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Interaction between vitamins C and E affects their tissue concentrations, growth, lipid oxidation, and deficiency symptoms in yellow perch (*Perca flavescens*)

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We have conducted studies with juvenile yellow perch (*Perca flavescens*) over a period of 20 weeks to address the question of the interaction between water- and lipid-soluble antioxidant vitamins. Fish ($2{\cdot}25 \pm 0{\cdot}14$ g) were divided into twelve groups, and triplicate groups were fed one of four casein-based, semi-purified diets formulated to contain low or high vitamin E levels of either 5 or 160 mg/kg without or with vitamin C supplementation (250 mg/kg). Diets were designated as −C−E, −C+E, +C−E, or +C+E, respectively. The fish fed the +C+E diet showed significantly higher weight gain, feed intake, and feed efficiency than the groups fed vitamin C-deficient diets. Total ascorbate concentrations of liver were significantly higher in fish fed vitamin C-supplemented diets than in fish fed the vitamin C-deficient diet after 16 and 20 weeks. The liver α-tocopherol concentrations were increased by supplemental vitamin C in vitamin E-deficient dietary groups which indicates a sparing or regenerating effect of vitamin C on vitamin E. Fish fed vitamin C-deficient diets (−C−E and −C+E) exhibited severe deficiency symptoms, such as scoliosis, lens cataracts, anorexia, and haemorrhages. The cumulative mortality was significantly higher in the −C−E groups. The thiobarbituric acid-reactive substances value was significantly higher in blood plasma of fish fed a diet unsupplemented with both vitamins. The findings in the present study with yellow perch support the hypothesis that vitamin C regenerates and/or spares vitamin E *in vivo*.

Yellow perch: Vitamin C: Vitamin E: Thiobarbituric acid-reactive substances: Deficiency symptoms

Vitamins C and E play important roles in numerous biological conditions, such as ageing, cataracts, DNA damage, atherosclerosis, diabetes, neurodegenerative diseases, cardiovascular diseases, and cancers. The major beneficial actions of vitamin C and E are due to their antioxidant properties that scavenge reactive oxygen species in biological fluid (Frei *et al*. 1990) and membranes (Burton *et al*. 1983). Tappel (1968) hypothesized that vitamin C might reduce tocopheroxyl radicals formed *in vivo* and numerous studies have been done to demonstrate this phenomenon (Packer *et al*. 1979; Niki *et al*. 1982; McCay, 1985; Chan, 1993). The interaction between the two vitamins occurs at the membrane–cytosol interface (Buettner, 1993), and vitamin C functions as a reducing agent of the membrane-bound oxidized vitamin E.

Diets with supplemental vitamin C resulted in higher vitamin E concentrations in tissues compared with those without supplemental vitamin C in rats (Igarashi *et al*. 1991; Ho & Chan, 1992), guinea-pigs (Bendich *et al*. 1984; Liu & Lee, 1998), and human subjects (Wantanowicz *et al*. 1984; Jacob *et al*. 1988; Stoyanovsky *et al*. 1995; Hamilton *et al*. 2000). However, results showing no effects of sparing or regenerating vitamin E by vitamin C have also been reported in rats (Chen, 1981), guinea-pigs (Burton *et al*. 1990), and human subjects (Jacob *et al*. 1996).

The interaction of the two vitamins was also examined in fish, such as rainbow trout (*Oncorhynchus mykiss*; Wahli *et al*. 1998), Atlantic salmon (*Salmo salar*; Hamre *et al*. 1997), and lake sturgeon (*Acipenser fulvescens*; Moreau *et al*. 1999). Hamre *et al*. (1997) found that a diet deficient in vitamin C resulted in significantly decreased liver vitamin E concentrations. The authors proposed that there would be two different interaction mechanisms: a synergistic effect of simultaneous protection in the water and lipid phases

Abbreviation: TBARS, thiobarbituric acid-reactive substances.

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against oxidation, and a regenerating effect of vitamin C on tocopheroxyl radicals.

