Effect of Different Astaxanthin Sources on Skin Pigmentation of Red Sea Bream (*Pagrus major*)

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Abstract: A study was conducted to investigate the effects of different dietary astaxanthin sources on skin pigmentation of red sea bream (*Pagrus major*). Red sea bream (initial weight 91.8 g) were randomly distributed in 60-*l* glass tanks and fed four different experimental diets supplemented with three kinds of astaxanthin sources, synthetic astaxanthin, *Phaffia* yeast and a marine bacterium (*Paracoccus* sp.), at a level of 30 mg astaxanthin/kg diet, and a diet without supplement was served as a control. Among the groups of fish fed astaxanthin diet, astaxanthin content in the skin of fish fed with *Paracoccus* sp. and *Phaffia* yeast were higher than fish fed with synthetic astaxanthin. The results demonstrated that astaxanthin derived from *Paracoccus* sp. and *Phaffia* yeast were effectively incorporated into the skin pigmentation of red sea bream and it might be suggested that the other carotenoids in *Paracoccus* sp. induced to enhance the skin pigmentation.

Key words: Red sea bream; Astaxanthin; *Paracoccus* sp.; *Phaffia* yeast

Introduction

Red sea bream, *Pagrus major*, is one of the most popular finfish for marine aquaculture in Japan. Due to its economic feasibility and the traditional food habits of the Japanese people, the aquaculture production of this fish is around 70.000－75.000 MT per year, being the second largest in Japan after *Seriola* group (Koshio 2002). This species is highly prized for the pigmentation of their skin, which is due primarily to the carotenoids, such as astaxanthin (Asx) (Tanaka et al. 1976). The market value of red sea bream is predominantly based on the visual appeal of their skin. Product appearance and quality implications play a significant role in maintaining the highest consumer acceptance. Shahidi et al (1998) associated the color of natural skin pigmentation with either acceptance or rejection by the consumer.

The skin color of red sea bream is important criterion. It has been found that this species loses their natural color when subjected to culture and this is caused by the lack of Asx in their artificial diet (Katayama et al. 1965). And the difference in skin color between cultured and wild red sea bream has lowered the price of cultured red sea bream. Therefore, to maintain skin color in farmed fish, the Asx should be supplemented in the diet.

Steven (1948) suggested that this species and other aquatic animal are unable to synthesize Asx *de novo* in their body, and only plants and protists (photosynthetic bacteria, algae, fungi) are capable of synthesizing carotenoid. In the natural aquatic environment, Asx is accumulated through the food chain from microalgae or phytoplankton, so called at the primary production level. Microalgae are consumed by zooplankton, insects or crustaceans which accumulate Asx, and they in turn are ingested

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by red sea bream and other fish (Kitahara 1984 and Foss et al. 1987).

Numerous trials have been conducted to improve fish pigmentation by using common Asx sources (Gomes et al. 2002; Choubert et al. 2006; Gouveia et al. 1998). In contrast, some new Asx sources have been discovered. *Paracoccus* sp. is a marine bacterium which produces Asx, and was suggested to be a candidate source of Asx (Yokoyama and Miki 1995). Therefore, the current experiment was designed to investigate the effects of supplementing red sea bream diet with some new potential Asx sources on skin pigmentation. The potential of *Paracoccus* sp. and *Phaffia* yeast as natural Asx sources was compared with synthetic Asx.

Materials and Methods

Experimental diets

A control diet was formulated to contain 42% crude protein and 13% crude lipid, without added Asx (Table 1). With this basal diet, three other diets were formulated to contain 30 mg Asx/kg diet from three different sources: synthetic Asx, *Phaffia* yeast and *Paracoccus* sp. Synthetic Asx was derived from Carophyll pink® 10%, and the Asx source which used in this study contains at least 10% Asx and consisted in stereoisomers (3*R*,3'*R*), (3*R*,3'*S*) and (3*S*,3'*S*) occur in a ratio of 1:2:1 (DSM Nutritional Product 1996; Bernhard 1990). *Phaffia* yeast product is generated from *Phaffia rhodozyma*. This product contained 4500 mg Asx/kg and was supplemented with 2000 mg ethoxyquin/kg. *Phaffia rhodozyma* contains predominantly (93%) the 3R, 3'R enantiomeric form of Asx and amounts to more than 70% of the total carotenoids are Asx (ECHCP 2002). *Paracoccus* sp. is one of the marine bacterium which produces Asx. Formerly, it was known as *Agrobacterium aurantiacum* and reclassified as *Paracoccus* sp. (Choi et al. 2005). The genus *Paracoccus* consists of Gram-negative, non-motile, rod-shaped and non-spore formers. Colonies are orange to red in color and capable of producing Asx (Lee et al. 2004). Asx content in *Paracoccus* sp. was 3300 mg Asx/kg and consisted in 3*S*,3'*S* isomers. *Paracoccus* sp. that used in this experiment was obtained in extract meal product and it is not alive bacterium. All of the ingredients were mixed and pelleted by using the laboratory pelletizer (AEZ12M, Hiraga-Seikakusho, Kobe, Japan), dried with a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan) and stored at 4℃. The diet was protected from light to avoid degradation of Asx.

