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Original Article

Effects of Natural (Red Bell Pepper & Tomato) and Synthetic (Astaxanthin & B-Carotene) Pigments on Flower Horn Fish (*Cichlasoma Sp.*) Blood Parameters

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ABSTRACT

Objective: In this survey, we have checked the effects of natural (red bell pepper and tomato) and synthetic (astaxanthin and β-carotene) pigments on bloods factors of Flower horn (*Cichlasoma sp.*). **Methods:** Five treatments (three replicates/treatment) were employed. Fishes were separated in fifteen equal groups and were fed for eight weeks with diets containing10 g kg^{-1} red bell pepper and tomato and 100 mg kg^{-1} astaxanthin and β-carotene. At the end of trial blood samples were taken from 5 fish that caught randomize by cutting the caudal stem. **Results:** Results of blood factors showed there is significant differences between WBCs, RBCs, Hb, HCT and Lymphocytes between treatments ($p<0.05$). The best result observed in treatment fed with red bell pepper. Between The factors of Monocytes and Neutrophils, there was no significant difference between treatments ($p>0.05$). The results showed that the use of red bell pepper in diets for Flower horn is effective and profitable in its ration.

1.INTRODUCTION

One of the most absorptive features of aquatic creatures is arguably their shining display of colors. The source of their colors comes from the foods in their natural encompassing (Bricaud *et al*. 1998). Fish color is primarily dependent on the presence of chromatophores that contain colored pigments. Carotenoids, which are produced primarily by phytoplankton and plants, are divided into two groups as carotenes and xantofilles. Although more than 600 carotenoids in nature have been defined, only a few of them are used in animal feeds, pharmaceuticals, cosmetics and food coloring (Bricaud *et al*. 1998; Ong & Tee 1992). The carotenoids in the normal and abnormal environment fishes are available and have beneficial effects on fish, including increased growth, boost the immune system by increasing antibodies are produced (Goodwin 1980; Zamanpor *et al*. 2005). Today, research on the use of pigments is prosper (Gouveia *et al.* 1997; Raymundo *et al*. 2005). Pigments play an important role in the diet of animals and animal feed industries. Carotenoids are group of natural pigments and micronutrient elements that is necessary to be added to fish diets (Talebi *et al*. 2013). Astaxanthin has been shown to increase egg survival and percentage of fertilized eggs, to protect eggs against extreme conditions (Craik 1985) and to stimulate growth (Torrissen 1984). A number of studies on astaxanthin were published by Christiansen who assayed the issue from the viewpoint of the influences of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (Christiansen & Torrissen 1997), growth and survival of Atlantic salmon juveniles (Christiansen & Torrissen

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1996), and first-feeding fry (Christiansen *et al*. 1995), antioxidant status and immunity in Atlantic salmon (Christiansen *et al*. 1995) and effects of astaxanthin and vitamin A on growth and survival during the first feeding of Atlantic salmon (Christiansen *et al*. 1994). Astaxanthin also is the major carotenoid pigment that found in aquatic animals (Guerin et al. 2003; Christiansen & Torrissen 1997) and important biological functions, including preventing the oxidation of unsaturated essential fatty acids PUFA, protection against the negative effects of ultraviolet light to produce vitamin A, an immune response, controls of growth and reproductive behavior and improve (Lorenz & Cysewski 2000; Torrisen *et al*. 1989). β-carotene is a pigment with chromatic spectrum yellow up to red and act as an antioxidant, vitamin A precursors (Gross 1991) and enhance immune system (Amar *et al*. 2003; Nakano *et al*. 1995). Besides pigmentation, carotenoids are involved in certain physiological functions, as informed by (Nakano *et al*. 1995) who studied the biochemical characteristics of the liver and blood in rainbow trout fed a diet supplemented by red yeast *Phaffia rhodozyma* containing astaxanthin as its principal carotenoid pigment or synthetic astaxanthin.