Pathological changes resulting from deficiencies of the two vitamins were studied in rainbow trout juveniles (Frischknecht *et al*. 1994; Wahli *et al*. 1998). In the study by Frischknecht *et al*. (1994), a significant increase in mortality, severe muscular dystrophy and splenic haemosiderosis were observed in the fish fed a diet deficient in both vitamins after 8–12 weeks. Fish fed a diet deficient in vitamin C but high in vitamin E exhibited the typical deficiency symptoms of vitamin C after 16–20 weeks, such as retarded growth rate, haemorrhages, gill alterations, and severe vertebral column deformations.

Yellow perch (*Perca flavescens*) is one of the most valuable fish species in North America, particularly in the Midwest as an aquaculture species (Stickney, 1993; Brown *et al*. 1996; Twibell & Brown, 2000; Twibell *et al*. 2000). Little nutritional information is available for this species, and no clinical deficiency symptoms of vitamins have been reported yet. The present study, therefore, was designed to investigate the interactions between vitamins C and E on growth, feed utilization, and deficiency symptoms in juvenile yellow perch. The interactions of the two vitamins, in terms of the sparing effects of vitamin C on vitamin E and synergism of their antioxidant actions, were also examined in the present study by determining the concentrations of each vitamin in the liver and thiobarbituric acid-reactive substances (TBARS) in blood plasma as a criterion for the lipid peroxidation.

Materials and methods

Design and diets

Four casein-based semi-purified diets were formulated to contain no vitamin C and low vitamin E (5 mg/kg; −C−E), no vitamin C and high vitamin E (160 mg/kg; −C+E), high vitamin C (250 mg/kg) and low vitamin E (+C−E), and high vitamin C and high vitamin E (+C+E). The composition of the basal diet for the four experimental diets is provided in Table 1. The basal diet was formulated to be isonitrogenous and isoenergetic according to previous work (Moreau & Dabrowski, 1996) in juvenile lake Sturgeon. In the basal diet, vitamin C was not detected and vitamin E (sum of α-, β-, γ-, and δ-tocopherols) concentration was 4·8 mg/kg. The detected vitamin E concentration of 4·8 mg/kg in the basal diet was due to the supplementation of *n*-3 highly unsaturated fatty acid concentrate and fish-protein hydrolysate (Table 1). Ascorbyl-2-monophosphate-Mg (Phospitan C; Showa Denko America, Inc., NY) and all-rac-α-tocopheryl phosphate-Na (Showa Denko America, Inc., NY) were used as the vitamin C and E sources, respectively. For the different levels of vitamins in experimental diets, cellulose was replaced by each vitamin. Five percent of fish-protein concentrate (CPSP 90; Sopropeche S. A., Boulogne-Sur-Mer, France) was supplemented in the diets to enhance palatability of the semi-purified diet to the juvenile yellow perch. The diets were cold-pelleted (2·0 mm diameter) with distilled water, freeze-dried to have less than 5 % moisture, crushed into desirable particle size (0·4–2·0 mm), and stored at −20°C until use.

Table 1. Composition of the basal semi-purified diet (no vitamin C and low vitamin E; −C−E)

Ingredient	Concentration (g/kg dry diet)
Casein (vitamin-free)*	360
Dextrin*	150
Maize starch†	105
Fish-protein hydrolysate‡	50
Egg white*	50
Gelatin*	40
Maize oil (tocopherol-stripped)*	60
Lard (tocopherol-stripped)*	50
n-3 Highly unsaturated fatty acid concentrate§	5
Vitamin mixture‖	40
Mineral mixture¶	30
Choline chloride*	1
Carboxymethylcellulose*	20
L-methionine*	5
Cellulose*	34

* ICN Biomedicals, Inc., Costa Mesa, CA.
† A. E. Staley MFG. Co., Decatur, IL.
‡ CPSP 90, Sopropeche S. A., Boulogne-Sur-Mer, France.
§ DHASCO and ARASCO (1:1, v/v), Martek Biosciences Corporation, Columbia, MD.
‖ Roche Performance Premix composition (per g vitamin mixture): vitamin A, 794 μg; vitamin D_3, 5·5 μg; vitamin B_{12}, 13 μg; riboflavin, 13·2 mg; niacin, 61·7 mg; d-pantothenic acid, 22·1 mg; menadione, 1·32 mg; folic acid, 1·76 mg; pyridoxine, 4·42 mg; thiamin, 7·95 mg; d-biotin, 0·31 mg. Hoffman-La Roche, Inc., Nutley, NJ.
¶ Se (5 mg) in the form of sodium selenite per kg Bernhart Tomarelli salt mixture (ICN Pharmaceuticals Inc., Costa Mesa, CA).