Fish, experimental conditions, and feeding

Red sea bream, *Pagrus major*, were obtained from Seiho Suisan Co. Ltd. (Mie, Japan) and fed commercial common carp feed (CP 35.1%, CL 10.5%, and CA 9.6%) without supplemental Asx. Sixteen fish (initial weight 91.8g) were randomly distributed in each well-aerated 60-*l* glass tanks. The feeding trial was conducted in two replicates with re-circulated artificial seawater system (Sea Life®, Tokyo, Japan) at a flow rate of 700 − 800 *ml*/min. The water renewal rate in the system was 50% a week. Important

Table 1. Formulation of the experimental diet of red sea bream

Ingredients (g/100g)	Diets[1]			
	C	SA	PY	PR
Jack mackerel meal[2]	45	45	45	45
Soybean meal[3]	10	10	10	10
Wheat flour[4]	21.5	21.5	21.5	21.5
Pregelatinized starch	5	5	5	5
Pollock liver oil	5	5	5	5
Soybean oil	3.5	3.5	3.5	3.5
Mineral mix.[5]	1	1	1	1
Vitamin mix.[6]	3	3	3	3
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	5.4	5.37	4.37	4.49
Synthetic Asx	0	0.03	0	0
Phaffia yeast	0	0	0.67	0
Paracoccus sp	0	0	0	0.91

[1] Diets were recognized by their astaxanthin source: C = Control, SA = Synthetic Asx, PY = *Phaffia* yeast, PR = *Paracoccus* sp.
[2] Crude protein, 66%; crude lipid 13%.
[3] Crude protein, 45%; crude lipid 3%.
[4] Crude protein, 17%; crude lipid 4%.
[5] P-free mineral mixture (g/100 g dry diet) contains: NaCl, 5.0; $MgSO_4.7H_2O$, 74.5; $FeC_6HO_7.7H_2O$, 12.5; Trace element mix., 5.0; Cellulose, 3.0.
Trace element mix (mg/g) contains: $ZnSO_4.7H_2O$, 353; $MnSO_4.5H_2O$, 162; $CuSO_4.5H_2O$, 31; $AlCl_3.6H_2O$, 10; CoCl $6H_2O$, 1; KIO_3, 3; Cellulose, 440.
[6] Vitamins mixture (mg/100 g dry diet) contains: Vitamin B_1, 6.0; Vitamin B_2, 10.0; Vitamin B_6. 4.0; Vitamin B_{12}, 0.01; Vitamin C, 500.0; Niacin, 40.0; Ca-pantothenate, 10.0; Inositol, 200.0; Biotin, 0.6; Folic acid, 1.5; p-amino benzoic acid, 5.0; Vitamin K_3, 5.0; Vitamin A, 4000.0IU; Vitamin D_3, 4000.0IU.

water quality parameters such as temperature, pH and salinity were monitored daily and dissolved oxygen was measured fortnightly. All the parameters were observed to be within the acceptable limit for fish culture. Average daily water temperature was 19.8℃. The fish were hand-fed each diet three times a day near satiation for 12 weeks.

Sample collection and chemical analyses

Initial weight data were recorded at the beginning of the experiment and growth of fish was measured every 3 weeks subsequently. Feed consumption was recorded weekly. After 12 week feeding, the fish were sampled to assess growth response and to determine the feed intake. The diets were analyzed for proximate composition as described by Watanabe (1988).

Five fish from each tank were randomly sampled for the skin, muscle and liver analyses. The skin was collected by cutting around the dorsal fin, while muscles were minced by a centrifugal mill (Retsch ZM 100, Germany) fitted with a 0.25-mm screen. Liver samples were ground by mortar. These samples were collected and kept at −20℃ until analysis.