Skin of tomato is containing 10.8g protein, 25.6g ash, 30g fiber in Per 100g and rich source of antioxidant and color compounds such as 12mg lycopene, 16mg vitamin C and 0.3mg β-carotene in per 100g wet weight. (Knoblich *et al*. 2005; Toor & Savage 2005; Tsatsaronis & Boskou 1975). Carotenoids found in tomatoes and other antioxidant compounds such as vitamins E, A and C cause tomatoes for high power to absorb free radicals that prevent of their reactions with biological macromolecules (Sanchez-Moreno *et al*. 2006). Red bell peppers is one of main vegetables, family of the *Solanaceae*, genus *Capsicumand* the species are *Capsicum annum*. Red bell peppers are a rich source of vitamins A and C that compounds are strong antioxidants such as vitamin C and carotene. Capsanthin in pepper, especially, common pepper has a chemical composition that stimulates the immune system and helps in attacking infectious agents (Mobli & Piraste 1994). In addition, red pepper (Capsicum annum), which are abundant and rich in carotenoid pigment, could be considered alternative sources. It was shown that red pepper could be used for pigmentation of Flower horn (Ghiasvand & Shapouri 2006; Talebi *et al*. 2013).

In this survey we used the ornamental fish commerce by several names, mainly flower horn, Luohan or Kirin cichlid (Nico *et al.* 2007; Ng & Tan 2010). Flower horns constitute a man-made hybrid complex, reportedly composite of parental species of the Neotropical cichlid genera "*Cichlasoma*", *Amphilophus* and *Paraneetroplus* (Vieja) (Nico *et al.* 2007; Ng & Tan 2010; McMahan *et al*. 2010). Due to its man-made origin this variety of fish does not have scientific name. Some of specialists think that this fish was created by physical-chemical shock induced mutations (Nico *et al.* 2007). Flower horns are named for their distinctive head shape and vivid colours, and have become favorite aquarium fish over the last decade. Flower horn is one of the most important commercial species cichlid. As a result of this, an intensive farming of the flower horn is increasing and economically important in several countries including Thailand where the flower horn is widely cultured (McMahan *et al*. 2010). This fish is a warrior fish and so jurisdiction even into other flowers. Therefor if some of them live together, they scotch each other. If fish sick, they lose their color while they are sickness and become dark. Therefore it's better to use carotenoids that increase immune system of fishes. In this survey has focused on the replacement of the currently used natural pigments by synthetic pigments. Only a few studies have reported on the relationship between pigments and immune system and even no investigations about effects of these pigments on blood factors have been published. In the present study, the effects of diets including various pigmentation on the growth and blood factors of Flower horn were examined.

2. MATERIALS AND METHODS

2.1. Experimental Procedure and Feeding Trial:

In this research, 75 cichlid fishes (Flower horn), which was purchased of Hatchery and education center Qom, were used. Their average living body weight was 20 ± 0.5 g, and average total length was 10 ± 1.5 cm. Their sex was not taken into consideration. Fiftheen aquariums, which had dimensions as 70 · 40 · 30 cm, were used. There were 5 fishes in each aquarium. The aquariums were placed side by side in two lines with an artificial photoperiod of 12L: 12D. 1 heater in each aquariums was used. The fish were fed twice in the morning and afternoon by 3 percent of the biomass.

The five different treatments (three replicates/treatment) used in the experiment were as follows:

Treatment 1: fed with commercial normal diet (control)

Treatment 2: Diet supplemented with astaxanthin 100 mg/kg

Treatment 3: Diet supplemented with β-carotene 100 mg/kg

Treatment 4: Diet supplemented with red bell pepper 10 g/kg

Treatment 5: Diet supplemented with tomato 10 g/kg

Table 1.
Nutritional composition of control diet (basal diet)

Chemical composition	Percentage (%)
Crude protein	47
Crude fat	8.5
Crude ash	10.5
Dry matter	6