Fish and feeding trial

Juvenile yellow perch averaging 2·25 ± 0·14 g initial weight were used as an experimental fish and the feeding trial was conducted in the Aquaculture Laboratory at the Ohio State University. Before the feeding trial, the fish were fed the basal diet (−C−E) for 2 weeks to adjust to the casein-based semi-purified experimental diet and to reduce possible body reserves of vitamin C. A total of 240 fish were randomly distributed into groups of twenty; three groups per treatment. Each experimental diet was fed to triplicate groups of fish with the feeding rates ranging from 4·0 % of fish weight at the beginning to 1·5 % at the end of the feeding trial. The feeding trial was conducted for 20 weeks in 40 litre glass aquaria, supplied with u.v.-irradiated and filtered semi-circulated water at a flow rate of 1·0–1·5 litres/min. All procedures and handling of animals were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, the Ohio State University. The fish were fed twice daily, 7 d per week. Supplemental aeration was also provided to maintain dissolved O_2 levels near saturation. The diurnal light–dark cycle was regulated at 12 h–12 h. Total fish weight in each tank was determined every 2 weeks to monitor their growth and to adjust the feeding rate. Feeding was stopped 24 h before weighing. The insides of the aquaria were scrubbed once per week in addition to the daily siphoning of faeces to minimize algae and fungal growth, which could provide a source of vitamin C.

Sample collection and analyses

At the end of the feeding trial all fish were weighed to measure growth rate and feed conversion ratio (dry feed

consumed/body weight gain), and cumulative survival was calculated. For the vitamin analyses, three fish were randomly selected from each group (total of nine fish per dietary treatment) and killed to collect livers. Blood was obtained from the caudal vein of fish randomly selected from each group (total of six fish per treatment) with heparinized syringes. Blood was stored on ice and then centrifuged at 1500 *g* for 10 min. Five fish per group (a total of fifteen fish per dietary treatment) were killed for the whole-body proximate analyses. Analyses of proximate compositions (crude protein, ash, and moisture) were performed by standard procedures (Association of Official Analytical Chemists, 1995).

Total and dehydro-ascorbic acid were analysed in liver samples by the dinitrophenylhydrazine colorimetric method with modifications for interfering substances (Dabrowski & Hinterleitner, 1989). Vitamin E (α-, β-, γ-, and δ-tocopherols) concentrations in diets and α-tocopherol in liver tissue were determined by HPLC (Cort *et al*. 1983; Zaspel & Csallany, 1983). TBARS in blood plasma were determined by the HPLC method of Tatum *et al*. (1990) with a slight modification. Before starting extraction of thiobarbituric acid–malonaldehyde adducts in plasma, samples were first mixed with 0·62 M-TCA (1:1, v/v) for deproteinization and followed by the method of Tatum *et al*. (1990). As a standard, 1,1,3,3-tetramethoxy-propane (Sigma Chemical, St Louis, MO) was used.

For the feed utilization, an instantaneous meal intake test was conducted three times during the 10th week, and once during the 16th and 20th week by feeding fish *ad libitum*. The respective experimental diets were fed to fish groups (three replicate tanks) as the morning meal on days 72, 114, and 140. The average feed amount that fish were fed *ad libitum* at a time was calculated from triplicate groups (from three tanks for each diet).

Statistical analysis

Each experimental diet was fed to three groups (tanks) of fish by a completely randomized design. Two-way ANOVA was used to test the effects of dietary vitamin C and E, and their interaction on growth performance (final body weight, feed conversion ratio, instantaneous meal intake, and survival), their accumulations in liver, and TBARS level in plasma. When differences were found in the two-way ANOVA, Tukey's multiple comparison test, honestly significant difference (HSD) of one-way ANOVA was used to compare the mean differences by the SPSS statistical package (version 10·0; SPSS Inc., Chicago, IL). Liver α-tocopherol concentrations were compared by Student's *t* test. Percentages were arcsine-transformed before analysis. Differences were considered significant at $P \leq 0{\cdot}05$.