Total carotenoids content in diets, skin, muscle and liver was determined by spectrophotometer after extraction with acetone. For carotenoid extraction, sample was weighted and 60 *ml* acetone and some sodium sulphate anhydrous were added. The mixture was ground and filtered through glass microfibre filters (GF/A, whatman paper) and rinsed with chloroform to increase the boiling point of the mixture. After mixing and phase separation between diethyl ether and water in separatory funnel, the upper layer was taken and placed in a round bottle flask to evaporate in a rotary evaporator at 35℃. The extract was concentrated and dissolved in benzene. Total carotenoids concentration was calculated from the absorbance of the benzene solution according to the method of McBeth (1972). The absorbance was measured by spectrophotometer (Shimadzu, Inc. Kyoto Japan), at wavelength of 460 nm and 480 nm for yellow and red carotenoids, respectively. Total carotenoids was quantified by using an equation as follow:

$$\underset{(ppm)}{\text{Total carotenoid}} = \frac{ABS}{E^{1\%}_{1cm}} \times \frac{\text{Dilution volume } (ml)}{\text{Sample weight (g)}} \times 1000$$

ABS = Optical density
E = Extinction coefficient value=1900 (Red carotenoid in benzene)
=2500 (Yellow carotenoid in benzene)

Asx content of the diet, skin, muscle and liver was determined by HPLC. The sample extract which was still diluted with benzene was re-evaporated and then dissolved in 1 *ml* n- hexane and 20 μl was used for injected into HPLC (Shimadzu, LC-10 AD). This system consisted of a 110×4.6 mm Lichosorb SI-60 (GL Sciences Shimadzu, Inc. Kyoto Japan), with temperature of 35℃, using 20% acetone in 80% n-hexane as a mobile phase and a flow rate of 1 *ml*/min. The retention times and peak areas of Asx were compared with those obtained from standard Asx (F. Hoffman-La Roche AG, Switzerland).

Statistical Analysis

Means and standard deviations were calculated for all fish for each parameter measured. All data were tested for normality and homogeneity of variance. Differences among groups were determined by one way ANOVA. When appropriate, means were compared by Duncan's multiple range test. Statistical significance was tested at a 0.05 probability level.

Table 2. Results of proximate analysis and astaxanthin content in the experimental diet

Proximate analysis (%)	Diets[1]			
	Control	SA	PY	PR
Moisture	7.0	8.6	5.4	6.1
Crude protein	41.5	40.5	41.9	41.7
Crude lipid	13.2	12.8	13.3	13.8
Crude ash	8.3	8.1	8.4	8.4
Carotenoid contents (mg/kg)				
Total carotenoids	2.03	34.8	74.5	258
Astaxanthin	1.00	31.0	35.8	37.7

[1]Diets were recognized by their astaxanthin sources : SA = Synthetic Asx, PY = *Phaffia* yeast, PR = *Paracoccus* sp.

Results

No significant differences were found in feed performance, such as specific growth

Table 3. Effect of feeding carotenoids supplements on growth and feed utilization parameters after 12 weeks of experiment

	Diets			
	Control	Synthetic Asx	*Phaffia* yeast	*Paracoccus* sp.
Initial (g)	93.1 ± 1.8[1]	92.5 ± 0.3	92.0 ± 0.5	91.1 ± 0.4
Final weight (g)	202 ± 7.6[b2]	191 ± 2.3[a]	195 ± 3.2[b]	198 ± 13[ab]
Feed intake (g)	179 ± 0.3[c]	167 ± 5.9[b]	151 ± 6.1[a]	174 ± 10[c]
Growth[3] (g)	108 ± 5.8[a]	98.0 ± 2.1[a]	103 ± 3.8[a]	107 ± 22[a]
SGR[4] (g)	0.92 ± 0.0[a]	0.86 ± 0.0[a]	0.90 ± 0.0[a]	0.92 ± 0.1[a]
FGR[5] (g)	1.60 ± 0.1[b]	1.71 ± 0.0[c]	1.46 ± 0.0[a]	1.60 ± 0.2[b]

[1] Values (mean ± SD of 16 fish) in the same column not sharing a common superscript letter are significantly different ($P < 0.05$).
[2] Different letter indicates significant differences among groups ($P < 0.05$).
[3] Growth (g) = Final weight − initial weight.
[4] SGR: specific growth rate = 100 × (ln final weight − ln initial weight)/no.days.
[5] FGR: feed gain ratio = feed intake (g)/weight gain.