The experimental diets were made by adding the requisite amounts of the different pigment sources to a commercial diet. For Preparation ration, natural pigments was dried in the shade, and powder. Initially 1g of liquid oil with pigments are well mixed and eked powder of basal diet. The amount of natural and synthetic pigments added to ration, were 10 g/kg and 100 mg/kg, respectively. For parallel actual food, 9g and 900mg of cellulose as filler added to treatment 2, and 1g of liquid oil eked control. Then the daily food of each aquarium with a digital scale was weight and packed. While water temperature was measured every day, pH values were measured in every 2 days for observing water parameters. At the end of experiment, Blood tests were done at the end of the period that included RBCs, WBCs, HCT, Hb, IgM, Lym and Mono.

2.2. Preparation of the Blood Samples:

Blood samples were taken from 5 fish that caught randomize. Time of fish handling was less than 1 min per fish. Whereupon, the total capture time avoided capture stress effects on analyzed parameters and no anesthetic was used (Pickering *et al*. 1982). The sampling was performed 20 hours after the last feeding during the forenoon, the samples were taken by cutting the stem caudal veins immediately after catching and stunning amount of 2cc. Blood taken from the fish into micro test tubes containing a drop of heparin (anticoagulant) was poured and shake gently until completely mixed blood and heparin (Jamalzade *et al*. 2001). Measurements of red blood cells (RBC) and white blood cells (WBC) by soluble Lewis, neubauer, Hemoglobin (Hb) was measured with by instrumental methods use SYSMEXLYS, Hematocrit (HCT) was determined by spinning blood samples in heparinized capillary tubes in micro-hematocrit and micro-centrifuge Hettichby far (14,000 rpm). For calibration and testing control used Binding Site of England was made.

2.3. Statistical Analysis

Statistical analysis consisted of one-way ANOVA, using the possibility level of 0.05 for refusal of the null hypothesis. All statistical analysis was performed using SPSS 19.0 and Excel 2013 software for Windows.

3. RESULT

Given the importance of water physicochemical parameters, including dissolved oxygen, temperature, pH and its effect on feeding, breeding all the time these factors were carefully controlled (Table 2). Results of water quality parameters, no significant difference was found during the breeding period ($p > 0.05$).

Table 2:
Water physicochemical parameters

Factor	Average	Min.	Max.
Oxygen	7.12±0.379	6.53	7.42
Temperature	28.60 ± 0.13	27.84	30.12
pH	7.9 ± 0.09	6.56	8.31

In this experiment, there was significant differences between blood factors including RBCs, Hemoglobin, Hematocrit, WBCs and Lymphocytes ($p<0.05$), but between Monocytes and Neutrophils, there was no significant differences between treatments (Table 3). Most RBCs, WBCs, Hb, HCT and Neu was relevant to treatment of red pepper. Most Mono and Lym, respectively related to tomato and astaxanthin. The Lowest RBCs, Hb, HCT and Lym-related control, and WBCs related tomato and the lowest Mono related to treatment β-carotene.

Table 3.
Mean blood parameters of the fishes fed various diets

Treatments Parameters	Control	Tomato	Red bell pepper	β-carotene	Astaxanthin
RBC (× 10 /μL)	881111±123012^{c}	1223421±176653^{b}	1438723±212078^{a}	1243213±141112^{b}	1312127±178.312^{ab}
WBC (/μL)	4212.9±836.5^{b}	4127.7±933.9^{b}	9738.6±1812.8^{a}	4315.1±799.3^{b}	9123.6±1016.8^{a}
Hb (gr/dl)	7.33±1.99^{ab}	9.16±1.33^{a}	10.23±1.98^{a}	9.53±1.12^{a}	9.43±0.96^{a}
Lym (%)	51.29±14.03^{c}	75.11±19.16^{ab}	87.14±13.81^{a}	73.29±8.53^{ab}	89.28±4.91^{a}
HCT (%)	26.33±5.99^{c}	34.79±5.12^{ab}	43.29±11.15^{a}	38.92±7.30^{ab}	33.89±6.39^{ab}
Neu (%)	2.32±1.04^{a}	2.83±1.29^{a}	3.11±1.58^{a}	2.02±1.17^{a}	2.58±0.79^{a}
Mono (%)	2.41±1.12^{a}	2.98±0.12^{a}	1.82±0.91^{a}	1.54±1.00^{a}	2.49±0.83^{a}