Results

Table 2 provides the results of growth, feed utilization performance, and cumulative survivals after 20 weeks of feeding. The fish fed the +C+E diet showed significantly higher weight gain and increased feed efficiency than the fish groups fed vitamin C-deplete diets (−C−E and −C+E). Significantly higher meal intake was found in the +C+E group than in −C+E and −C−E groups. Survival was significantly lower in fish fed the diet deficient in both vitamin C and E than in fish fed diets supplemented with either vitamin C or E. However, no significant difference was found in hepatosomatic index between the treatments. A two-way ANOVA test (Table 4) showed that weight gain was significantly affected by either vitamin C ($P=0{\cdot}006$) or E ($P=0{\cdot}046$), but there was no significant interaction between the two vitamins ($P=0{\cdot}16$). Feed conversion ratio and instantaneous meal intake were significantly affected by dietary vitamin C, but not by vitamin E ($P<0{\cdot}05$).

In liver (Table 3), total ascorbic acid (sum of reduced and dehydro-ascorbic acid) concentration was significantly higher in fish fed vitamin C-supplemented diets after 16 and 20 weeks. The liver vitamin C concentration was not affected by dietary vitamin E. Liver α-tocopherol concentration was not significantly affected by dietary supplementation of either vitamin C or E after 20 weeks (Table 4). However, a Student's *t* test showed that α-tocopherol concentration was significantly ($P<0{\cdot}05$) elevated by approximately 140 % (from 10·4 (SD 3·50) to 25·2 (SD 5·56) nmol/g liver) in vitamin E-deficient dietary groups,

Table 2. Weight gain, feed conversion ratio, instantaneous meal intake, hepatosomatic index and survival of yellow perch juveniles fed four experimental diets for 20 weeks*

(Mean values of triplicate groups and standard deviations)

Diet...	−C−E		−C+E		+C−E		+C+E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial body wt (g)†	2·30	0·08	2·28	0·19	2·28	0·14	2·12	0·14
Final body wt (g)†	16·6^{b}	0·66	17·3^{b}	2·26	19·2^{ab}	1·23	21·9^{a}	2·02
Feed conversion ratio‡	1·27^{a}	0·08	1·29^{a}	0·05	1·11^{ab}	0·01	1·06^{b}	0·11
Instantaneous meal intake (g/100 g body wt)§	1·47^{b}	0·28	1·34^{b}	0·21	1·61^{ab}	0·17	2·02^{a}	0·03
Hepatosomatic index (%)‖	1·80	0·06	1·88	0·16	1·76	0·12	1·91	0·32
Survival (%)	82·2^{b}	4·6	95·8^{a}	7·2	97·4^{a}	4·5	100^{a}	0·0

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 590.
† Weight gain (%) = (final weight − initial weight) × 100/initial weight.
‡ Feed conversion ratio = dry feed intake (g)/wet weight gain (g).
§ Means of three separate tests.
‖ Hepatosomatic index (%) = liver weight × 100/body weight.

Table 3. Total ascorbate (TAA), dehydroascorbate (DHAA), and α-tocopherol concentrations in liver of yellow perch fed four experimental diets for 16 and 20 weeks*

(Mean values of triplicate groups and standard deviations)

Diet...	−C−E		−C+E		+C−E		+C+E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16 weeks								
TAA (nmol/g)	18·2[b]	10·2	12·0[b]	2·30	252·2[a]	43·3	258·4[a]	30·3
DHAA (nmol/g)	11·2[b]	0·73	13·4[b]	3·40	153·4[a]	19·9	149·3[a]	10·4
α-Tocopherol (nmol/g)	10·4[b]	3·50	39·4[ab]	32·6	25·2[b]	5·56	62·8[a]	18·9
20 weeks								
TAA (nmol/g)	12·4[b]	3·60	11·2[b]	9·02	291·9[a]	58·4	326·0[a]	87·9
DHAA (nmol/g)	9·36[b]	3·08	7·81[b]	12·1	151·8[a]	62·7	175·4[a]	99·2
α-Tocopherol (nmol/g)	11·1	6·36	34·3	13·9	28·6	21·3	48·1	21·6
TBARS (pmol/ml)	29·1[a]	8·29	8·18[b]	5·93	2·33[b]	0·50	2·60[b]	1·24

TBARS, thiobarbituric acid-reactive substances.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, See Table 1 and p. 590.

but not significantly elevated in vitamin E-supplemented dietary groups after 16 weeks. Also, increases of the liver α-tocopherol concentrations by approximately 150 and 40 % were observed by vitamin C supplementation in vitamin E-devoid and -supplemented dietary groups, respectively, after 20 weeks, although they were not significant ($P>0·05$).

