A Review of Astaxanthin as a Carotenoid and Vitamin Source for Sea Bream

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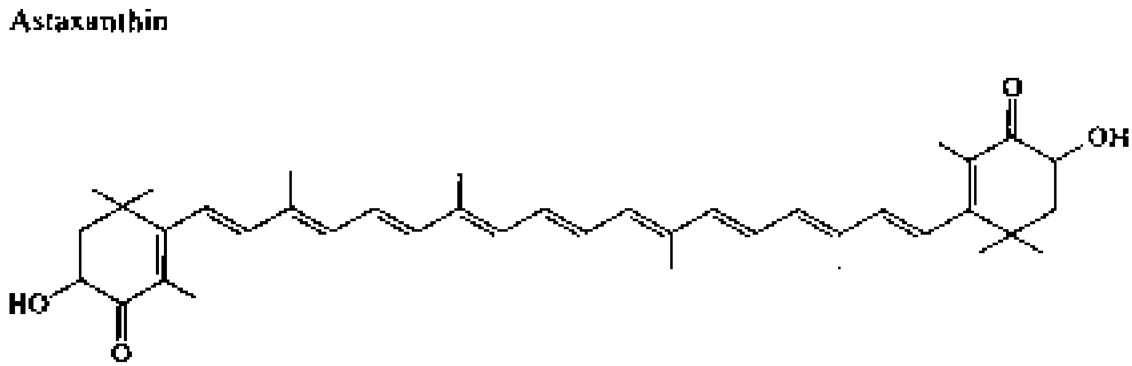
Sea Bream (*Chrysophrys major*, *Pagrus major*, Tai, Red Snapper) are highly prized for the pigmentation of their skin, which is due primarily to the carotenoid, astaxanthin (Tanaka et al, 1976). Sea Bream are unable to synthesize astaxanthin *de novo,* only plants and protists (bacteria, algae, fungi) are capable of synthesizing carotenoids (Steven, D.M. 1948), therefore the pigment must be available in either their native habitat or manufactured diet. In the natural aquatic environment, astaxanthin is biosynthesized in the food chain within microalgae or phytoplankton, the primary production level. The microalgae are consumed by zooplankton, insects or crustaceans which accumulate astaxanthin, and in turn are ingested by Sea Bream and other fish (Kitahara 1984 and Foss et al., 1987).

Katayama et al 1965, discovered that the faded color of cultured Sea Bream was caused by the lack of astaxanthin in their artificial diet. Sea Bream that are cultured without a supply of astaxanthin contain only 5% of the carotenoid content of their wild counterpart. Carotenoids are lost from the flesh and skin due to insufficient dietary intake, metabolic degradation and excretion. The stomach contents of wild Sea Bream were analyzed and found to contain *Squilla oratoria* and other crustacea that supply the necessary astaxanthin. Sea Bream which were provided with dietary carotenoids such as beta-carotene, lutein, canthaxanthin, and zeaxanthin were unable to convert these pigments to astaxanthin. Thus, to yield the reddish pigmentation of cultured Sea Bream, it is necessary to provide a source of dietary astaxanthin (Tanaka et al., 1976 and Katayama et al., 1972, Nakazoe et al, 1984).

Figure 1



The astaxanthin accumulated in the skin of Sea Bream is an esterified form of the carotenoid, meaning that fatty acids are bonded to the hydroxyl groups at either one or both of the ring structures (Nakazoe et al, 1984). The carotenoids in the skin are localized in the xanthophores while the non-carotenoid pigments are localized in erythrophores (Goodwin, 1984). The astaxanthin composition of *Haematococcus* algae meal (NatuRose™) and crustaceans (krill and crawfish) are also primarily an esterified form (Lambertsen and Braekken, 1971, Maoka et al., 1985, Foss et al., 1987), with *Haematococcus* having optically pure (3S, 3'S)-chirality (Grung et al., 1992 and Renstrom et al., 1981). In contrast, the free form of astaxanthin is present in the flesh of salmonids species such as trout and Atlantic salmon (Kitahara 1984 and Foss et al., 1987).

In an independent study conducted by Nisshin Flour Milling Co., trials were conducted to test the ability of *Haematococcus* algae meal to pigment the skin of sea bream. The carotenoid in the skin of the sea bream increased from 1.71 ppm to 7.9 $\mu g/cm^3$ after 100 days and was considered to be effective.

Ito et al. tested the effects of feeding fish different astaxanthin sources. Separate groups of fish weighing an average of 270 grams were fed either 100 ppm free astaxanthin or astaxanthin ester. In the group fed free astaxanthin, the carotenoid content of the skin improved for 1 month, but reached a saturation point and did not increase further. In the group fed astaxanthin ester, the carotenoids in the skin was significantly higher after the first and second sampling periods. After two months the group fed astaxanthin esters had a 1.7-fold higher astaxanthin content in the skin than the group fed free astaxanthin (13.23 mg/kg compared to 7.94 mg/kg, respectively). Thus, dietary astaxanthin esters are more efficiently utilized than free astaxanthin for deposition and coloration of skin.