Data are presented as Mean ± SE. Each replicate consists of measurements from 5 fish. The means with different letters in each column denote a significant difference (P<0.05)

4. DISCUSSION

It's very important to rises immune system for fish production, since its impairment leads to disease circumstance or death (Maule *et al*. 1989). The use of immune stimulants can increase munitions against diseases, upgrade activities in the nonspecific defense mechanism (Anderson 1992). Nutrition has an efficacy on health and immune responses of fishes as referred by some authors (Landolt 1989; Blazer 1992; Lall & Olivier 1993). Fish are often exposed to stressors under culture conditions, which cause a series of physiological responses, known as stress, which are divided in primary, secondary and tertiary responses (Wedemeyer 1996; Barton 2002). Primary responses are mediated by the neuro-endocrine system with a fast splash of stress hormones, catecholamine and cortisol into the fish circulatory system. Catecholamines (adrenaline and noradrenalin) are released and synthesized through cromafin cells and cortisol by the interrenals cells. Secondary responses are the different biochemical and physiological effects correlated with stress and mediated by the stress hormones. Tertiary responses affect the animal as a whole, compromising growth, disease resistance and reproductive success. The effects of stress can be minimized through rising immune system (Rehulka 2000). Carotenoids are known to have a positive role in the mediator metabolism of fish (Segner *et al*. 1989). Fish, like other animals can't produce carotenoids in normal conditions, so the carotenoids should be added as a food supplement (Wozniake 1996). In nature, carotenoids have been implicated in various incumbencies such as pigmentation, antioxidant activity, immune stimulation and reproduction, and they play a positive role in intermediary metabolism (Segner *et al*. 1989; Torrisen *et al*. 1989). In addition, all fish species do not show the same pathways for the metabolism of carotenoids, and therefore, there is no global transformation of carotenoids in fish tissues (Chatzifotis *et al*. 2005). Pigments also increase the immune system and rise the resistance which with findings of Bjerkeng *et al*. (2000), Torrissen *et al*. (1996), Amaninejad *et al*. (2009) and Talebi *et al*. (2013) was similar. Therefore, in the present study, some physiology stress parameters were analyzed to try the effect of pigments in Flower horn feed. Values for Hb, RBC, WBC, HCT and total Lymphocyte, Monocyte and Neutrophil of Flower horn fed with the experimental diets for 8 weeks. Talebi *et al* (2013) surveyed Effect of red bell pepper and astaxanthin on Growth, Pigmentation and Blood Factors of Rainbow Trout and they expressed there was significant difference between the experimental treatments (p<0.05). The highest weight gain was displayed by 55mg/kg red pepper, followed by the groups of 33mg/kg red pepper, 44mg/kg red pepper, astaxanthin and control group (P<0.05). The most SGR, BWI, GR and survival rate related to 55mg/kg red pepper. Also Results showed that coloration of the fish was fed with red pepper significant change in their meat than the control were created and she revealed that red bell pepper increased immune system resistance. Ghiasvand & Shapouri (2006) examined effects of natural (red bell pepper and tomato) and synthetic (astaxanthin and β-carotene) pigments on colour of the Albino Oscar (*Astronotus ocellatus sp*., Agassiz, 1831) and she showed a significant correlation between treatments fed diets containing different source carotenoid with other treatments (P<0.05). The treatment of astaxanthin showed higher concentrations of carotenoids, a relatively changed of color was observed in the skin of other fish that were fed containing natural pigments. In this survey we proved that red bell pepper has positive affective on immune system, and in previous studies have proved that this vegetable increase growth parameters and coloration (Ghiasvand & Shapouri 2006; Talebi *et al*. 2013), then it can be alternative of synthetic pigments. Amaninejad *et al*, (2009) acclaimed that carotene in algae cause increased immune system resistance.

