Comparison of ascorbate content in hatchery-reared and wild black sea bream, *Acanthopagrus schlegeli* (Pisces)

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Summary

Calcium ascorbate (CaA) has been mainly used as vitamin C source in fish feed in Japan. However CaA was instable in a composed diet and decomposed during storage. We compared ascorbate level in the brain, liver, muscle and eye between wild and hatchery-reared fish. Ascorbate was primarily accumulated in the brain. Hatchery-reared fish fed with ordinary composed diet incorporated less ascorbate than wild fish. However, ascorbate fortified diet elevated the level to that of wild fish. Although natural feed organisms ingested by wild fish contained low amounts of ascorbate, wild fish could effectively incorporate ascorbate in the organs. The low ascorbate in hatchery-reared fish might be explained by their poor ability to absorb dietary ascorbate and/or utilize the ascorbate inside their bodies. The current results suggested the importance of supplementation with a suitable amount of ascorbate in composed diet to exert the effects of vitamin C.

Introduction

Ascorbate influences such physiological activities as collagen synthesis, stress response, disease resistance and lipid metabolism in fishes[1-5]. Although the importance of dietary ascorbate has been well determined in many fishes, requirements for ascorbate in individual fish organs have not been adequately determined. Sufficient ascorbate accumulation induces the activation of physiological functions. However, the low stability of calcium ascorbate (CaA) which is widely used in fish feed in Japan might weaken the physiological activities of cultured fish.

Accordingly, for better understanding of ascorbate status in the fish, we compared ascorbate level and distribution in some organs between hatchery-reared and wild black sea bream. Furthermore, the absorption process of dietary ascorbate was examined by ascorbate level in the gut content.

Materials and Methods

Feed organism

Feed organisms for larval stage, rotifer (*Brachionus plicatilis*) and *Artemia salina* nauplius, were supplied by Hiroshima Prefectural Sea Farming Association.

Fish and diet

Hatchery-reared fish and composed diet were obtained from hatcheries, fish farms and the fishery research laboratory of Kyushu University. Wild fish were caught by seine net and line fishing in the Seto Inland Sea, Hiroshima Prefecture.

Fish were dissected immediately after sacrifice, and the organs and gut contents were frozen with dry ice or liquid nitrogen for transport to the laboratory. The samples were stored in a deep freezer at −80℃ until ascorbate

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analysis. Ascorbate content of feed samples was determined during 1 week after being kept in a freezer (－20℃).

To determine stability of CaA in fish diet, sample of composed diet stored in a freezer (－20℃), refrigerator (5℃) and at room temperature (16－20℃) were subjected to ascorbate analysis.

Ascorbate analysis

The ascorbate analysis was conducted by HPLC (Hitachi 655A－11) using a reversed-phase column and a UV detector (Hitachi 655A－21; Tokyo, Japan), as described previously[5]. As authentic ascorbates, L (+)-ascorbic acid (Kanto Chem. Ltd. Tokyo, Japan) and L-ascorbyl 2-monophosphate-Mg (APM; Wako Pure Chem. Ltd, Osaka, Japan) were used.

Results

Figure 1 shows the ascorbate content in feed organisms during seed production. Hatched fish larvae were fed with cultured rotifer as the first feed organism, and then switches to *Artemia salina* nauplius. Rotifers were reared by feeding with micro-algae every 8 h. Rotifer reared with a cold-stored Latin showed relatively constant ascorbate level from 4 h after feeding. Ascorbate content of *Artemia* reared with the different diet showed high variation.

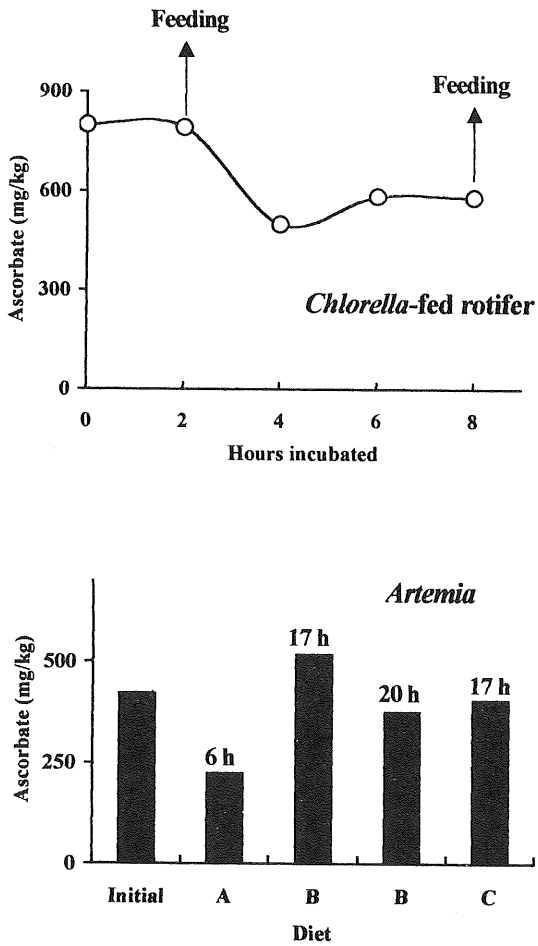

Fig. 1 Ascorbate content (mg／kg) in feed organisms. In the lower figure, the numbers show hours incubated.
Diet A: Fish liver oil-fed
Diet B: *Schizochytrium* sp.-fed
Diet C: *Euglena* and fish liver oil-fed

