L-Ascorbyl-2-Sulfate Alleviates Atlantic Salmon Scurvy (43781)

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Abstract. **Duplicate lots of 150 Atlantic salmon (*Salmo salar*), average weight 0.5 g, were fed NRC diet H-440 base containing L-ascorbic acid (C_1) or L-ascorbyl-2-sulfate (C2S); or L-ascorbyl-2-monophosphate (C2MP): at 0 or 100 mg C_1; 50, 100, 300 mg C2S; or 50, 100 mg C2MP per kg dry diet in 12°C freshwater tanks. After 12 weeks, negative controls (no vitamin C) exhibited reduced growth, scoliosis, lordosis, and petechial hemorrhages typical of fish scurvy. All other lots grew normally. Four 100-fish lots of scorbutic salmon, average weight 3.3 g, were placed on recovery diets of 0, 50, or 300 mg C2S, or 100 mg C2MP per kg dry diet. After 5 weeks, fish fed either level of C2S intake had recovered and resumed growth. Negative controls continued to develop acute scurvy. The 41 survivors in this no-vitamin-C group all had advanced scurvy, whereas all fish in both C2S-fed recovery groups appeared normal. Tissue assays for C vitamers disclosed normal levels of C_1 and C2S in the recovery groups. All other test treatment lots containing C_1, C2S, or C2MP had fish with normal appearance and no significant differences in growth response for the 17-week test period. C2S at 50 mg or more per kg diet as the sole vitamin C source promoted normal growth in young Atlantic salmon for more than 20-fold increase in weight.**
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L-ascorbyl-2-sulfate (C2S) is a heat- and oxidation-resistant compound which can be converted into ascorbic acid by C2sulfatase in rainbow trout tissues (1). Efficacy of C2S as a vitamin C source for rainbow trout (*Oncorhynchus mykiss*) was reported in 1993 (2), but other reports questioned the efficacy of C2S (3–6). Some vitamin C activity for C2S was reported for channel catfish (*Ictalura punctatus*) (7). C2S metabolism in fish has been reviewed (8, 9) but no growth studies with C2S as the sole vitamin C source for Atlantic salmon (*Salmo salar*) have been reported when highly purified test diets were used. Therefore, this study was designed to induce scurvy in Atlantic salmon fry with NRC diet H-440 base devoid of vitamin C, and to compare growth response among three common vitamin C sources: dipotassium L-ascorbyl-2-sulfate dihydrate (C2S); L-ascorbic acid (C_1); and magnesium L-ascorbyl-2-monophosphate (C2MP).

Materials and Methods

Dipotassium L-ascorbyl-2-sulfate dihydrate (ASTOS) was provided by Pfizer, Inc. L-ascorbic acid was provided by Hoffmann-LaRoche, Inc. Magnesium L-ascorbyl-2-monophosphate (PHOSPHATAN C) was provided by Pfizer, Inc.

Duplicate lots of Atlantic salmon fry, average weight 0.5 g, were fed NRC diet H-440 base (NRC/NAS Nutrient Requirements of Trout, Salmon, and Catfish. National Academy Press. Washington, DC. p50, 1973) containing C_1 at 0 or 100 mg; or C2S at 50, 100, or 300 mg; or 50 pr 100 mg C2MP per kg dry diet for 17 weeks in 12°C freshwater tanks. The diet was made by combining all dry ingredients with an equal weight of 90°C water and mixing until temperature dropped to 50°C, at which time the liquified mass was poured into shallow trays and refrigerated immediately until it hardened (Table I). The vitamin C additions were made by dissolving weighed quantities of the specific vitamer in 10 ml of distilled water, which was then

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Table I. Composition of Experimental Diets Used to Feed Atlantic Salmon Fry

Vitamin free casein	350 g
Gelatin	150 g
Corn oil	60 g
Cod liver oil	30 g
White dextrin	280 g
α-Cellulose	80 g
Vitamin mix[a]	10 g
Mineral mix[b]	40 g
Total dry ingredients	1000 g

[a] Vitamin mix provided thiamin HCl 50 mg, riboflavin 200 mg, pyridoxine HCl 50, choline chloride 5000 mg, nicotinic acid 750 mg, Ca-pantothenate 500 mg, inositol 2000 mg, biotin 5 mg, folic acid 15 mg, ascorbic acid varied mg, vitamin B_{12} 100 μg, mendadione (K) 40 mg, and dl-α-tocopherol acetate 400 mg per kg dry diet.

