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# Muscle Coloration of Rainbow Trout with Astaxanthin Sources from Marine Bacteria and Synthetic Astaxanthin

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## Abstract

This study was performed to investigate the effects of marine bacteria (*Paracoccus* sp.) and synthetic astaxanthin (Asx) as Asx sources on coloration (skin and muscle) of rainbow trout (*Onchorhynchus mykiss*). In the 12-week trial duplicate groups of 30 fish (28.1 ± 0.3g) were fed one of the four experimental diets. Three experimental diets contained 30 mg Asx/kg diet of synthetic Asx, marine bacteria or combined synthetic Asx and marine bacteria. One diet was served as a control diet. The fish fed diet supplemented both marine bacteria and synthetic Asx provided the highest total carotenoids and Asx content in the skin and muscle.

**Keywords:** Rainbow trout; Asx sources; Marine bacteria; Synthetic Asx

## Introduction

One of the most important quality parameters of salmonid fish is their flesh color. Consumers prefer to select the salmonids that have more reddish or pink colored muscle. Carotenoids are responsible for the typical color of salmonid muscle, and especially Asx, one of the carotenoids, is the most efficient one used for salmonid coloration [1, 2]. Carotenoids is not only used for coloration but it is also benefits to human beings such as decrease the risk of some cancer cases, cardiovascular disease and some other disease diseases [3-5]. Fish, as like other animals, are unable to synthesize carotenoids de novo [6]. Therefore feeding fish with carotenoid pigments is regarded as the most important to provided red or pink muscle of farmed salmon [7].

Asx can be produced synthetically and are commonly used for coloration for salmonids. However, consumers still concerns about the use of synthetic additives, so scientists may preferences to observe the alternative natural Asx sources to decrease this negative image of product.

Recently, most of promising alternatives to synthetic Asx for salmonids coloration are *Haematococcus* algae [8,9], *Phaffia rhodozyma* [10], Longistilla, *Pleuroncodes planipes* [11] and *Chlorella vulgaris* [12]. In addition, red pepper (*Capsicum annum*) and marigold flower (*Tagetes erecta*), which are abundant and rich in carotenoid pigments, could be considered alternative sources [13]. Another Asx source that has been recommended to be natural Asx sources is marine bacteria (*Paracoccus* sp). However, it is still least information about this source on coloration of salmonids. To the best of our knowledge, no investigations on marine bacteria have been published on coloration of rainbow trout. In the present study, the effects of diets incorporated two Asx sources from synthetic Asx and marine bacteria on the coloration of rainbow trout were examined as additives in rainbow trout diets.

## Materials and Methods

### Experimental diets

Two Asx sources were used in this experiment. One of them was synthetic Asx that come from carophyll-pink containing 100.000 mg Asx/kg (supplied by DSM Nutritional Products Ltd, Switzerland). And the other one was marine bacteria that generated from *Paracoccus* sp., that produces Asx and 4-ketozeaxanthin.

Four experimental diets were used in this study. A control diet (no Asx supplementation), containing 32% crude protein, 12% crude lipid, 7% crude ash and 97% of dry matter. Three of experimental diets were supplied with 30 mg Asx/kg of synthetic Asx (SA), marine bacteria (MB) and combined synthetic Asx and marine bacteria (CSB) (Table 1).

The ingredients were thoroughly mixed mechanically (ACM-50 LAT, Aikohsha Mfg. Co. Ltd., Tokyo, Japan) and distillated water was added prior to pelletizing (AEZ12M, Hiraga Seisakusho, Kobw, Japan). Pellets were dried in a vacuum freeze-drier (REL-206, Kyowa Vacuum Tech. Co. Ltd., Tokyo, Japan) and stored at 5°C until used.

### Fish, feeding and experimental conditions

The rainbow trout used in this study were obtained from eyed egg of rainbow trout from Oizumi Station of Tokyo University of Marine Science and Technology and hatched under laboratory conditions at the Laboratory of Fish Nutrition, Tokyo University of Marine Sciences and Technology. The fish were raised on a non-carotenoid-supplemented commercial rainbow trout diet until they grew to the designated sizes. Fish with initial body weight of 28.1 ± 0.3 g were housed in 60 L glass rectangular tanks (duplicate tanks per treatment) at a density of 30 fish per tank and feeding was conducted for 12 weeks. The fish were hand fed to near satiation in two times per day, six days a week. The tanks were well aerated and had continuous dechlorinated tap water supply in a semi-recirculating system at a rate of 0.6-1.0 l/min and the average temperatures for each experiment were 16.6 ± 0.7°C.

