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Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout

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Abstract

Effects of carotenoid sources on pigmentation, sensory properties and fatty acid composition of rainbow trout (*Onchorhynchus mykiss*) were investigated. The fish (120.51 \pm 0.75 g) were fed with diets containing 1.8% marigold flower, 5% red pepper, 70 mg kg⁻¹ commercial astaxanthin and compared with a control group for 60 days. Commercial astaxanthin provided the highest carotenoid accumulation in the fish, and this was followed by red pepper and marigold flower (p < 0.05). Dietary carotenoid sources did not significantly affect fatty acid composition of the fish fillets. Trout muscle coloured with commercial astaxanthin was more preferred than the others by the sensory panellists.

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1. Introduction

Consumers are becoming more concerned about how fish are produced and which type of feed ingredients are used. Fish nutrition has an important impact on several parameters directly influencing the quality of fish, some of which are colour and appearance, sensory property and nutritional quality. The need for improved knowledge of fish nutrition is therefore of great importance.

The colour of salmon flesh is one of the most important quality parameters (Sigurgisladottir, Torrissen, Lie, Thomassen, & Hafsteinsson, 1997) because consumers have a preference for red or pink-coloured products of salmonid fishes (Gormley, 1992; Hatano, Takahashi, Takama, & Nakajima, 1987; Ostrander, Martinsen, Liston, & McCullough, 1976; Rounds, Clenn, & Bush, 1992; Sigurgisladot-

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tir, Parrish, Lall, & Ackman, 1994; Skonberg, Hardy, Barrows, & Dong, 1998). Therefore feeding fish with carotenoid pigments is regarded as the most important management practice for marketing of farmed salmon (Moe, 1990). Carotenoids are responsible for the typical colour of salmonid muscle, and especially astaxanthin, one of the carotenoids, is the most efficient one used for salmonid pigmentation (Ando, Osada, Hatano, & Saneyoshi, 1992; Storebakken & No, 1992; Torrisen, Hardy, & Shearer, 1989). In addition to pigmentation, carotenoids also have some other significant benefits to human beings; they decrease the risk of some cancer cases, cardiovascular diseases, and some other diseases (Gaziano & Hennekens, 1993; Mayne, 1996; Ziegler, 1989).

Fish are unable to synthesize carotenoids *de novo* (Goodwin, 1984), and these compounds must be obtained through the diet. Carotenoid pigments can be produced commercially and are commonly used for pigmentation of salmonids. However, alternative natural carotenoid sources have also been studied because of public concerns about the use

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of synthetic additives. Red pepper and marigold flower, which are cheap, abundant, and rich in carotenoid pigments, can be considered as alternative sources. While no study on marigold flowers has yet been encountered in the literature, it was shown that red pepper could be used for pigmentation of salmonids (Carter, Palafox, & Islas, 1994; Yanar, Kumlu, Çelik, Yanar, & Tekelioğlu, 1997), However, effects of these carotenoid sources on sensory properties and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*) have not yet been studied. Moreover, present knowledge is largely restricted to commercial carotenoids.

The objective of this study was to investigate changes in fatty acids, sensory properties and pigmentation of rainbow trout fed with red pepper and marigold flower, and compared with commercial astaxanthin.

2. Materials and methods

2.1. Experimental procedure

The experiment was conducted in floating net cages $(1 \text{ m} \times 1 \text{ m} \times 1 \text{ m})$ for 60 days in the Sir Dam Lake/Turkey. Thirty rainbow trout, *Onchorhynchus mykiss* $(120.51 \pm 0.75 \text{ g})$ in weight) per cage were stocked in two replicates for each experimental feed. The fish were hand-fed at 3% of their biomass twice a day for 60 days. Throughout the experiment, temperature, dissolved oxygen level and pH of the rearing water were 10.14 ± 1.91 °C, $8.49 \pm 2.10 \text{ mg/1}$ and 7.53 ± 0.91 , respectively.

2.2. Feeding trial

Four different pelleted feeds used in the experiment were: Diet CO for the control group (basal diet, no addition of carotenoids), Diet AS, supplemented with 70 mg kg^{-1} the commercial astaxanthin (Carophyll-pink, Hoffman La Roche, Switzerland), Diet RP, supplemented with 5% red pepper meal, Capsicum annum (containing 70 mg kg^{-1} total carotenoid) and Diet MF, supplemented with 1.8% marigold flower meal, Tagetes erecta (containing 70 mg kg⁻¹ total carotenoid). The basal diet contained 10% crude fat, 13% crude ash, 45% crude protein, 88% dry matter, 3% crude fibre (supply was obtained from Pinar AS, Turkey). To moderate the dietary protein imbalance, due to plant additives between the diet groups, fish meal, including 65% protein, was added to the required level. The ingredients were first turned into a homogeneous doughy consistency by adding water and converted into pellet form by being pressed through a sieve of 4 mm holes in a grinding machine. The pellets were stored in refrigerator containers at -20 °C. Pellets were thawed before they were given to the fish.

2.3. Proximate composition analysis

In both plants, ash and moisture contents were, determined as described by AOAC (1984) methods. Lipid content was determined by the method of Bligh and Dyer (1959). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldhal's method ($6.25 \times N$) (AOAC, 1984). Crude cellulose was determined by the AOAC (1990) method (Table 1).

2.4. Carotenoid analysis

The carotenoid content of samples was extracted by the method of Torrissen and Nævdal (1984). Four fish samples were used for carotenoid analysis. The total carotenoid concentration in the flesh was determined spectrophotometrically in acetone using $E_{(1\%,1 \text{ cm})} = 1900$ (Foss, Storebakken, Schiedt, Liaen, & Austreng, 1984) at 474 nm, and for both plant meals, $E_{(1\%,1 \text{ cm})} = 2500$ (Schiedt & Liaaen-Jensen, 1995) at 450 nm. The total carotenoid concentrations of red pepper and marigold flower meal were determined to be 1400 and 3890 mg kg⁻¹, respectively, and these amounts were taken into account while adding to the basal diet (Table 1).

2.5. Fatty acid analysis

Lipids were extracted by the method of Bligh and Dyer (1959) and stored under nitrogen at -20 °C for further analyses. The fatty acids in the total lipid were saponified into the free form by saponification with 0.5 N methanolic NaOF, followed by esterification with 14% BF₃ (w/v) in methanol (IUPAC, 1979). Esterified samples were analysed using a Thermoquest Trace gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column $(30 \text{ m} \times 0.25 \text{ mm i.d.}, 0.20 \text{ }\mu\text{m film thickness of polyethyl-})$ ene glycol) (Supelco Inc., Bellefonte, PA, USA) and a flame-ionization detector (FID). Helium (30 ml min^{-1}) was used as the carrier gas. The samples were injected at 120 °C. After 2 min the temperature was raised at $5 \,^{\circ}\text{C} \,^{\text{min}^{-1}}$ to 220 $\,^{\circ}\text{C}$ where it was kept for 8 extra minutes. The temperatures of the injector and the detector were set at 240 and 250 °C, respectively. Fatty acid methyl esters were identified by comparing their retention times with those of the commercial fatty acid methyl ester standards. The relative concentrations of each fatty acid were expressed as percentages of their total.

Table 1 Proximate composition and total carotenoid content of natural carotenoid sources

Carotenoid sources	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Total carotenoid content (mg kg ⁻¹)
Marigold flower	14.17	9.35	15.3	3890
Red pepper	13.68	14.13	19.10	1400

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