Table 1 shows the ascorbate content in various composed diets. Larval diets, B-1 and B-2, were lowest in total ascorbate content. In larval diets, CaA and APM were supplemented as vitamin C sources. However, APM was not found in some juvenile diets. A vitamin mixture included a fairly high amount of CaA but not APM.

Table 1 Ascorbate content (mg/kg) of artificial diets used in hatcheries

Dietary type	Maker	Ascorbate (mg/kg)#		
		APM*	Ascorbic acid	Total
Larval diet	A	91	6,450	6,540
	B-1	17	36	53
	B-2	14	10	24
Juvenile diet	C	295	15	310
	D-1	0	972	972
	D-2	0	373	373
Parental diet	E	148	0	148
Vitamin mixture	F	0	66,500	66,500

#Content was expressed as free ascorbic acid
* L-ascorbyl 2-monophosphate Mg

Table 2 shows the ascorbate content in diets and the fish organs. Although ascorbate was undetectable in diet No.1, a low level of ascorbate was found in the fish organs. Wild fish less than 30 g of body weight accumulated higher amount of ascorbate than that of cultured fish. Fortification of ascorbate with APM or a vitamin mixture markedly affected ascorbate content in the organ and reached a level close to the value in wild fish. Dietary ascorbate seemed to be preferentially incorporated in the brain, but the level was relatively higher in wild fish.

Table 2 Comparison of ascorbate content in hatchery-reared and wild fish

No.	Body weight (g)	n	Ascorbate (mg/kg)				
			Feed	Brain	Liver	Muscle	Eye
Hatchery-reared							
1	4.1 ± 1.5	4	0	71.8 ± 25.5	11.4 ± 5.6	7.7 ± 2.8	16.3 ± 7.6
2*	4.1 ± 2.3	4	246	210.0 ± 44.9	32.1 ± 1.6	16.2 ± 2.3	48.7 ± 8.4
3*	12.0 ± 6.1	4	972	259.0 ± 32.6	91.6 ± 27.3	17.0 ± 3.5	39.0 ± 12.3
4	23.5 ± 4.0	4	49.1	205.0 ± 5.4	18.3 ± 8.4	2.2 ± 1.5	11.6 ± 2.6
5	49.3	2	65.0	208.0	19.2	3.6	13.1
6*	51.0 ± 6.9	4	373.0	231.0 ± 37.9	51.7 ± 11.8	14.4 ± 2.6	30.9 ± 4.1
Wild							
1	7.2 ± 1.6	4		196.0 ± 38.5	49.9 ± 15.4	17.3 ± 6.1	36.5 ± 0.7
2	29.3 ± 6.9	4		338.1 ± 52.8	45.1 ± 15.1	12.0 ± 4.9	20.5 ± 5.1
3	270 ± 85	4		260.0 ± 3.3	42.2 ± 2.1	6.6 ± 1.2	16.2 ± 3.5
4	450	1		217.0	32.7	12.8	17.8
5	484 ± 81	5		286.0 ± 90.7	37.9 ± 16.2	6.5 ± 3.9	8.8 ± 2.7

Values are mean and SD.
*Fish fed with ascorbate fortified diet

Figure 2 shows the stability of CaA in a composed diet during storage under the different conditions. Ascorbate in the diet was almost fully degraded during 2 week storage at 5℃ or room temperature. Preservation for 3 weeks at -20℃ resulted in a loss of 53% of ascorbate in the diet.

Figure 3 shows ascorbate in the gut content of hatchery-reared and wild fish. The gut content was taken out from the stomach and intestine (anterior, middle, posterior). Ascorbate level in the gut content was remarkably

higher in the hatchery-reared fish. Although ascorbate content in the wild fish gut was less than that with 100 mg／kg diet, accumulation of ascorbate in the organs was highly abundant.