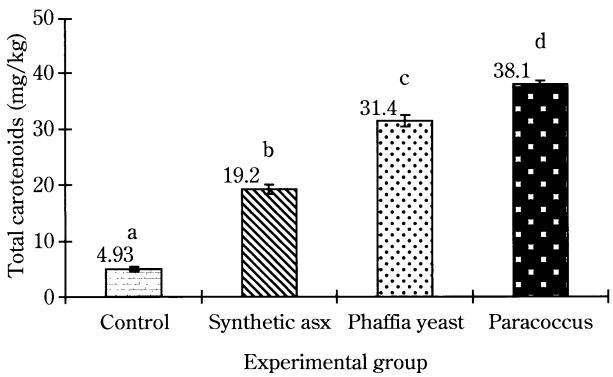

Fig. 1. Total carotenoid content in the skin of red sea bream after 12 weeks of experiment.

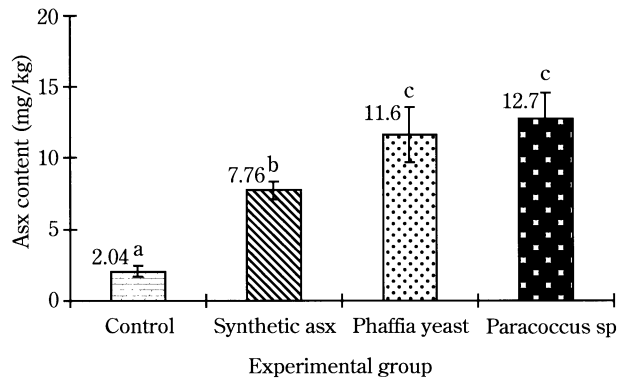

Fig. 2. Asx content in the skin of red sea bream after 12 weeks of experiment.

rate (SGR) and feed gain ratio (FGR) among the dietary treatments ($p > 0.05$) (Table 3). However, total carotenoids content in the skin of red sea bream fed the diets containing Asx from natural sources (*Paracoccus* sp. and *Phaffia* yeast) was significantly higher than that of fish fed synthetic Asx (ANOVA, $p < 0.05$) (Fig. 1). In contrast, total carotenoids content in the muscle and liver of red sea bream was not affected by the dietary Asx sources. The total carotenoids in the muscle of red sea bream fed control, synthetic Asx, *Phaffia* yeast and *Paracoccus* sp. diets were 1.5, 1.7, 1.2 and 1.0 mg/kg wet weight, respectively. On the other hand, the total carotenoid contents in the liver of red sea bream fed control, synthetic Asx, *Phaffia* yeast and *Paracoccus* sp. diets were 2.2, 2.5, 2.5 and 2.0 mg/kg wet weight, respectively. However, Asx contents were not detected in the muscle and liver.

The use of the different Asx sources had significantly ($p < 0.05$) effect on the Asx content in the pigmentation in skin of red sea bream (Fig. 2). Fish fed diet with Asx sources contained higher levels of skin Asx than fish fed control diet. Further, the skin Asx contents of fish fed *Paracoccus* sp. and *Phaffia* yeast (as natural Asx sources) were higher than that of fish fed the synthetic Asx ($p < 0.05$).

Discussion

Carotenoids are known to have a positive role in the intermediary of fish (Tacon, 1981; Segner et al. 1989), that can enhance nutrient utilization and may ultimately result in improved growth (Amar et al. 2001). In the present study, fish fed diets supplemented with Asx sources did not show any difference in growth and FGR from control group. These results are in accordance with another study carried out with *Pagrus pagrus* fed different carotenoid sources for 105 days (Kalinowski 2005) and agreed well with the growth performance of gilthead sea

bream (*Sparus auratus*) fed different carotenoid sources for 9 weeks (Katayama et al. 1976).

