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Examination of the qualitative ability of some cold water marine teleosts to synthesise ascorbic acid

Anne Mæland *, Rune Waagbø

Institute of Nutrition, Directorate of Fisheries, P.O. Box 185, N-5002 Bergen, Norway

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Abstract

Activity of L-gulonolactone oxidase (GLO), which is involved in ascorbic acid (AA) synthesis, was measured in kidney and liver tissues of seven teleosts, Atlantic sterlet, *Acipenser ruthenus*, and Atlantic dogfish, *Squalus acanthias*. GLO activity was also measured in liver and kidney tissues of Atlantic halibut, *Hippoglossus hippoglossus*, and Atlantic salmon, *Salmo salar*, fed AA unsupplemented and AA supplemented diets. AA synthesis was not detected in both tissues of the seven teleosts but was found in the kidneys of sterlet and dogfish. The respective GLO activities were 240 and 513 μg AA synthesised g^{-1} kidney tissue h^{-1}. Cold water marine teleosts such as herring, *Clupea harengus*, eel, *Anguilla anguilla*, salmon, cod, *Gadus morhua*, mackerel, *Scomber scombrus*, halibut, and turbot, *Scophthalmus maximus*, lack GLO activity and therefore dietary AA supplementation is required in their culture. AA deficient as well as AA supplemented diets did not induce GLO activity in salmon or halibut.

Keywords: Vitamin C; L-Gulonolactone oxidase; Ascorbic acid synthesis; Marine fish species; Aquaculture; Tissue ascorbic acid; Depletion; Diet

1. Introduction

Ascorbic acid (AA) is synthesised de novo from glucose in livers or kidneys of most vertebrates [2,10,14]. The AA producing tissues contain the enzyme, L-gulonolactone oxidase (EC 1.1.3.8, GLO). L-Gulonolactone oxidase catalyses the final step in AA synthesis where L-gulonolactone is converted to 2-oxo-L-gulonolactone, which spontaneously isomerises to L-ascorbic acid. Species that are not able to synthesise AA lack this enzyme [25].

All mammalian species, with exception of primates and guinea pigs synthesise AA in the liver [2,10,15]. In reptiles, amphibians and primitive bird species AA synthesis occurs in the kidneys [2,15]. Teleosts, as salmonids and channel catfish, *Ictalurus punctatus*, are not able to synthesise AA [12,20,27]. However, AA synthesis has been found in kidneys and/or livers from several warm water teleost species such as common carp, *Cyprinus carpio*, mullet, *Mugil cephalus* and goldfish, *Carrasius auratus* [20,23,27]. GLO activity has been found in kidneys of primitive actinopterigian fishes [6,13], some elasmobranchs and African lungfish, *Protopterus aethiopicus* [24]. The site of AA synthesis in white sturgeon, *Acipenser transmontanus*, is the posterior half of the kidney while the head kidney has the highest AA concentration [12].

AA status in mammalians affects the GLO activity. Exogenous intake of high doses of AA reduced the GLO activity in mouse liver [26]. This may be different in fish since increased AA status in Siberian sturgeon, *Acipenser baeri*, had no effect on the GLO activity [13].

Teleosts develop AA deficiency symptoms such as reduced growth and survival, scoliosis, lordosis, haemorrhages and reduced immune competence when AA is

* Corresponding author. Tel.: +47 5523800; fax: +47 55238095; e-mail: anne.meland@nutr.fiskeridir.no

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absent in the diet [9,18,22]. Atlantic turbot deficient on AA develops tyrosine crystals in kidney and spleen [3]. In Ayu, *Plecoglossus altivelis*, depleted of AA a lack of schooling behaviour was observed [11]. However, turbot juveniles did not develop any deficiency symptoms when fed AA unsupplemented diet over a period of 3 months (R. Waagbø, pers. commun.).

Eel, salmon, cod, turbot and halibut are among commonly used fish species in aquaculture. Storage and feeding periods in sea cages could be relevant also for other wild caught fish species (mackerel, herring). The AA requirement for most of the marine cold water teleosts presented in this experiment are not established. To the best of our knowledge, nothing is known so far regarding the ability of these teleosts to synthesise AA. GLO may influence the level of dietary supplementation of AA to cover the requirement and maintenance of AA status in the tissues.

