.00	8.00	12.00	12.00	11.80	11.00	10.71	10.52
Humidity (%)	5.07	5.40	5.65	5.83	4.98	4.98	4.74	5.07	5.02	4.17

[1] Vitamin supplement composition (IU / kg of food): Vitamin A 1600000, Vitamin D3400000, Choline Chloride 12000, Niacin 4000, Riboflavin 8000, Pyridoxine 4000, Folic Acid 2000, Vitamin B12 8000, Inositol 20000, Vitamin C60000, Vitamin K32000, Vitamin E 40000.

[2] Mineral supplement composition (g / kg of food): zinc 12.5, iron 26, manganese 15.8, copper 4.2, cobalt 0.48, selenium 2, iodine 1.

Estimation of pigmentation of fillet and skin

For this purpose, from each replication, five fish were randomly selected for slaughter on day 45. After weighing, a part of the Fillet was cut between the dorsal fin to the pectoral fin along with the skin. Each sample was placed in ice with numbered nylons and frozen for 24 hours. Then it was evaluated by Minolta CR-400 colorimetric device based on three indicators L (light intensity), A (redness), and B (yellowing) in two parts of each Fillet and skin (Ünal Şengör *et al.*, 2019).

Data analysis method

The data of this experiment were evaluated based on a completely randomized design (CRD). After adjusting the data and performing the initial calculations in Microsoft Excel software, the GLM procedure of SAS software was used for statistical analysis of the research data. In cases where there was a significant difference between the treatments, the comparison of the mean of the treatments was performed using LSMEANS command, PDIFF statement, and Tukey test at a 5% probability level.

Results

Rainbow trout Fillet and skin colorimetry

The results for rainbow trout Fillet and skin colorimetry are shown in Table 2. Analysis results for beetroot; Showed that the highest index of Fillet redness was related to the treatment of 10% of beetroot and the highest index of yellowing and brightness of Fillet was related to the control treatment, although the difference between all treatments in the studied indicators was significantly different. ($p<0.05$). Also, the highest index of skin redness was related to the 10% beetroot treatment, and the highest index of yellowing and lightness of the skin was related to the control treatment. Among all treatments in the studied indices was not significant at the level of 5% ($p>0.05$). Examination of the results revealed that in the case of safflower; Numerically, there was no significant difference between the treatments of redness, yellowing, and brightness of the Fillet color ($p>0.05$).

Table 2: The effect of different experimental groups on colorimetric indices of rainbow trout meat and skin.

Experimental groups	Flesh			Skin		
	L*	a*	b*	L*	a*	b*
Control	5.14	1.47	10.11	40.83	-2.04	9.19
Safflower (5%)	60.33	8	19.17	66.83 ab	-2.83	17.00
Safflower (10%)	59.67	8.5	17.17	59.17 b	.050	9.50
Safflower (15%)	59.83	8.16	18.17	62.67 ab	-2.00	11.7
Beetroot (5%)	60.3	7.5	19.8	66.8	-2.3	8.2
Beetroot (10%)	59.2	8.5	18.0	54.0	-1.8	11.5
Beetroot (15%)	59.7	7.3	19.3	65.3	-2.2	5.7
Scarlet firethorn (5%)	48.04	2.76	8.95	39.79	-1.85	8.87
Scarlet firethorn (10%)	48.58	3.40	8.56	37.70	-1.84	10.44
Scarlet firethorn (15%)	49.90	2.89	9.01	43.01	-1.88	10.58
P-Value	0.253	0.249	0.407	0.484	0.945	0.447
SEM	0.58	0.34	0.33	1.20	0.13	0.45

In each column, the means with non-common English letters are statistically significantly different from each other ($p\leq0.05$) and the means without English letters are not significantly different from each *other*. *p*-value: probability level (significance level), SEM: mean standard error, L*: brightness; a*: redness; b*: yellowing

Regarding skin discoloration, the results of Table 2 showed that statistically, the treatment of 10% safflower on the factors of redness, yellowing, and L * or skin brightness was significantly different from other treatments ($p \leq 0.05$) (Figs. 1 and 2). According to the results of Table 2, in relation to scarlet firethorn; fillet brightness index (L*), fillet red/green index (a*), Fillet yellow/blue index (b*), skin brightness index (L*), red/green skin index (a*) and yellow/blue index Skin (b*) was not affected by different treatments ($p<0.05$).

Figure 1: Color change of rainbow trout skin fed by safflower (A: 10% treatment, B: 15% treatment, C: 5% treatment).

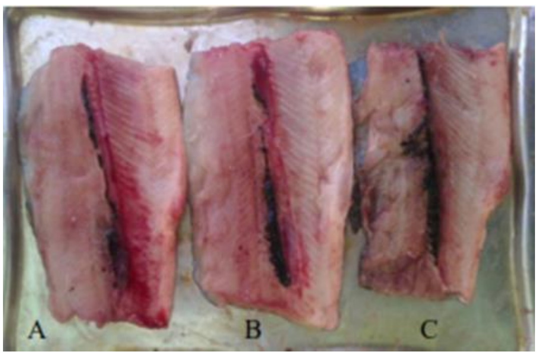

Figure 2: Color change of rainbow trout meat fed by safflower (A: 10% treatment, B: 15% treatment, C: 5% treatment).