Carotenoid pigmentation in fish is affected by dietary Asx source, dosage level, duration of feeding and dietary composition (Bjerkeng 2000). Results on this experiment showed that fish fed diet supplemented with Asx resulted more reddish skin color; however, the skin was colorless in the fish fed control diet. Among the three sources of Asx, fish groups fed *Paracoccus* sp. and *Phaffia* yeast, derived from natural Asx, exhibited more reddish skin coloration than that of synthetic Asx, suggesting better utilization of the natural Asx. Numerous studies have shown that natural Asx sources were more suitable carotenoid sources in fish feed compared with synthetic Asx. For instance, Asx supplement from shrimp shell meal in *Pagrus pagrus* commercial diets could significantly improve skin pigmentation (Kalinowski et al. 2005). In another study with goldfish, the best coloration obtained, as ascertained by total carotenoids content, was achieved with using *Chlorella vulgaris* biomass, and the red hue was maximum when used *Haematococcus pluvialis* biomass (Gouveia et al. 2003; Harker et al. 1996). It was also reported that the presence of phospholipids in the algae might increase the absorption of carotenoids since dietary phospholipids stimulated the absorption of dietary fatty acids in post-larval turbot (Guerden et al. 1998).

Paracoccus sp., one of the natural Asx sources containing free Asx, was considered to be more efficient for skin pigmentation in fish from the result of current study (Yokoyama and Miki 1995). It is a new and interesting phenomenon because it was reported that the ester form of Asx was more highly accumulated in the skin than free form (Nakazoe et al. 1984; Lorenz 1998). Red sea bream fed a diet containing 100 mg/kg Asx in ester form presented a significantly higher carotenoid accumulation in the skin after one month feeding and 1.7-fold higher Asx content after two month feeding than the free form (Ito et al. 1986). However, it was reported that Australian snapper fed the diet supplemented with unesterified and esterified forms of Asx provided similar contents of Asx accumulated in the skin (Booth et al. 2004). The accumulation of Asx in the skin of fish fed *Paracoccus* sp. was higher than *Phaffia* yeast. It might be due to the form 3*S*,3'*S* of Asx produced by *Paracoccus* sp., which is known as the carotenoid of alga *Haematococcus pluvialis*, while *Phaffia* yeast contains only predominantly (97%) of the 3*R*,3'*R* enantiomeric form. *H. pluvialis*, as like *Paracoccus* sp., which contains 3*S*,3'*S* form was superior as Asx source for skin pigmentation of red sea bream (Guerin and Hosokawa 2001). In this experiment, fish fed *Phaffia* yeast could also accumulate Asx in the skin. It might be due to proper preparation to remove the cell wall before inclusion into the feed (Johnson et al. 1980). However, some studies on rainbow trout (Choubert and Heinrich 1993; Gouveia et al. 1996b) using a natural Asx, *H. pluvialis* biomass, showed no significant differences in flesh pigmentation from fish fed *Phaffia* yeast and synthetic Asx. This might be due to thickness of cell wall in *Phaffia* yeast. Johnson et al. (1980) demonstrated that the most efficient deposition of Asx in trout occurred when the cell wall of *Phaffia rhodozyma* was partially removed by enzymatic digestion.

In addition, higher Asx content in supplemented diets with *Paracoccus* sp. and *Phaffia* yeast (Table 2) lead to significantly higher Asx accumulation in skin than the fish administered diet with synthetic Asx. This finding is in agreement with the report of Bjerkeng (2000) who suggested that Asx accumulation in the skin was not only affected by carotenoid sources but by the dosage level. Among the experimental diets, total carotenoids content in the *Paracoccus* diet was higher than those of *Phaffia* yeast and synthetic Asx diets. Thus, it might be considered that the other carotenoids except Asx in the diet supplemented with *Paracoccus* sp. induced to enhance the accumulation of Asx more than the other diets.

This study showed that fish fed diets supplemented with Asx did not show any pigmentation in the muscle of red sea bream. Generally, red sea bream could not accumulate carotenoids in their muscle (Fujita et al. 1983; Ito et al. 1986). As like gilthead sea bream; it is well in

accordance with the market image that red sea bream has white muscle (Katayama et al. 1965). Whereas, hepatic Asx contents were not affected by the various dietary Asx sources.

The data obtained in this study demonstrated that *Paracoccus* sp. and *Phaffia* yeast might be better Asx sources to enhance the skin pigmentation in red sea bream.

References

Amar, E. C., V. Kiron, S. Satoh and T. Watanabe (2001) Influence of various dietary synthetic carotenoids on biodefense mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* **32** (Suppl. 1), 162-1.

Bernhard, K. 1990. Synthetic astaxanthin. The route of a carotenoid from research to commercialization. In: "Carotenoids: Chemistry and Biology", N. I. Krinsky et al. (editors), Plenum Press, New York, pp. 337-363.