### Sampling and analytical methods

Fish were weighed at start and every three week, and also five fish were taken out for analysis. The fish were starved for 24 h and

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anesthetized with ethylene glycol monophenyl ether (300 ppm) before being weighed. Sampled fish were dissected to remove skin and fillet the flesh from anterior to nearly caudal fin one side of the fish. To measure Asx and carotenoids content, the removed skin and muscle were divided in three region: (I) anterior (pectoral fin to dorsal fin); (II) middle (dorsal fin to anus) and (III) posterior (anus to near to caudal). Skin and fillet samples were minced with a grinder, and samples of approximately 1-2 g were taken for analysis, 50 ml acetone and then about 2 g of anhydrous sodium sulphate were added to the samples. Total carotenoids content in diets, skin, and muscle 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 microfiber 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°C. The extract was concentrated and dissolved in benzene. Total carotenoids concentration in the diet, skin and fillet was determined spectrophotometerically in benzene using E (1%, 1cm)=2500 at 460 nm for yellow carotenoid, and E (1%, 1cm) = 1900 at 480 nm for red carotenoid. The proximate analysis and total carotenoid concentrations of experimental diets were showed in the (Table 2).

Asx content in the experimental diet, skin and muscle were 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 Inc.), with temperature of 35°C, using 20% acetone in 80% n-hexane as a mobile phase and a flow rate of 1 ml $min^{-1}$.The peak times and areas were compared with those obtained from standard and authentic Asx (F.Hoffman La-Roche, Switzerland).

### Statistical analysis

Growth data and pigment levels of the fish taken separately from each treatment diet for each sampling time (on day 0, 20, 40 and 60) were analysed by analysis of variance (one- way ANOVA), and the significant differences in ANOVA were ranked with Duncan multiple comparison test at the 5% level of significance in SPSS.

## Results and Discussion

Growth and feed utilization efficiency presented in Table 3 indicate that there were no significant differences in mean final weight, weight gain or specific growth rate (SGR). There were also no significant differences in feed conversion ratio (FCR).

All of the region of skin and muscle showed that the fish fed control diet was lower in the total carotenoids and Asx content than that of fish fed diet contained Asx sources (Figures 1 and 2). Total carotenoids and Asx content in the anterior skin of fish fed with CSB diet was higher than that of two groups of fish fed diet contained Asx.

Total carotenoids in the muscle also showed that fish fed diet supplemented with CSB was higher in value that another two experimental groups, however, the fish fed MB diet was higher in Asx content in the muscle than that of fish fed SA and CSB diets (Figures 3 and 4).

In the skin region, the fish fed MB and CSB diets tended to have higher values in total carotenoids and Asx content than that of fish fed SA diet (Figure 5). Similar with skin values, total carotenoids and Asx content in the middle muscle of fish fed MB and CSB diets were higher than that of fish fed SA diet. Total carotenoids and Asx content in the posterior skin of fish fed diet supplied with combined marine bacteria and synthetic Asx tended to have higher than that of fish fed MB or SA diets. Also, it was observed that posterior muscle had higher accumulated total carotenoids and Asx in the fish fed CSB diet than that of fish fed MB or SA diets, except in the 9 week of experiment fish fed MB diet had higher Asx values than that on another two experimental groups.

## Discussion

Supplementing dietary Asx sources did not significantly improved

| Ingredients (%) | Diets[1] | | | |
|---|---|---|---|---|
| | C | SA | MB | CSB |
| Jack mackerel meal | 30 | 30 | 30 | 30 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Wheat flour | 10 | 10 | 10 | 10 |
| Pregelatinized starch | 10 | 10 | 10 | 10 |
| Pollock liver oil | 4 | 4 | 4 | 4 |
| Soybean oil | 5 | 5 | 5 | 5 |
| Mineral mix. | 1 | 1 | 1 | 1 |
| Vitamine mix. | 3 | 3 | 3 | 3 |
| Choline chloride | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamine E (50%) | 0.1 | 0.1 | 0.1 | 0.1 |
| Cellulose | 16.4 | 16.4 | 15.7 | 16.2 |
| Synthetic Asx | 0 | 0.03 | 0 | 0.015 |
| Marine bacteria | 0 | 0 | 0.75 | 0.375 |

[1] Diets were recognized by their Asx source: C = Control diet, SA = Synthetic Asx ; MB = Marine bacteria and CSB= Combined SA and MB.