Treatments in this study, which were fed with red pepper, had high immune system compared to control diet. Most RBCs, WBCs, Hb, HCT, Lym and Neu showed in treatment red bell pepper and astaxanthin. The result of this survey is oppose with Rehulka (2000) that essayed effect of astaxanthin on rainbow trout blood parameters, it was found that the fish fed a diet containing 49.8 mg astaxanthin/kg for 84 days had similar growth and significantly ($P<0.05$) lower red blood cell count (RBC: 1.06 vs. 1.15 T/l), hematocrit (PCV: 0.386 vs. 0.422), hemoglobin (Hb: 71.8 vs. 76.5 g/l) and mean cell hemoglobin concentration (MCHC: 0.19 vs. 0.18) when compared to fish fed a diet without astaxanthin supplement. The fish fed the astaxanthin-containing diet had lower ($P<0.05$) plasma triacylglycerol (TGL: 3.69 vs. 4.85 mmol/l), lower ($P<0.01$) plasma calcium (Ca^{2+}: 2.75 vs. 2.92 mmol/l) and significant ($P<0.01$) negative correlation between the levels of plasma cholesterol and plasma uric acid ($r_=-0.960$ vs. $r_=-0.489$). Mac Carthy et al. (47) number of RBC (1.7-1.2) ×106 per cubic millimeter count, hemoglobin 9-7 gr/dl and hematocrit 32-45% reported. Haley and Weiser (1985) reported the average number of RBCs (1.5±0.16) ×106 per cubic millimeter count in rainbow trout. Nakano et al. (1995) had studied the effects of red yeast containing astaxanthin and synthetic astaxanthin on the serum enzyme activities GPT ALT and GOT AST. As the author asserts, the levels of both enzymes in the fish fed a diet containing red yeast or synthetic astaxanthin were significantly lower than those of the fish fed a control diet. The mean amount of serum lipid peroxide in the fish given a diet containing red yeast and synthetic astaxanthin was also lower than that of the control fish.

The plant pigment was found to be more efficacious than astaxanthin although they contained equal amounts of carotenoids. The logical reason for this may be differences in carotenoid sources. This development is important for joinery aquaculture. In conclusion, treatments that fed with red pepper in terms of coloration, growth (Ghiasvand & Shapouri, 2006) and immune resistance were in better than the control (Ghiasvand & Shapouri 2006; Talebi *et al*. 2013). Also plant pigments were more cheap can be tabernacle to synthetic carotenoids. According to this study, red pepper can be useful, it should be mentioned that can't be used for high doses of plant pigments. The main reason for this has been considered due to containing high level cellulose of the plant and can decrease or stop the growth (Talebi *et al*. 2013). It is essential to research the other variables that may be effective on the blood parameters, such as the species of fish, the size of fish, type of pigments, and the duration of feeding on pigment sources.

REFERENCES

Amaninejad P, Emadi H, Emtiazjoo M, Hosseinzadehsahafi, H (2009) Effects of Dunaliella microalgae (*Dunaliella salina*) on different immune indices (complements c, and antioxidant capacity) in rainbow trout (*Oncrohynchusmykiss*). Scientific and Research Journal of Marine Biology. Islamic Azad University, Ahvaz. 6:3-21.

Amar E.C, Kiron V, Satoh S, Watanabe T (2003) Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss, Walbaum*) associated with dietary intake of carotenoids from natural products. Fish and Shellfish Immunology. 16:527-537.

Anderson D.P (1992) Immune stimulants, adjuvants, and vaccine carriers in fish: application to aquaculture. Annual Review of Fish Diseases. 2:281-307

Barton B.A (2002) Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology. 42:517-525.