Bjerkeng, B (2000) Carotenoid pigmentation of salmonid fishes recent progress. In: Cruz-Suárez, L. E., Rique-Marie, D., Tapia- Salazar, M., Overa-Novoa, M. A., y Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola 19-22 Noviembre, 2000. Mérida, Yucatán.

Booth, M., R. Warner-Smith, G. Allan, and B. Glencross (2004) Effects of dietary astaxanthin source and light manipulation on the skin color of Australian snapper *Pagrus auratus* (Bloch and Schneider, 1801). *Aquac. Res.* **35**, 458-464.

Choi, S., Y. Nishida, S. Matsuda, S. Adachi, H. Kasai, X. Peng, S. Komemushi, W. Miki and N. Misawa (2005) Characterization of β-caroten ketolases, CrtW, from marine bacteria by complementation analysis in *Escherichia coli*. *Marine Biotechnology*; **7**, 515-522.

Choubert, G., O. Heinrich (1993) Carotenoid pigments of the green alga *Haematococcus pluvialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. *Aquaculture* **112**, 217-226.

DSM Nutritional Products (1996) CAROPHYLL Pink 10% CWS. DSM Nutritional products Ltd. Bessel, Switzerland.

European Commission Health and Consumer Protection Directorate-General (ECHCP) (2003) Update of the opinion of the Scientific Committee on Animal Nutrition on the use of astaxanthin-rich *Phaffia rhodozyma* in feeding stuffs for salmon and trout.1-5.

Foss P., B. Renstrom and S. Liaaen-Jensen (1987) Natural Occurrence of enantiomeric and meso astaxanthin in crustaceans including zooplankton. *Comp. Biochem. Physiol.* **86B**, 313-314.

Fujita T., M. Satake, T. Watanabe, C. Kitajima, W. Miki, K. yamaguchi and S. Konosu. 1983. Pigmentation of cultured red sea bream with astaxanthin diester purified from Krill oil. *Nippon Suisan Gakkaishi*, **49**(12), 1855-1861.

Geurden, I., P. Bergot, L. Schwarz and P. Sorgeloos (1998) Relationship between dietary phospholipid classes and neutral lipid absorption in newly-weaned turbot, *Scophthalmus maximus*. *Fish Physiol. Biochem.* **19**, 217-228.

Gomes, E., J. Dias, P. Silva, L. Valente, J. Empis, J. B Gouveia and A. Young (2002) Utilization of natural and synthetic sources of carotenoids in the skin pigmentation of gilthead seabream, *Sparus aurata*. *E. Food Res. Tech.* **214**, 287-293.

Gouveia, L., E. Gomes and J. Empis (1996b). Potential use of a microalgal, *Chlorella vulgaris* in the pigmentation of rainbow trout, *Onchorhynchus mykiss* muscle. *Leben Unter Fors*, **202**, 75-79.

Gouveia,L.,G. Choubert, E. Gomes, P. Rema and J. Empis (1998) Use of *Chlorella vulgaris* as a carotenoid source for salmonids: Effect of dietary lipid content on pigmentation, digestibility and muscular retention. *Aquacult. Int.*, **6**, 269-279.

Gouveia, L., G. Choubert, E. Gomes, N. Pereira, J. Santinha and J. Empis (2002) Colouringation of gilthead seabream, *Sparus aurata* (Lin 1875), using *Chlorella vulgaris* microalga. *Aquacult. Res.*, **33**, 987-993.

Gouveia, L., P. Rema, O. Pereira and J. Empis J (2003) Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. *Aquaculture Nutrition*, **9**; 123-129.

Grung, M., F. M. L. D'Souza, M. Borowitzka and S. Liaan-Jensen (1992) Algal carotenoids 51, secondary carotenoids 2, *Haematococcus pluvialis aplanospores* as a source of (3*S*,3'*S*)-astaxanthin esters. *J. Appl. Phycol.*, **4**, 165-171.

Guerin, M. and H. Hosokawa (2001) Pigmentation of red seabream with natural astaxanthin derived from the alga *Haematococcus pluvialis*: comparison with synthetic astaxanthin. Aquaculture 2001 conference, World Aquaculture Society, Lake Buena Vista, Florida, January 2001.