[b] Mineral mix contained salt mix #2 USP XII 100 g plus $AlCl_3$ 15 mg, $ZnSO_4$ 300 mg, CuCl 10 mg, $MnSO_4$ 80 mg, KI 15 mg, $CoCl_2$ 80 mg, and Na_2SeO_3 10 mg.

added to the liquified diet during the last phase of the mixing process. When solidified, the diet was cut into 3-cm cubes, placed in sealed glass jars, and kept at −20°C until fed. Daily rations were thawed and grated to appropriate size, and fish were fed to satiation at 07:00, 12:00, and 16:00 hr 6 days per week. Each tank of fish was bulk weighed and counted monthly after a one day fast. Tanks were cleaned daily and were thoroughly scrubbed at weigh periods. Mortalities were removed when observed, and daily feed intake was recorded. Each lot of 150 fish was confined in a 300-liter circular tank supplied with 1–2 liters of 12°C dechlorinated municipal water per min. Each tank was equipped with center screen and external standpipe. All fish handling procedures were performed under approved animal care procedures of the University of Washington and U.S. Public Health Service. Fish were exposed to a natural photoperiod in an outdoor shed.

At the 12-week weigh period, all lots were sampled then reduced to 100 fish for the remaining 5 weeks on test. Four groups of 100 Atlantic salmon, average weight 3.3 g, previously fed for 12 weeks on the no-C diet, were placed on repletion diets containing no-C, 50 mg C2S, 300 mg C2S, or 100 mg C2MP per kg dry diet. Feeding continued for 5 weeks, at which time fish in all tanks were weighed, counted, and examined for signs of scurvy. Anesthetized fish were exsanguinated, eviscerated, then liver and carcass were frozen on dry ice, sealed and stored at −60°C until assayed. Tissues were prepared and assayed for C_1 and C2S levels using the method of Felton and Halver (10) (assays for C_1 and C2S were supported by Pfizer, Inc., Food Science Group, New York, NY).

Results

At Week 12 of the experiment, negative controls (no-C group) showed reduced growth with about 10% of population exhibiting signs of scoliosis, lordosis, and petechial hemorrhages typical of fish scurvy (Table II). All other lots receiving any vitamin C source grew at the same rate, and no signs of scurvy were detected. After anesthesis, necropsy showed normal external and internal tissues.

After 2 weeks on the repletion program (Week 14 of the experiment), all fish on any vitamin C source were feeding avidly and petechial hemorrhages had disappeared. After 5 weeks on the repletion program, all lots of fish fed any vitamin C source appeared normal, without visual signs of scurvy in any fish. Some

Table II. Growth of Atlantic Salmon on C Treatments

Added to diet (mg/kg diet)	Found[a]	Average wt 12 weeks[b] (n = 150 × 2)	Average wt 17 weeks[b] (n = 100)	Dead	Scurvy
100 mg C_1	24 ± 0.5	5.3 ± 0.5 g[b]	12.0 ± 0.5 g[b]	8	0
50 mg C2S	49 ± 5	5.1 ± 0.2	12.9 ± 0.1	2	0
100 mg C2S	79 ± 4	4.9 ± 0.3	11.7 ± 0.1	5	0
300 mg C2S	282 ± 11	4.9 ± 0.2	12.7 ± 0.6	4	0
50 mg C2MP	44 ± 2	5.4 ± 0.5	12.2 ± 0.2	2	0
100 mg C2MP	87 ± 3	4.8 ± 0.2	12.4 ± 0.6	6	0
0	<2	3.4 ± 0.5	3.8	59	all
Repletion of 0 groups beginning at 12 weeks (n = 100)[c]					
0		3.29 g	3.78 g	59	all
50 mg C2S		3.30	5.24	1	0
300 mg C2S		3.26	7.45	6	0
100 mg C2MP		3.30	6.39	2	0