The aim of this study was to determine whether marine cold water teleosts actual for aquaculture have the ability to synthesise AA, as evaluated by GLO activity in liver and kidney tissue. The study included an examination of the impact of dietary AA supplementation on the potential GLO activity in Atlantic halibut and Atlantic salmon. Atlantic dogfish and sterlet, as well as rat were used as positive controls.

2. Materials and methods

2.1. Fish and sampling

In the present study GLO activity was examined in kidney and liver tissue of the following species:

Squaliformes	Atlantic dogfish	*Squalus acanthias* L.
Acipenseriformes	Sterlet	*Acipenser ruthenus* L.
Clupeiformes	Atlantic herring	*Clupea harengus* L.
Anguilliformes	European eel	*Anguilla anguilla* L.
Salmoniformes	Atlantic salmon	*Salmo salar* L.
Gadiformes	Atlantic cod	*Gadus morhua* L.
Perciformes	Atlantic mackerel	*Scomber scombrus* L.
Pleuronectiformes	Atlantic halibut	*Hippoglossus hippoglossus* L.
	Atlantic turbot	*Scophthalmus maximus* L.

Five fish of each species were sampled. Sterlet was obtained from a local zoo store and Atlantic herring was obtained from Bergen Aquarium (Norway). Wild Atlantic dogfish, European eel, Atlantic cod and Atlantic mackerel were collected from local fishermen and Atlantic turbot was obtained from Stolt Sea Farm, Øyestranda (Norway). All of the fish were immediately after killing kept frozen on dry ice and kept at -80°C until analysed. Livers and kidneys were dissected prior to analysis. Rat liver tissue was used as positive control for GLO activity.

At Austevoll Aquaculture Research Station, Institute of Marine Research (IMR), Norway, 50 young Atlantic halibut juveniles were kept in a 100-l glass-fibre tank for 4 weeks and fed in excess by automatic feeders formulated diet with no supplementation of AA. Thereafter, the halibuts were randomly distributed into two tanks and fed in excess diets supplemented and unsupplemented with AA kg^{-1} for 4 weeks. At Matre Aquaculture Research Station, IMR (Norway) Atlantic salmon fry were distributed into two glass-fibre tanks (1 m $\times$ 1 m $\times$ 0.35 m) and fed in excess by automatic feeders formulated diets supplemented and unsupplemented with AA kg^{-1} for 24 weeks. At the end of these feeding periods five fish per tank were anaesthetised in metomidate HCl solution (7 g l^{-1}) and immediately transferred to dry ice.

The fish meal-based diets fed to the salmon and halibut were produced according to Sandnes et al. [18]. The diets were supplemented with 0 or 100 mg crystalline L-ascorbic acid kg^{-1}, packed in plastic bag and stored under CO_2 atmosphere at -20°C until use. The AA content in the unsupplemented and supplemented halibut diets were 0 and 40 mg kg^{-1}, respectively. The respective salmon diets contained 0 and 106 mg kg^{-1}.

2.2. Analytical methods

GLO activity in livers and kidneys were determined according to Dabrowski [4] with the following modifications. Samples of tissue (0.1–0.5 g) were homogenised with five-fold ice-cold 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 0.2% sodium deoxycholate by use of a Potter–Elvehjem homogeniser equipped with a teflon pestle. The homogenate was centrifuged for 10 min at 15000 $\times$ g (4°C). An aliquot of 63 μl of the supernatant, the enzyme extract, was added to 1 ml phosphate buffer and 63 μl 50 mM glutathione (Sigma, St. Louis, MO, USA) and the reaction was initiated by adding 125 μl 100 mM L-gulonolactone (Sigma). For each sample a blank was made by replacing the substrate, L-gulonolactone, with 50 mM phosphate buffer. The samples were incubated at 22°C for 3 h and the enzyme reaction was terminated by addition of 0.5 ml 20% meta-phosphoric acid (MPA). After 5 min on ice the samples were centrifuged for 10 min at 1800 $\times$ g (4°C) followed by AA determination in the supernatants as described below. GLO activity was calculated as μg AA synthesised h^{-1} g^{-1} tissue.