In another report, Red Sea Bream (*Chrysophrys major*) were fed diets containing either β-carotene, zeaxanthin, lutein, canthaxanthin, free astaxanthin, or astaxanthin ester. Feeding either β-carotene or canthaxanthin resulted in a decrease in the carotenoid level of the integuments. Other groups of fish were fed a total of 21.4 mgs of zeaxanthin, 21.4 mgs. of lutein, and 18.5 mgs. of free astaxanthin resulting in total carotenoid accumulations of 482.1 μg, 256.2 μg, and 235.5 μg respectively. However, when only 9.68 mgs. of astaxanthin ester was fed, the total carotenoid accumulation was 542.0 μg. Thus, half as much dietary astaxanthin ester resulted in 2.3-fold higher carotenoid deposition than free astaxanthin (Nakazoe et al., 1984).

The principal sources of astaxanthin for commercially cultured Sea Bream have been processed crustacean wastes from krill, shrimp, crab and crawfish, the yeast *Phaffia rhodozyma*, or chemically synthesized astaxanthin. Crustacean waste products (oils and meals) generally contain less than 1000 ppm of astaxanthin, which necessitates exceedingly high inclusion rates (10-25%) into feeds for efficient pigmentation. Additionally, crustacean sources contain high amounts of moisture, ash and chitin which limits the percentage of these products that can be included in feeds. The other natural of astaxanthin is derived from *Phaffia rhodozyma*, however it produces only free astaxanthin (at concentrations of about 2000-4000 ppm), which has been demonstrated to be inferior for the pigmentation of Sea Bream.

***Haematococcus* algae meal (NatuRose™) produces astaxanthin esters at concentrations of 15,000 ppm and greater, the astaxanthin and its mono- and diesters from *Haematococcus* have optically pure (3S, 3'S)-chirality (Grung et al., 1992 and Renstrom et al., 1981). The astaxanthin ester composition of *Haematococcus* algae meal is similar to that of crustaceans (Lambertsen and Braekken, 1971, Maoka et al., 1985, Foss et al., 1987). Additionally, the composition of *Haematococcus* algae meal is balanced, and supplements the normal feed with essential nutrients Table 1.**

Other than pigmentation, studies indicate that carotenoids also have a biological function involved in growth and reproduction. It has been demonstrated that Atlantic salmon fry have an increased growth rate when carotenoids are supplied in their diet. The mobilization of carotenoids and their transport from the flesh to the skin and ovaries during maturation further indicates a role as a fertilization hormone, function in respiration, photoprotective element, stress protection from elevated temperatures or ammonia, and a provitamin A. A further role of carotenoids may be the protection of lipid tissues from peroxidation *in vivo*, since cold water fish such as salmonids have a high level of polyunsaturated fats in their membranes (Tacon A., 1981; Craik J., 1985; Torrissen O.J., 1984; Burton G.W., 1984). Furthermore, a report in World Aquaculture (Volume 23(3) September 1992, p.59) documents a study by Dr. Takeshi Watanabe who demonstrated the essential role of astaxanthin in the diet of red seabream broodstock. This may represent the first indisputable proof for the requirement of carotenoids for the growth and survival of eggs and larvae.

Interestingly, a recent groundbreaking study in Norway by Christiansen and his colleagues demonstrated that Atlantic salmon fry have a definitive growth and survival

requirement for astaxanthin their diet. Fish fed diets with astaxanthin below 5.3 ppm were found to have marginal growth, those fed levels above 5.3 ppm had significantly higher lipid levels accompanied by lower moisture levels. When fry were fed astaxanthin concentrations below 1 ppm, survival rates plummeted. More than 50% of the fry fed diets with less than 1.0 ppm astaxanthin died during the experimental period, survival of those groups receiving higher concentrations had survival rates greater than 90%. Thus, Atlantic salmon have the distinction as being the first salmonid species for which astaxanthin has been shown to be an essential vitamin, with absolute minimum levels being about 5.1 ppm. Higher astaxanthin levels of 13.7 ppm in the feed continued to improve the fish lipid levels another 20% to the plateau point. Their results also strongly suggested a provitamin A function for astaxanthin over the same fry-feeding period (Christiansen R., O. Lie, and O.J. Torrissen, 1995).

Table 1	**Typical**
protein	**23.62**
carbohydrates	**38.0**
fat	**13.8**
iron (%)	**0.73**
magnesium (%)	**1.14**
calcium (%)	**1.58**
biotin (mg/lb)	**0.337**
L-carnitine (ug/g)	**7.5**
folic acid (mg/100g)	**1.30**
niacin (mg/lb)	**29.8**
pantothenic acid (mg/lb)	**6.14**
vitamin B1 (mg/lb)	**2.17**
vitamin B2 (mg/lb)	**7.67**
vitamin B6 (mg/lb)	**1.63**
vitamin B12 (mg/lb)	**0.549**
vitamin C (mg/lb)	**58.86**
vitamin E (IU/lb)	**186.1**
ash	**17.71**

Summary

Studies with Sea Bream demonstrate that dietary astaxanthin esters result in pigment depositions of 1.7 to 4-fold higher than either free astaxanthin or other pigment sources. Other carotenoids such a beta-carotene, zeaxanthin, lutein, and canthaxanthin are not converted to astaxanthin and may impart an unfavorable color. Although the reason for the preference is not clear, astaxanthin esters are an effective and superior source of pigmentation for this species.

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