Bricaud A, Morel A, Babin M, Allali K, Claustre H (1998) Variations of light absorption by suspended particles with Chlorophyll a concentration in oceanic (case 1) waters: analysis and implications for bio optical models. Journal Geophysical Research. 103(13):31033-31044.

Blazer V.S (1992) Nutrition and disease resistance in fish. Annual Review of Fish Diseases. 2: 309-323.

Bjerkeng B, Storebakken T, Liaaen-Jensen S (2000) Response to carotenoids by rainbow trout in the sea: resporption and metabolism of dictary astaxanthin and canthaxanthin. Aquaculture. 91:153-162.

Chatzifotis S, Pavlidis M, Jimeno CD, Vardanis G, Sterioti A, Divanach P (2005) The effect of different carotenoid sources on skin coloration of cultured red porgy (Pagrus pagrus). *Aquaculture Research.* 36:1517-1525.

Christiansen R, Torrissen O.J (1996) Growth and survival of Atlantic salmon (*Salmo salar L.*) fed different dietary levels of astaxanthin. Aquaculture Nutrition. 2:55-62.

Christiansen R, Torrissen O.J (1997) Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar L.*). Aquaculture. 153:51-62.

Christiansen R, Glette J, Lie O, Torrissen O.J, Waagbo R (1995) Antioxidant status and immunity in Atlantic salmon (*Salmo salar L.*), fed semi-purified diets with and without astaxanthin supplementation. Journal of Fish Diseases. 18:317-328.

Christiansen R, Lie O, Torrissen O.J (1995) Growth and survival of Atlantic salmon (*Salmo salar L.*), fed different dietary levels of astaxanthin: first-feeding fry. Aquaculture Nutrition. 1:189-198.

Christiansen R, Lie O, Torrissen O.J (1994) Effect of astaxanthin and vitamin A on growth and survival during first feeding of Atlantic salmon (*Salmo salar L.*) Aquaculture Fish Manager. 25:903-914.

Craik J.C.A (1985) Egg quality and egg pigment content in salmonid fishes. Aquaculture. 47:6168.

Goodwin T.W (1980) The biochemistry of carotenoids, 2 nd ed., Chapman and Hall, London. 291 pp.

Gouveia L, Gomes E, Empis L (1997) Use of Chlorella vulgarisin diets for rainbow trout (*Oncorhynchus mykiss*) to enhance pigmentation of muscle. Journal Applied Aquaculture-Culture. 7:61-70.

Ghiasvand Z, Shapouri M (2006) The Effect of Natural and synthetic Pigments on the colour of the Albino Oscar (*Astronotus ocellatus sp.*, Agassiz, 1831). Journal of Sea Biology. 2:75-83.

Gross J (1991) Pigments in vegetables. New York. Van Nostrand Reinhold. 8: 99-102.

Guerin M, Huntley M.E, Olsubjectaizola M (2003) Haematococcus astaxanthin: applications for human health and nutrition. Trends in Biotechnology. 21: 210-216.

Haley P.J, Wieser M.G (1985) Erytrocytevolum distribution in rainbow trout. Veterinary Research. 46(10):2210-2212.

Jamalzade H, Keyvan A, Jamili S.H, Oryan S.H, Saeedi A (2001) Study of some blood indices on *Salmo trutta Caspius*. Journal of Fisheries. 1:25-26.

Knoblich M, Anderson B, Latshaw D (2005) Analyses of tomato peel and seed byproduct and their use as a source of carotenoids. Journal of the Science of Food and Agriculture. 85:1166-1170.

Pickering A.D, Pottinger T.G, Christie P (1982) Recovery of the brown trout (*Salmo trutta L.*), from acute handling stress: a time-course study. Journal of Fish Biolog. 20: 229-244.

Landolt M (1989) The relationship between diet and the immune response of fish. Aquaculture. 79:193-206.

Lall S.P, Olivier G (1993) Role of micronutrients in immune response and disease resistance in fish. In: INRA Ed., Fish Nutrition in Practice Les collogues. 61:101-118.