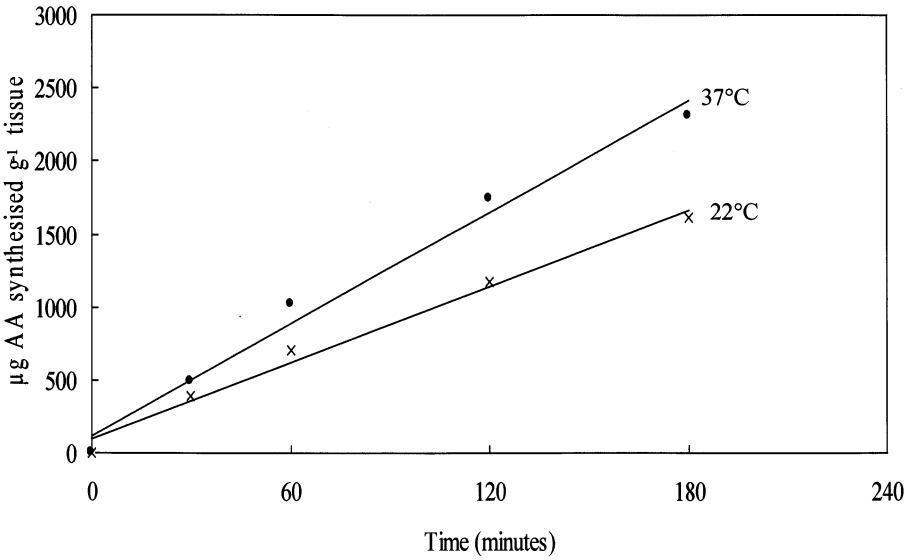

Fig. 1. Comparison of GLO activity in rat liver at 22 and 37°C at time 0, 30, 60, 120 and 180 min.

Linearity of the GLO activity in rat liver tissue was analysed by terminating the enzyme reaction after 0, 30, 60, 120 and 180 min at 22 and 37°C. A comparison of GLO activity at 22 and 37°C were performed in five samples of sterlet kidneys and rat livers incubated for 3 h.

AA content of the liver and kidney tissues from the five individuals per species, the dietary groups of salmon and halibut and the diets was analysed by means of an HPLC method using electrochemical detection of AA. The HPLC system consisted of a double piston pump (LKB, Bromma, Sweden), auto injector (Gilson, Villiers-le-Bel, France), an amperometric electrochemical detector (Hewlett-Packard, Waldbronn, Germany) with a glassy carbon working electrode and an integrator (Shimadzu, Kyoto, Japan). The potential of the glassy carbon working electrode was set at $+0.6$ V versus an AgCl reference electrode. Current range and response time was set at 0.5 μA and 8 s, respectively. The injection volume was 20 μl (Rheodyne loop 7010 injector, CA, USA) and flow rate was 0.8 ml per min. The stationary phase was an ODS-Hypersil (C18) 5 μm analytical column (100 mm $\times$ 4.6 mm) connected to a similar guard column (20 mm $\times$ 4.0 mm) both obtained from Hewlett-Packard.

The mobile phase consisted of 1.1 mM tetra-butyl-ammonium-hydrogen-sulphate of LiChropur quality (Merck, Darmstadt, Germany), 16 mM sodium acetate trihydrate, 0.54 mM EDTA and 10 mM potassium chloride. The chemicals were dissolved in double-distilled water and adjusted to pH 4.8 with anhydrous acetic acid followed by vacuum-filtration through a 0.2-μm filter. Prior to use the mobile phase was degassed with helium.

Six working standards containing 0.1–1.0 μg AA ml^{-1} were prepared in mobile phase with added 0.5% (w/v) MPA and 0.1% D,L-dithiothreitol (DTT). The working standard solutions were kept in cryotubes at -80°C until use.