Vitamin E isomers, such as α-tocopherol, β-+γ-tocopherols, δ-tocopherol, and tocopheryl phosphates (dietary source) were determined in diets and liver. However, the concentrations of β-+γ-tocopherols, and δ-tocopherol were very low in the diets (ranged from 0·01 to 0·5 μg/g diet). Concentrations in the liver of fish after 16 and 20 weeks were much lower than those in diets or not detectable.

The TBARS value was significantly lower in blood plasma of fish fed diets supplemented with either vitamin C or E and with both vitamins compared with that of fish fed the diet deficient in both vitamins C and E (Table 3). Plasma TBARS concentration was significantly affected by dietary supplementation of vitamin C ($P=0·001$) and E ($P=0·008$). The interaction between the two vitamins was also significant ($P=0·007$), showing a positive effect of either supplemental vitamin C or E (Table 4). However, no synergistic effect of the two vitamins on TBARS value was found.

From the 13th week, fish began to show some deficiency symptoms, and at the 20th week the fish fed the diets deficient only in vitamin C (−C−E and −C+E) exhibited severe deficiency symptoms, such as haemorrhages (Fig. 1 (A)), scoliosis (Fig. 1 (B); vitamin C-specific), anorexia, and lens cataracts (Fig. 1 (C)). The cumulative percentages of fish showing deficiency symptoms by the 20th week were 28·3 (SD 5·77) and 16·7 (SD 2·89) for diets −C−E and −C+E, respectively. The above deficiency symptoms were not found in fish fed vitamin C-supplemented diets (+C−E and +C+E), regardless of vitamin E status.

Table 4. Statistical results by two-way ANOVA test showing the main effect of dietary vitamin C or E, and their interaction with each dependent variable* (*P* values and F ratios)

Dependent variables	Error between subject effects	Statistical results by ANOVA†					
		Vitamin C		Vitamin E		Interaction	
		P	F ratio	*P*	F ratio	*P*	F ratio
Final weight	22·3	0·006	13·6	0·046	5·57	NS	2·41
FCR	0·04	0·002	20·0	NS	0·12	NS	0·95
IMI	1·83	0·01	11·5	NS	1·18	NS	4·73
Survival	483·7	0·019	8·6	0·032	6·7	NS	1·95
TAA	697·2	<0·001	94·1	NS	0·29	NS	0·33
DHAA	837·5	0·002	20·0	NS	0·18	NS	0·07
α-Tocopherol	2312·8	NS	2·54	NS	4·76	NS	0·04
TBARS	211·1	0·001	29·7	0·008	12·1	0·007	12·7
TAA (16 weeks)	179·9	<0·001	239	NS	0·00	NS	0·16
DHAA (16 weeks)	31·3	<0·001	449	NS	0·02	NS	0·24
α-Tocopherol (16 weeks)	2931·2	NS	3·03	0·017	9·12	NS	0·15

FCR, feed conversion ratio; IMI, instantaneous meal intake; TAA, total ascorbic acid; DHAA, dehydro-ascorbic acid; TBARS, thiobarbituric acid-reactive substances.
* For details of diets and procedures, see Table 1 and p. 590.
† Degree of freedom was 1 in each case.