Harker, M., A. J. Tsavalos and A. J. Young (1996) Autotrophic growth and carotenoid production of *Haematococcus pluvialis* in a 30 liter air-lift photobioreactor. *J. Ferment. Bioeng.*, **82**, 113-118.

Ito, Y., T. Kamata and M. Sameshima (1986) Studies on the improvement of body color of red sea bream *Pagrus major* by astaxanthin and astaxanthin dipalmitate. *Suisanzoshoku*, **34**, 77-80.

Johnson, E. A., T. G. Villa and M. Lewis (1980) *Phaffia rhodozyma* as an astaxanthin source in salmonids diets. *Aquaculture*, **20**, 123-134.

Kalinowski, C. T., L. E. Robaina, H. Fernandez-Palacios, D. Schuchardt and M. S. Izquierdo (2005) Effect of different carotenoid sources and their dietary levels on red porgy, *Pagrus pagrus*, growth and skin color. *Aquaculture*, **244**, 223-231.

Katayama, T., N. Ikeda and K. Harada (1965) Carotenoids in Sea Breams, *Chrysophrys major* Temminck and Schegel. *Nippon Suisan Gakkaishi*, **31**, 947-952.

Katayama, T., K. Shintani, M. Shimaya, S. Imai and C. O. Chichester (1972) The transformation of labeled

astaxanthin from the diet of Sea Bream, *Chrysophrys major* Temminck and Schegel, to their body astaxanthin. *Nippon Suisan Gakkaishi*, **38**, 1399-1403.

Kitahara, T. (1984) Carotenoids in the Pacific salmon during the marine period. *Comp. Biochem. Physiol.*, **78B**, 859-862.

Koshio, S. (2002) Red sea bream, *Pagrus major*. In: Webster, C. D., Lim, C. E. (Eds.), Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, New York, USA, pp. 51-63.

Lee, J. H, Y. S. Kim, T. J. Choi, W. J. Lee and Y. T. Kim (2004) *Paracoccus haeundaensis* sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, **54**, 1699-1702.

Lorenz, T. R. (1998) A review of astaxanthin as a carotenoid and vitamin source for sea bream. Naturose Technical Bulletin vol.052. Cyanotech, Hawaii, USA.

McBeth, J. W. (1972) Carotenoids from nudibranchs. *Comp. Biochem. Physiol.* **41B**, 55-68.

Nakazoe, J., S. Ishii, M. Kamimoto and M. Takeuchi (1984) Effects of supplemental carotenoid pigments on the carotenoid accumulation in young sea bream, *Chrysophrys major. Bull. Tokai Reg. Fish. Res. Lab.*, **113**, 29-41.

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

Shahidi, F., A. Metusalach and J. A. Brown (1998) Carotenoid pigments in sea foods and aquaculture. *Crit. Rev. Food Sci. Nutr.*, **38**, 1-6.

Steven, D. M. (1948) Studies on animal carotenoids. I. Carotenoids of the brown trout (*Salmo trutta* Linn.) *J. Exp. Biol.*, **25**, 369.

Tacon, A. G. J. (1981) Speculative review of possible carotenoid function in fish. *Prog. Fish-Cult.*, **43**, 205-208.

Tanaka Y., T. Katayama, K. L. Simpson and C. O. Chichester (1976) The carotenoids in marine red fish and the metabolism of the carotenoids in Sea Bream, *Chrysophrys major* Temminch and *Schegel. Nippon Suisan Gakkaishi*, **42,** 1177-1182.

Watanabe, T. (1988) Fish Nutrition and Mariculture. Japan International Cooperation Agency, Yokosuaka. 1988. 233 p.

異なるアスタキサンチン源のマダイ皮膚色素に及ぼす影響

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合成および天然由来の異なるアスタキサンチン（Asx）源のマダイの色揚げ効果について検討した。飼料中の Asx 含量を30 mg/kg となるように合成アスタキサンチン，パフィア酵母および Asx 産生海洋細菌（*Paracoccus* sp.）を添加した飼料をマダイ（91.8 g）に12週間給餌した。その結果，パフィア酵母および *Paracoccus* sp.を添加した飼料で，皮膚の Asx 含量が合成 Asx 添加区に比較し高くなった。なかでも，*Paracoccus* sp.を添加した区で最も高くなった。*Paracoccus* sp.には Asx 以外のカロテノイドも豊富に含まれることより，他の色素もマダイの色揚げに関与しているのではないかと推察された。