Samples of 1 g wet weight were weighed accurately and mixed with 3 ml ice-cold 5% MPA with added 0.54 mM EDTA and 0.1% DTT using a Potter-Elvehjem homogeniser. The samples were centrifuged at 4°C at $1800 \times g$ for 10 min. Volumes of 1.5 ml of the supernatants were mixed well with 3 ml chloroform/methanol solution (2:1, v/v) before centrifugation for 10 min as described above. Aliquots of the water phase were mixed with mobile phase buffer with added 0.5% MPA and 0.1% DTT to give an AA concentration between 0.1 and 1 μg ml^{-1}. The sample solutions were then filtered through a 0.45-μm filter into auto-sampler cups. The samples were kept on ice until start of the analysis.

2.3. Statistics

The results are given as mean values $\pm$ SD. Amount AA synthesised g^{-1} rat liver versus incubation time was subjected to linear regression. Comparison between enzyme activity at 22 and 37°C in rat liver and sterlet kidney was carried out using Kolmogorov–Smirnov two-sample test carried out at the significance level $P < 0.05$. The statistical tests were carried out by Statistica™, 4.5 software (Statsoft, USA, 1993).

3. Results

The linearity of GLO activity in rat liver at 22 and 37°C showed regression coefficients of 0.99 and 0.98,

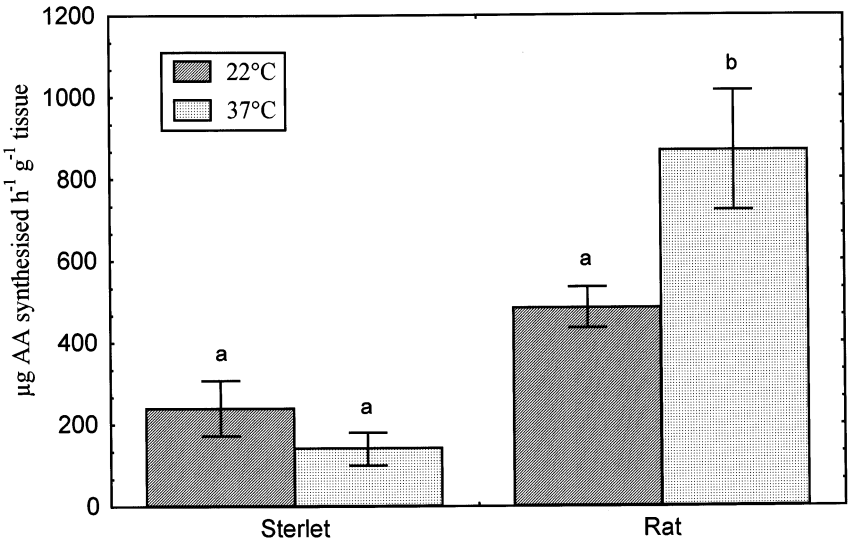

Fig. 2. Comparison of GLO activity in rat liver and sterlet kidney at 22 and 37°C after an incubation time of 3 h. Different letters indicate significant differences within the species ($P < 0.05$, $n = 5$)

respectively (Fig. 1). Even though the formation of AA proceeded almost in a straight line up to 180 min, approximately 20–25% reduction in enzyme activity occurred after 180 min compared to after 60 min.

The comparison of enzyme activity in sterlet kidney incubated at 22 and 37°C for 3 h showed higher, although not significant, activity at 22°C (Fig. 2). Significantly higher GLO activity was observed in the rat liver at 37°C compared to 22°C.

The mean weight of the five fish per species is given in Table 1, and ranged between 7.3 and 1992 g for the salmon and the cod, respectively.

GLO activity in kidneys of dogfish and sterlet were 513 and 240 μg AA synthesised h^{-1} g^{-1} tissue, respectively (Table 1). In kidneys from herring, eel, salmon, cod, mackerel, turbot and halibut no GLO activity was detected. No GLO activity was detected in livers from any of the fish species (Table 1). Nor were any GLO activities observed in salmon or halibut fed AA supplemented or unsupplemented diets.

AA content in kidneys ranged between 3 μg AA g^{-1} tissue in salmon fed no supplementary AA to approximately 78 μg AA g $tissue^{-1}$ in dogfish and eel (Fig. 3). In liver tissue the AA content ranged between 5 μg AA g^{-1} in salmon fed AA-deficient diet and 95 μg AA g^{-1} tissue in mackerel (Fig. 3). The AA contents in both kidney and liver from salmon and halibut were influenced by their respective dietary AA regimes.