**Table 1:** Formulation of the experimental diet of rainbow trout

| | Diets[1] | | | |
|---|---|---|---|---|
| | C | SA | MB | CSB |
| Moisture | 3.05 | 3.46 | 2.91 | 2.53 |
| Crude protein | 32.0 | 32.0 | 32.4 | 32.5 |
| Crude lipid | 11.7 | 11.9 | 12.4 | 12.2 |
| Ash | 6.87 | 6.85 | 6.71 | 6.78 |
| Total carotenoids | 2.31 | 30.6 | 68.3 | 51.5 |
| Astaxanthin | 0 | 29.4 | 38.5 | 37.7 |

Diets were recognized by their Asx source: C = Control diet, SA = Synthetic Asx, MB = Marine bacteria, CSB = Combined SA and MB. Asx content in all of the pigment diets were 30 mg Asx/kg.

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

| | Diets | | | |
|---|---|---|---|---|
| | C | SA | MB | CSB |
| Initial (g) | 27.6 ± 0.1[1] | 28.0 ± 0.1 | 28.8 ± 0.9 | 28.3 ± 0.6 |
| Final weight (g) | 142 ± 10 | 142 ± 5.2 | 152 ± 17 | 145 ± 19 |
| Growth (g)[2] | 114 ± 11 | 114 ± 5.3 | 123 ± 18 | 117 ± 19 |
| Feed intake (g) | 111± 8.2 | 106 ± 3.8 | 107 ± 20 | 109± 17 |
| SGR (g)[3] | 1.95 ± 0.09 | 1.93 ± 0.05 | 1.98 ± 0.17 | 1.94 ± 0.18 |
| FGR (g)[4] | 0.97 ± 0.02 | 0.93 ± 0.07 | 0.86 ± 0.04 | 0.93 ± 0.01 |

[1] Values are mean ± S.D of three groups per treatment.

No significantly differences (P>0.05) were observed among treatments means

[2] Growth (g) = Final weight – initial weight

[3] SGR: specific growth rate = 100 x (ln final weight – ln initial weight)/no.days

[4] FGR: feed gain ratio= feed intake (g)/weight gain

**Table 3:** Effect of feeding Asx supplements on growth and feed utilization parameters after 12 weeks of experiment]

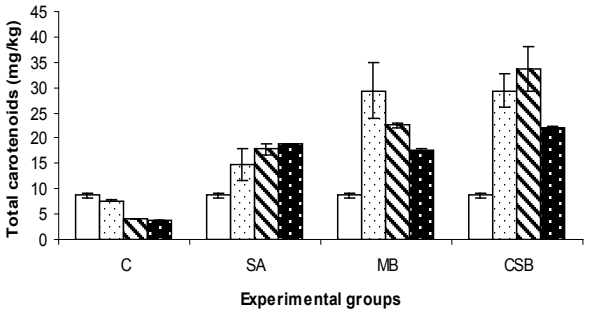


**Figure 1:** Total carotenoids content in the skin of rainbow trout during the experimental period.(□)Initial ; (□): 6 weeks;(▒) : 9 weeks; (■): 12 weeks.

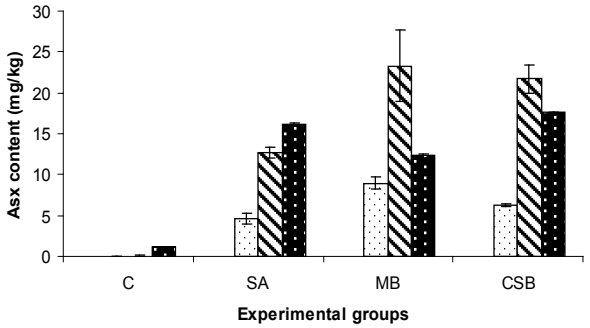


**Figure 2:** Asx content in the skin of rainbow trout during the experimental period.(□)Initial ; (□): 6 weeks;(▒) : 9 weeks; (■): 12 weeks.

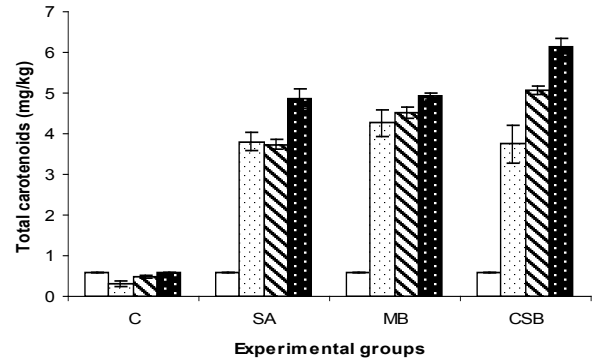


**Figure 3:** Total carotenoids content in the muscle of rainbow trout during the experimental period..(□)Initial ; (□): 6 weeks;(▒) : 9 weeks; (■): 12 weeks.

either growth rate or feed efficiency of rainbow trout. Our results are consistent with the previous salmonid studies. Buttle et al. [14] reported that supplementing Asx and cantaxanthin into the diet did not affect the growth of farmed Atlantic salmon (*Salmo salar* L).