[a] mg/kg dry diet ± SEM; n = 3; assay after 30 days storage at −20°C in sealed jars. Assays by Felton Consultancy, School of Fisheries HF-15, Seattle, WA 98195. s = 2 mg/kg

[b] ±SEM

[c] Repletion study began at 12 weeks with 4 lots of 0 C groups, average weight 3.3 g. All visual scurvy disappeared by Week 17 (5 weeks on repletion diets) in lots receiving any vitamin C source.

shortening of opercles remained but new tissue was visible at the edge of the opercles. All petechial hemorrhages had disappeared. Negative control (no-C group) continued to develop scurvy, and, after 5 weeks, the remaining 41 surviving fish all exhibited acute scurvy (Fig. 1). Representative samples of liver and eviscerated carcass were analyzed by HPLC after extraction of tissues with 5% trichloracetic acid and confirmed that vitamin C_1 was present in tissues of repleted fish (Table III) (10).

Those lots of fish continued on any vitamin C source grew to over 20-fold increase in weight in 17 weeks without any significant difference ($P = 0.05$) in weights between dietary treatments fed any C source at 50 mg or more vitamer C per kg dry diet. Mortality was insignificant between any treatment containing any vitamin C source (Table II).

Discussion

L-ascorbyl-2-sulfate (C2S) added to diet H-440 base as the vitamin C source at 50 mg or more per kg dry diet (24 mg C_1 equivalent) promoted normal growth in Atlantic salmon reared in 12°C freshwater for a 17-week period and over a 20-fold increase in weight without visible signs of scurvy. Equivalent growth was obtained with 100 mg C_1 or 50 mg or more of C2MP as the vitamin C source. No significant difference in growth response over the 17-week period was observed between lots of fish receiving any vitamin C source. HPLC analysis for liver and carcass C_1 and C2S also supported the growth response conclusions. These findings are in contrast to the reports on poor utilization of C2S by salmonids (3–6). The repletion experiments demonstrated that C2S could be used by Atlantic salmon as an effective vitamin C source for the recovery from scurvy and return to normal growth rates when 50 mg or more was present in the diet. The dietary requirement of juvenile Atlantic salmon in freshwater systems has not been quantified, and reports may be influenced by the stability of the vitamin C source used. L-ascorbic acid has a very short half-life in most diet preparations and storage conditions (11, 12), and most of the active reducing compound