Lorenz R.T, Cysewski G.R (2000) Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. Trends Biotechnology. 18:160-167.

MacCarthy D.H, Stevenson J.P, Roberts M.S (1973) Some blood parameters of Rainbow trout (*Salmo gairdneri*). Journal of Fish Biology. 5:1-8.

Maule A.G, Tripp R.A, Kaattari S.L, Schreck C.B (1989) Stress alters the immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology. 120:135-142.

McMahan C.D, Geheber A.D, Piller K.R (2010) Molecular systematics of the enigmatic Middle American genus Vieja (Teleostei: Cichlidae). Molecular Phylogenetics and Evolution. 57:1293-1300.

Mobli M, Piraste B (1994) Vegetable production. Isfahan University of Technology. 877 pp.

Nakano T, Kanmuri, T, Takeuchi M (1995) Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin. Journal of Agriculture and Food Chemistry. 43:1570-1573.

Nico LG, Beamish W.H, Musikasinthorn P (2007) Discovery of the invasive Mayan Cichlid fish (*Cichlasoma urophthalmusn*) in Thailand, with comments on other introductions and potential impacts. Aquatic Invasions. 2:197-214.

Ng H, Tan H.H (2010) An annotated checklist of the non-native freshwater fish species in the reservoirs of Singapore. Cosmos. 6:95-116.

Ong A.SH, Tee ES (1992) Natural sources of carotenoids from plants and oils. Meth Enzymology. 213:142-167.

Raymundo A, Gouveida L, Batista A.P, Empis J, Sousa I (2005) Fat mimetic capacity of Chlorella vulgaris biomass in oil in water food emulsions stabilized by pea protein. Food Research Institute. 38:961-965.

Rehulka J (2000) Influence of astaxanthin on growth rate, condition and some blood indices of Rainbow Trout (*Oncorhynchus mykiss*). Aquaculture Elsevier. 190:27-47.

Sanchez-Moreno C, Plaza L, Ancos B, Cano M.P (2006) Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. Food Chemical. 98:749-756.

Segner H, Aren P, Von-Poeppinghaussen K, Schmidt H (1989) The effect of feeding astaxanthin to *Oreochromis* *niloticus* and *Colisa labiosa* on the histology of the liver. Aquaculture. 79:381-390.

Talebi M, Khara H, Zoriehzahra J, Ghobadi Sh, Khodabandelo A, Mirrasooli E (2013) Study on effect of red bell pepper on growth, pigmentation and blood factors of rainbow trout (*Oncorhynchus mykiss*). World Journal of Zoology. 8 (1):17-23.

Torrissen O.J (1984) Pigmentation of salmonids-effect of carotenoids in eggs and start-feeding diet on survival and growth rate. Aquaculture. 43:185-193.

Torrisen O.J, Hardy R.W, Shearer K.D (1989) Pigmentation of salmonids-carotenoid depositions and metabolism. Critical Reviews in Environmental Science and Technology. 1(2):209-225.

Toor R.K, Savage G.P (2005) Antioxidant activity in different fractions of tomatoes. Food Research Institute. 35:487-494.

Tsatsaronis G.C, Boskou D.G (1975) Aminoacid and mineral salt content of tomato seed and skin waste. Journal of the Science of Food and Agriculture. 29:421-423.

Torrisen O.J, Hardy R.W, Shearer, K.D (1989) Pigmentation of salmonids-carotenoid deposition and metabolism. Critical Reviews in Environmental Science and Technology. 1:209-225.

Torrissen O.J, Hardy R.W, Shearer K.D, Scott T.M, Stone F.E (1996) Effect of dietary lipid on apparent digestibility coefficients for canthaxanthin in Rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 88:351-362.

Wedemeyer G.A (1996) Physiology of fish in intensive culture systems. Chapman & Hall, New York. 232pp.

Wozniake M (1996) The role of carotenoids in fish. Protectio Aquarum ET Piscatoria. Acta Societatis Botanicorum Poloniae. 22:65-75.