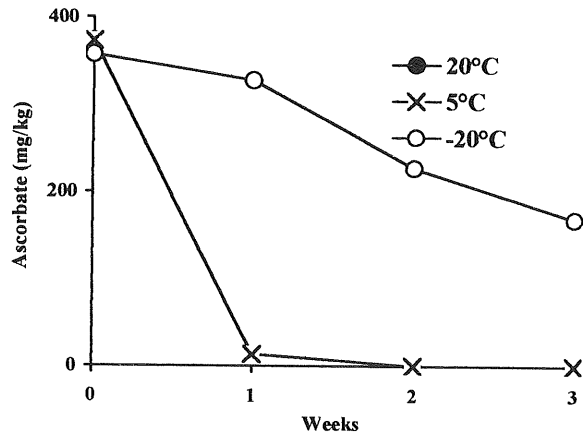

Fig. 2 Stability of Ca-ascorbate (mg／kg) in composed diet during storage

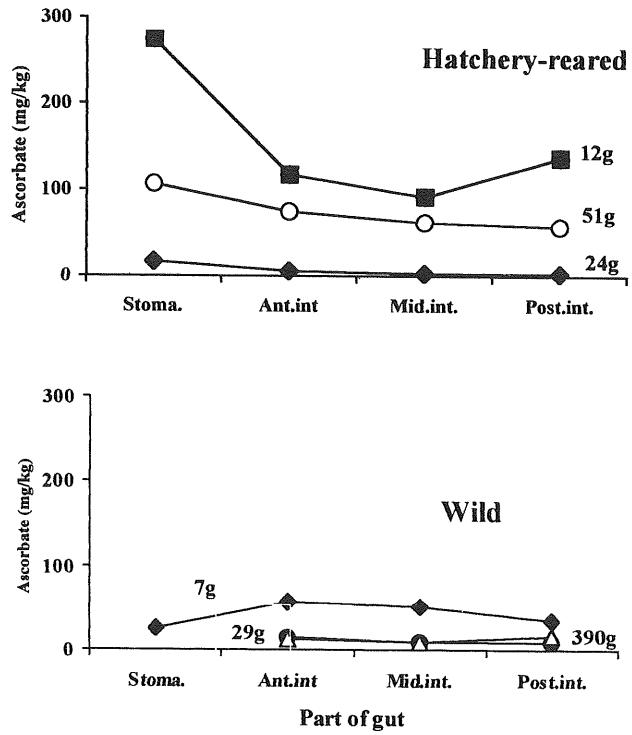

Fig. 3 Ascorbate (mg／kg) in gut content of hatchery-reared and wild fish
Body weight of fish was shown in the figures. Ascorbate content in diet was as follows: BW 12 g: 972 mg／kg; BW 51 g: 373 mg／kg; BW 24 g: 49.1 mg／kg

Discussion

The optimal level of dietary ascorbate has been reported as 50 - 100 mg／kg diet in rainbow trout *Oncorhynchus mykiss*[1], channel catfish *Ictalurus punctatus*[6] and Korean rockfish *Sebastes schlegeli*[7] to sustain normal growth and survival, and to prevent deficiency symptoms. Kosutarak *et al*[8] reported that 50 mg APM／kg diet did not

induce any differences in weight gain, feed conversion efficiency, mortality or tolerance against temperature stress compared with the higher APM supplement.

Ascorbate content of the rotifer fed on *Chlorella* reached 1300 μg/g[9] and 2300 μg/g[10]. *Artemia* enriched with ascorbyl palmitate raised the ascorbate level to 1750 μg/g on a dry basis[9]. The present findings were lower than the data showed above. The effect of ascorbate fortification should be evaluated from the level incorporated by larval fish.

In fish diets, CaA and APM are actually used in composed diet in Japan. But CaA is unstable against oxidation[11], as shown in the present study. Thus, in rearing fish it is important to pay attention to the preservation characteristics of fish diet, dietary ascorbate and accumulation in fish.

Ascorbate measurement in the gut content showed that cultured juveniles seemed to absorb ascorbate in the middle intestine, while the wild fish did so at the posterior intestine. Low ascorbate level in feed organisms and high accumulation of ascorbate in wild fish would be accounted for by an effective absorption mechanism different from that in cultured fish.

The ascorbate contents of the various organs were lower in the fish fed on unfortified diets, when compared with wild fish. Ascorbate was preferentially accumulated in the brain which is in accordance with the study of Ikeda et al[12]. Ascorbate might accelerate transmission of substances in relation to the central nervous system[13]. Besides the body pool, there appeared to be a largely independent ascorbate pool in the brain, assuming particular importance in stress situations[14] Thus, the current results suggested that the low ascorbate accumulation in the cultured fish might have been due to low ability in absorption and/or exposure to oxidation under stressful conditions which might induce high ascorbate consumption. Comparison of the status of vitamin C and E in cultured and wild yellowtail *Seriola quinqueradiata* revealed that cultured fish suffered more from oxidization stress, judging from the low ascorbate and tocopherol contents[15].

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