In our experiment, fish skin and muscle color parameters reacted according to fish feed. The fish fed combined synthetic Asx and marine bacteria showed higher in color efficacy in all area of fish than fish fed diet supplemented synthetic Asx or marine bacteria alone. These results are similar to those reported by Torrisen et al. [15] it suggested that combination of Asx and canthaxanthin in ratio of 75% Asx and 25% canthaxanthin could improve carotenoid deposition and retention compared to those upon the use of Asx or canthaxanthin alone. And it might be caused that the combination of two Asx sources (synthetic Asx and marine bacteria) contained more kinds of carotenoids. According to Agus et al. suggested that combined supplementation with Asx and other carotenoids were a better means for skin coloration of red sea bream.

Skin and fillet coloration in the fish fed marine bacteria was higher in total carotenoids and Asx content than fish fed on synthetic Asx. It might be occurred because total carotenoids content in marine bacteria diet was higher than synthetic Asx diet. Yokohama et al. [16] reported that marine bacteria contained free form of Asx was recommended to be good coloration source. The highly fillet coloration of rainbow trout fed diet supplemented with marine bacteria might be related with chemical structure of Asx in marine bacteria. Free Asx is more effectively utilized than cantaxanthin, the former making flesh more reddish than cantaxanthin at comparable flesh concentration [2]. Marine bacteria, as like as natural Asx sources, contained Asx as the 3S, 3'S stereoisomer and primarily as monoester (>90%), with diesters comprising ~8% and the free molecule ~1% [17]. It tends to produce higher coloration in rainbow trout compared to synthetic Asx provided at the same dietary concentration. In addition, highly skin coloration on marine bacteria might be caused effects of other carotenoids in marine bacteria to skin and fillet coloration of rainbow trout. According to Yokoyama and Miki [18] that besides Asx, marine bacteria contained also β-Carotene, Echinenone, β-Cryptoxanthin, 3-Hydroxyechinenone, Zeaxanthin, Adonirubin, Adonixanthin, and Cis-Adonixanthin and other rest carotenoids.

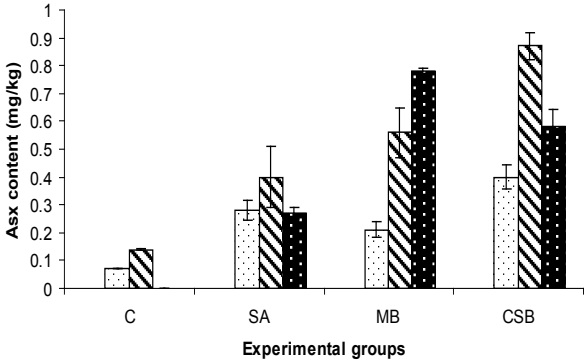


**Figure 4:** Asx content in the muscle of rainbow trout during the experimental period.(□)Initial ; (□): 6 weeks;(▒) : 9 weeks; (■): 12 weeks.

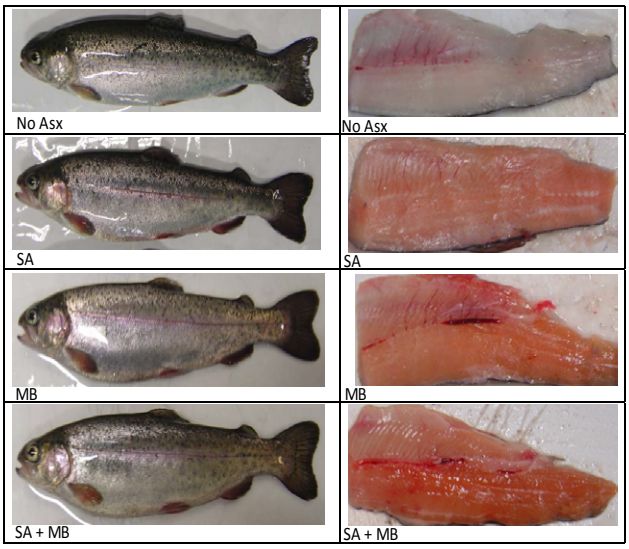


**Figure 5:** Condition of muscle coloration at the end of experiment.

The posterior and middle cut areas of fillet of rainbow trout were commonly higher than anterior part of fillet. It is similar with previous result reported that caudal part may contain 30-40% more carotenoids than the back and neck parts of the fillets [19]. Refsgaard et al. [20] reported a 19% difference in Asx concentration in favor of the caudal part. No and Storebakken [21] found that Asx deposition in the caudal (tail) was higher than in the anterior (front) part.

This study has demonstrated that supplementation of marine bacteria into the diet may significantly influence fillet coloration of rainbow trout.

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