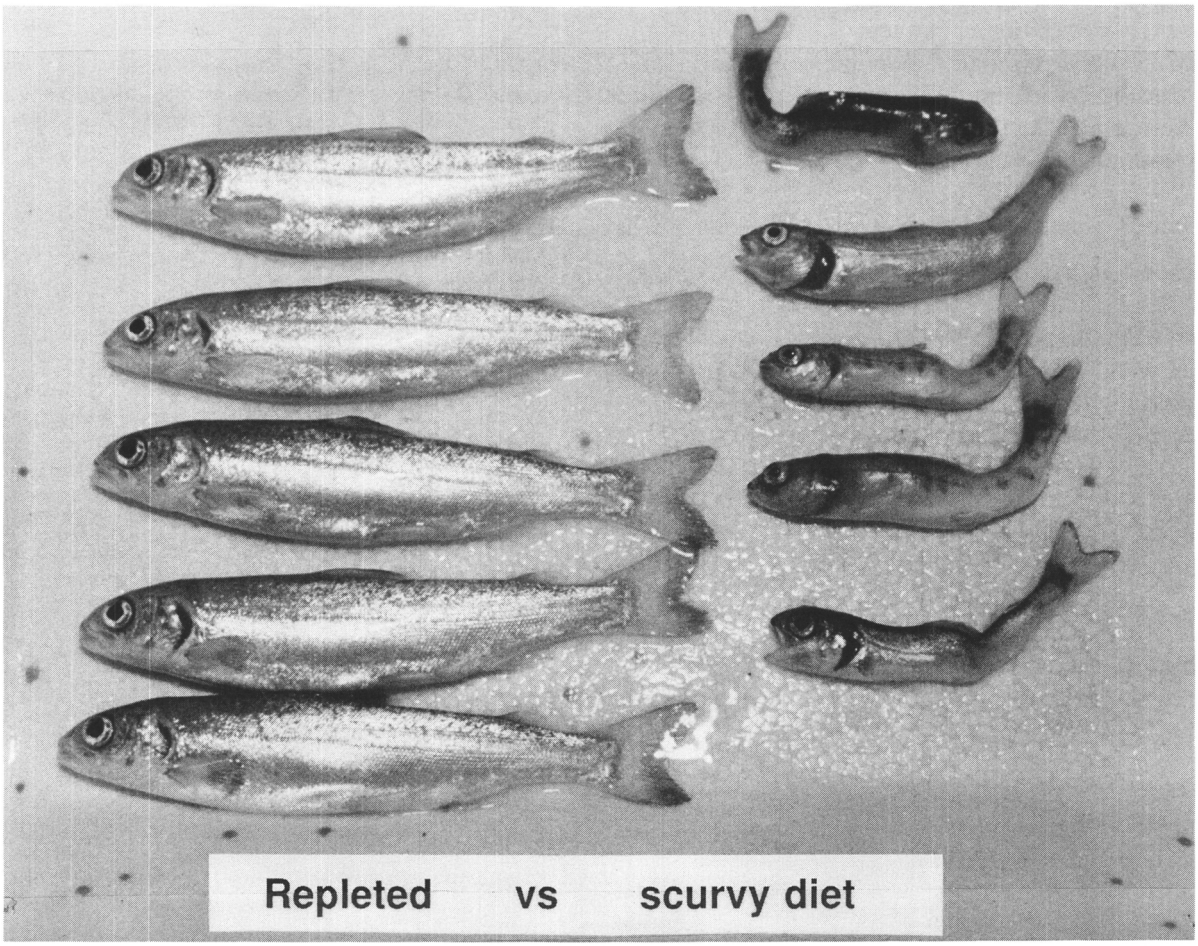

Figure 1. Atlantic salmon repleted for 5 weeks with 50 mg C2S; 300 mg C2S; or 100 mg C2MP/kg dry diet versus salmon continued for 5 additional weeks on the no-C diet. No differences in response among diet treatments containing any C source were observed. Differences between the no-C group and the C repleted groups were obvious.

Table III. Tissue assays[a]

Diet[b]	Liver C_1[a]	Liver C2S[a]	Carcass C_1[a]	Carcass C2S[a]
12-Week trial (n = 2 × 150, T = 12°C)				
0	17.1	n.d.	23.0	22.6
100 mg C1	12.9	2.3	18.4	16.4
50 mg C2S	10.2	2.3	16.3	14.2
100 mg C2S	10.2	2.6	22.7	11.2
300 mg C2S	12.4	2.4	19.9	15.8
5-Week repletion study (n = 1 × 100, T = 12°C)				
0	14.5	10.3	21.8	<2
50 mg C2S	23.1	<2	19.9	3.5
300 mg C2S	12.3	<2	21.4	4.5

[a] Assays provided by Felton Consultancy, School of Fisheries HF-15, Seattle, Wa 98195. μg/g Tissue; n = 3; s = 2 μg/g tissue.
[b] mg/kg Dry diet added during diet preparation.

may be lost by the time it is ingested, digested, and absorbed in the gut. Types of diets used may also have a significant effect upon amounts of ascorbic acid present when commercial ingredients and trace mineral supplements are incorporated into the diet formula and ascorbic acid is exposed to potential oxidizing catalysts or oxydized compounds in many commercial diet preparations. The less reactive and more stabile C2S survives mixing, manufacturing, and storage procedures, and should be present in the gut for absorption as an effective vitamin C source for young Atlantic salmon.

Conclusions

L-ascorbyl-2-sulfate at 50 mg or more per kg dry diet as a sole vitamin C source promoted growth of juvenile Atlantic salmon for more than a 20-fold increase in weight. C2S at 50 mg or more per kg dry diet in a repletion diet alleviated scurvy symptoms rapidly and returned the Atlantic salmon to positive growth. Tissue assays of repleted lots demonstrated the presence of ascorbic acid and C2S in these tissues.

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