Discussion

The purpose of selecting the source of pigment in this study was to create color in the skin and flesh of rainbow trout, after using it in food and reviewing the results, it was found that:

In the case of safflower

After using it in feed and reviewing the results, it was found that numerically, the red color index of Fillet is related to treatments 10, 15, 5, and control, respectively. Also, the yellowness and brightness index of Fillet color was related to control treatments, 5, 15, and 10%, respectively. There was no statistically significant difference between the treatments ($p>0.05$). The results of the recent study are consistent with the experience of Lorenz (1998) who added 3, 6, and 9% levels of red bell pepper powder to the goldfish diet. In the study of Diler and Gokoglu (2000) who added levels of 0, 2.5, and 5% red pepper to the diet of rainbow trout, a significant difference was observed between the treatments that caused the pigmentation of fish Fillet. This result may be due to differences in pigment source, culture time, and other farming conditions. Regarding the color change of skin, the results showed that numerically, the red index of skin color is related to treatments 10, 15, 5, and control, respectively. However, due to the fact that rainbow trout have green skin, the experiment showed that the highest green color is related to the control treatment and the lowest is related to the 10% safflower treatment. Also, numerically, the yellow index of skin color was related to control treatments, 5, 15, and 10%, respectively. As the results showed, statistically, the treatment of 10%

safflower on the L* factor or skin brightness was significantly different from other treatments ($p \leq 0.05$). In a study by Ghiasvand and Shapouri (2006) who added white carrots and red peppers to their diets, they found no significant effect between treatments that did not match the results of a recent study, the cause of this discrepancy could be due to tested fish and the type of food used. On the other hand, in a study conducted by Mashalchi *et al*. (2010), 200 mg/kg of *Dunaliella salina* was given to the white scar fish (*Astronorus ocellatus*). The colorimetric test showed that there was a significant difference in the skin color of the fish. This result is consistent with the results of a recent study.

In the case of beetroots

Nnumerically, the red color index of Fillet is related to treatments 10, 5, 15 and control, respectively. Also, the yellowness and brightness index of Fillet color was related to control treatments, 5, 15, and 10%, respectively. The results of the recent study are consistent with the experiences of Ninwichian *et al.*(2020) who added 2, 4 and 6% of tomato powder to the goldfish diet. In the study of Diler and Gokoglu (2000) who added levels of 0, 2.5 and 5% red pepper to the diet of rainbow trout, there was a significant difference between the treatments that caused the pigmentation of fish Fillet. This result can be due to differences in pigment source and culture time and other culture conditions. Regarding color change of skin, the results showed that numerically the skin redness index is related to treatments 10, 15, 5 and control, respectively. However, due to the fact that rainbow trout have green skin, the experiment showed that the highest green color are related to the control treatment and the lowest is related to the 10% beetroot treatment. Also, numerically, yellowing index, skin color brightness was related to control treatments, 5, 15 and 10%, respectively. However, no statistically significant difference was observed between the experimental treatments ($p > 0.05$). The research of Ghiasvand and Shapouri (2006) who added white scarlet, carrot and red pepper to their diets stated that they did not find a significant effect between treatments, which is consistent with the results of a recent study. On the other hand, in a study conducted by Lee *et al*. (2010), levels of 0, 8, 16% pepper were given to *Zacco platypus,* Colorimetric experiments showed that with these levels, there was a significant difference in the skin color of the fish. This result contradicts the results of a recent study, which may be due to the type of fish tested and the source of pigment, culture time, and other farming conditions.

In the case of Scarlet firethorn

After using Scarlet firethorn in the diet and reviewing the results, it was found that in terms of brightness, redness, and yellowing of Fillet and skin, although the 10% treatment is numerically better, there is no significant difference between all treatments (*p*>0.05). The results were consistent with studies by Ghiasvand and Shapouri (2006) who added carrots and red peppers to the diet of scarlet white fish, and Lorenz (1998) who added red bell pepper powder to the goldfish diet. However, the findings of this study are consistent with the results of Lee *et al*. (2010) study, which examined the effect of red pepper on the skin pigmentation of *Zacco platypus* and observed a significant difference between the pigmentation between treatments. But the findings of this study were inconsistent with the findings of Mashalchi *et al*. (2010) who used the alga *Dunaliella salina* to feed on white scar fish (*Astronorus ocellatus*), which may be due to the type of fish, the source of the pigment and the duration of feeding.

The results of this study on the effect of beetroot on skin color and carcass of rainbow trout showed that the highest Fillet redness index was related to the treatment of 10% beetroot, although the difference between all treatments, in this case, was not significant. Also, the highest index of skin redness was related to the 10% treatment of beetroots, which did not show a statistically significant difference between the treatments. Findings of this study regarding the effect of safflower on skin color and Fillet of rainbow trout showed that there was no significant difference in Fillet color of the studied treatments but it was seen that there was a significant difference in skin brightness for 10% treatment safflower compared to the control group. As a result, the 10% level had the best redness of the Fillet and skin. The results of this study on the effect of scarlet firethorn on the skin color and carcass of rainbow trout indicated that there was no significant difference in the brightness of skin and Fillet. However, since the condition of treatments of 5 and 10% of scarlet firethorn was better numerically and at the same time their consumption in the whole period was much better than the control treatment, it is possible to use it in the diet of rainbow trout.

The results showed that in all studied treatments, 10% safflower treatment gave the best results.

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