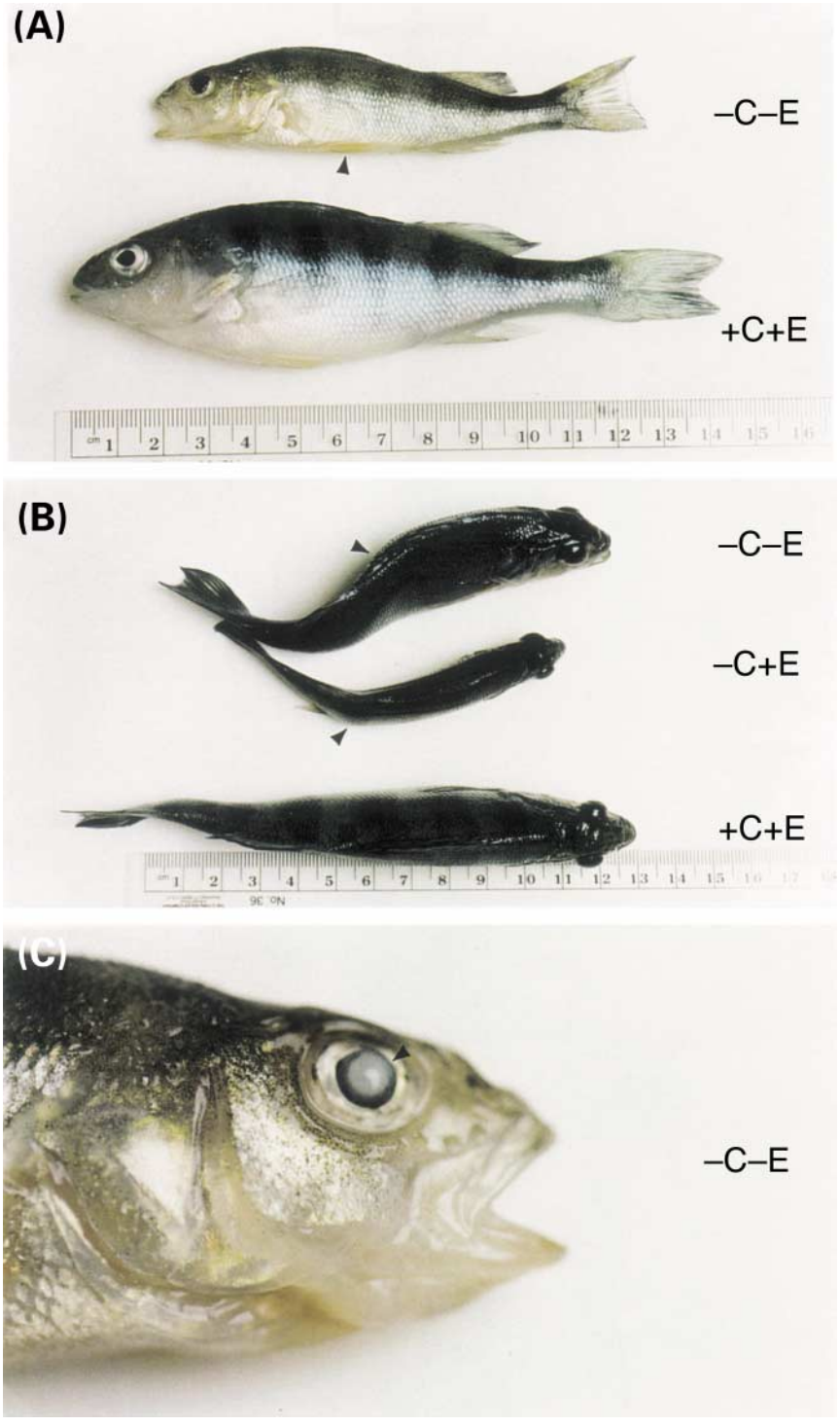

Fig. 1. Deficiency symptoms of vitamin C and/or E in yellow perch fed one of four casein-based semi-purified diets. The diets were formulated to contain low and high vitamin E levels of 5 and 160 mg/kg without or with vitamin C supplementation (250 mg/kg) designated as diets −C−E, −C+E, +C−E, or +C+E, respectively. (A), Growth depression and haemorrhages (►) on fins of fish fed −C−E diet (upper) and normal growth of fish fed +C+E (lower). (B), The broken vertebrae (scoliosis) of fish fed diets −C−E (upper) and −C+E (middle) and normal shape of fish fed +C+E diet (lower). (C), Extreme close-up of lens cataract (►) of fish fed −C−E diet for 20 weeks.

Discussion

The present study showed a typical growth trend in which growth rate is higher in the fish fed vitamin C-supplemented diets than in fish fed a diet devoid of vitamin C. The vitamin C level in the supplemented diets in the present study was approximately six times higher than the recommended requirement level in other fish species (National Research Council, 1993). The increased growth rates and feed efficiency in several fish species fed diets

sufficient in vitamin C are well documented elsewhere (Dabrowski *et al*. 1990, 1996; Lee *et al*. 1998; Shiau & Hsu, 1999). In the present study, the effects of supplemental vitamin C and E on how fish respond to the diets in terms of feed acceptance were tested. The results of the tests (Table 2; instantaneous meal intake) revealed that yellow perch are more affected by vitamin C than E, in terms of feed acceptance. This might mean that sufficient dietary vitamin C can increase the palatability to yellow perch that subsequently results in increased feed intake and enhanced appetite. The lower survivals in both vitamin C and E-deficient groups were due to the deficiency symptoms of the two vitamins that resulted in impaired metabolism.

In the present study, total ascorbate concentration in the liver increased in response to the dietary vitamin C supplementation (Table 3). There were no signs of dietary vitamin E effects on the liver total ascorbate or dehydroascorbate concentrations. Similar results were found in lake sturgeon (Moreau *et al*. 1999). Moreau *et al*. (1999) found that ascorbate concentrations in the liver and posterior kidney were correlated with dietary vitamin C regardless of dietary vitamin E level. However, there are other works that have reported elevated ascorbate concentration in liver of Atlantic salmon (Hamre *et al*. 1997) and rats (Tanaka *et al*. 1997), and human plasma (Hamilton *et al*. 2000) by supplementation of vitamin E.

Numerous studies have been carried out on vitamin C and E and their interactions in mammals (McCay, 1985). However, there are several inconsistent results in terms of the sparing effect of vitamin C on vitamin E in human and animal models (Chen, 1981; Bendich *et al*. 1984; Wantanowicz *et al*. 1984; Jacob *et al*. 1988, 1996; Burton *et al*. 1990; Igarashi *et al*. 1991; Stoyanovsky *et al*. 1995; Hamilton *et al*. 2000). In fish, literature is scarce on the interactions of the two vitamins (Gatlin *et al*. 1986; Hamre *et al*. 1997; Wahli *et al*. 1998; Moreau *et al*. 1999). In Atlantic salmon, Hamre *et al*. (1997) reported that liver vitamin E concentrations were not affected by vitamin C except in the vitamin C-deficient dietary group. Vitamin E data in yellow perch liver (Table 3) are well supported by Hamre *et al*. (1997) and Wahli *et al*. (1998). In the present study, α-tocopherol concentrations in liver were elevated by approximately 150 % by the supplementation of vitamin C in vitamin E-deficient dietary groups after 16 and 20 weeks. Therefore, the hypothesis that vitamin C regenerates vitamin E by reducing the tocopheroxyl radical *in vivo* (Tappel, 1968; McCay, 1985; Mukai *et al*. 1991) may be supported in yellow perch. The findings in the present study suggest that the sparing or regenerating effects of vitamin C on vitamin E in fish may possibly be conditional depending on the vitamin E status in tissues. This phenomenon, which showed conditional sparing effects of vitamin C depending on vitamin E status, was found in other studies in fish (Hamre *et al*. 1997; Wahli *et al*. 1998; Moreau *et al*. 1999). Moreau *et al*. (1999) also found the sparing effect of vitamin C for vitamin E in liver of juvenile lake sturgeon fed diets similar to those used with the yellow perch except for a five times higher dose of vitamin C. In that study (Moreau *et al*. 1999), dietary vitamin C supplementation (1250 mg/kg diet) increased liver α-tocopherol by 46 % in vitamin E-supplemented groups. In the vitamin E-deficient groups, however, vitamin C decreased liver α-tocopherol significantly, and the authors discussed that the decreased level was attributed to the pro-oxidant effect of high vitamin C dose in the diet. Yellow perch, a teleost, cannot synthesize vitamin C *in vivo*, unlike lake sturgeon (Dabrowski, 1990, 1994; Moreau & Dabrowski, 1998), which can synthesize vitamin C *in vivo* due to the presence of gulonolactone oxidase (*EC* 1·1·3·8), an enzyme involved in ascorbic acid synthesis. The negative effects of a high dose of vitamin C in the juvenile lake sturgeon were then explainable. In the present study, however, we did not find any negative effect of vitamin C. This result was in agreement with Hamre *et al*. (1997) who used a teleost, Atlantic salmon, which cannot synthesize vitamin C *in vivo*.

TBARS has been the most frequently used indicator for determination of protective actions of the two antioxidant vitamins against lipid peroxidation (Harats *et al*. 1990; de Zwart *et al*. 1999). Our result in yellow perch showed similar trends easily found in studies of human and animal models (Harats *et al*. 1990; Sakuma *et al*. 1997; Liu & Lee, 1998; Naidoo & Lux, 1998). In the present study, TBARS in plasma of fish fed the diet deficient in both vitamins C and E was 12-fold greater ($P<0·05$) than that of fish fed diets supplemented with either vitamin C or E, or both (Table 3). Also, TBARS concentration was more affected by dietary vitamin C ($P=0·001$) than by vitamin E ($P=0·008$) (Table 4). This result is in agreement with the study of Naidoo & Lux (1998) that showed the reduction of TBARS in human blood plasma by combined administration of both vitamin C and E, or vitamin C alone. Tanaka *et al*. (1997) also reported a trend of TBARS level similar to that found in yellow perch in plasma and liver tissues of rats suggesting that vitamin C deficiency can increase oxidative stress *in vivo* more than vitamin E deficiency. However, there also were reports of negative (pro-oxidant) or unclear effects of vitamin C or both vitamin C and E on TBARS in human subjects (Mulholland *et al*. 1996; Nyyssonen *et al*. 1997) and fish (Moreau *et al*. 1999).

The deficiency signs found in the present study were very clear and similar to those found in other fish species (Poston, 1967; Halver *et al*. 1969; Moreau & Dabrowski, 1996; Hamre *et al*. 1997). Scoliosis found from the 15th week in the present study is one of the representative deficiency symptoms of vitamin C in fish (Poston, 1967; Halver *et al*. 1969) due to a failure of the normal production of collagen (Halver, 1988; National Research Council, 1993; Dabrowski, 2001). Other deficiency symptoms, such as anorexia and haemorrhages, were also reported as the deficiency signs of either vitamin C or E, or both vitamins by other fish species (National Research Council, 1993; Dabrowski, 2001). We also found that a dietary deficiency of vitamin C can cause lens cataracts in yellow perch (Fig. 1 (C)). The eye abnormalities caused by vitamin C deficiency were previously reported in fish species, such as rainbow trout (Halver *et al*. 1975), red drum (Collins *et al*. 1993), and oscar (Fracalossi *et al*. 1998). Dabrowski & Wieser (1990) also discussed that a high concentration of vitamin C in the fish eye reflects the importance of vitamin C in this organ and demonstrated that dietary vitamin C deficiency in cyprinid

fish resulted in significant depletion of ascorbate concentrations in all eye compartments; retina, lens, and humour.

In the present study, supplemental dietary vitamin C protected yellow perch against vitamin E-deficiency symptoms, such as poor growth, anorexia, and haemorrhages, which were observed in the groups fed diets deficient in both vitamin C and E. These results are well supported by the other studies in rainbow trout (Frischknecht *et al*. 1994; Wahli *et al*. 1998) and Atlantic salmon (Hamre *et al*. 1997). The liver vitamin C has been used as an indicator of vitamin C deficiency in fish (Hilton *et al*. 1977; Lim & Lovell, 1978; Hardie *et al*. 1991; Fournier *et al*. 2000). Overt deficiency symptoms were found in fish that had vitamin C concentrations less than 170 and 114 nmol/g liver in channel catfish and rainbow trout, respectively (Hilton *et al*. 1977; Lim & Lovell, 1978). In the present study, the fish groups that showed deficiency symptoms also exhibited very low concentrations of liver vitamin C (9·84 nmol/g liver; Table 3).

In conclusion, our findings in yellow perch may support the hypothesis that vitamin C spares and/or regenerates vitamin E *in vivo*. Supplemental vitamin C and/or E reduce lipid peroxidation *in vivo*. However, there were no synergistic effects of both vitamins. The deficiency symptoms of vitamin E, with respect to antioxidant properties may be reduced or prevented by dietary supplementation of vitamin C. These findings need to be considered in further studies of the dietary requirement for the two vitamins in fish, especially in yellow perch.

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