4. Discussion

In the present study mean GLO activity in the sterlet was 240 μg AA synthesised h^{-1} g^{-1} tissue, which is similar to enzyme activities found in other acipencerides [6,13]. The GLO activity at 25°C in these experiments ranged between 9 and 50 μg AA synthesised h^{-1} g^{-1} tissue in Siberian sturgeon [13] and between 400 and 1000 μg AA synthesised h^{-1} g^{-1} tissue in lake sturgeon, *Acipenser fulvescens*, and white sturgeon, respectively [6]. GLO activity in the kidneys of the elasmobranch species gummy shark, *Mustelus manazo*, and stringray, *Dasyatis akajei*, was approximately 14 and 4 μg AA synthesised h^{-1} mg^{-1} protein, respectively [24]. In the present experiment, mean enzyme activity in the kidneys of the dogfish was 513 μg AA synthesised h^{-1} g^{-1} tissue.

This study showed that no GLO activity exists in the kidneys or livers of the teleost species examined. Similar results have been obtained for several teleosts of different orders [15,20,24]. However, there are indications that some teleosts as tilapias, cyprinids and mullet may have the ability to synthesise AA [20,23,27]. In carp, there was reported GLO activity in both kidney and liver [20,27]. On the contrary, no GLO activity in tissue from carp was confirmed in the later study [4,5]. It has been demonstrated that carp larvae require dietary supplementation of AA, as judged from deficiency signs occurring when fed low dietary AA [7].

The AA content in the AA-supplemented diet for the halibut was 40 mg kg^{-1}, which was lower than expected (supplemented 100 mg AA kg^{-1}). The free form of AA that has been used in the present experiment is known to be unstable when exposed to oxygen and metal ions [19] and AA concentration decrease in the feed under production and storage. The AA content in the AA-supplemented salmon diet was 106 mg kg^{-1}.

No GLO activity was found in liver and kidney from halibut and salmon fed diets with or without AA. Atlantic salmon develops deficiency symptoms when

Table 1
Mean weight ± SD and L-gulonolactone oxidase activity ± SD in kidneys of some teleosts, sterlet and dogfish ($n = 5$)

Species	Body weight (g)	GLO activity (μg AA synthesised h^{-1} g^{-1} tissue)	
		Kidney	Liver
Picked dogfish (*Squalus acanthias*)	1548 ± 118	513 ± 224	N.D.
Sterlet (*Acipenser ruthenus*)	11.4 ± 2	240 ± 68	N.D.
Atlantic herring (*Clupea harengus*)	122 ± 39	N.D.[a]	N.D.
Eel (*Anguilla anguilla*)	402 ± 135	N.D.	N.D.
Atlantic salmon (*Salmo salar*)+	11.3 ± 2	N.D.	N.D.
Atlantic salmon (*Salmo salar*)−	7.3 ± 4	N.D.	N.D.
Cod (*Gadus morhua*)	1992 ± 385	N.D.	N.D.
Mackerel (*Scomber scombrus*)	358 ± 40	N.D.	N.D.
Atlantic halibut (*Hippoglossus hippoglossus*)+	23.3 ± 5	N.D.	N.D.
Atlantic halibut (*Hippoglossus hippoglossus*)−	24.4 ± 4	N.D.	N.D.
Turbot (*Scophthalmus maximus*)	24.0 ± 6	N.D.	N.D.

The + and − signs indicate that the fish was fed a diet with or without AA supplementation, respectively.
[a] Not detected.

the diet is low in AA [8,17]. The decrease in AA concentration in liver and kidney of the halibut, as well as the salmon, fed AA-deficient diets show the necessity of dietary AA supplementation to maintain tissue AA. As in the present study, AA depletion in organs occurs when diets are deficient of AA [1,16,18]. The minimum dietary AA requirement in Atlantic halibut remains to be established.

In rats and mice, the GLO activity at 37°C ranges between 400 and 3000 μg AA synthesised h^{-1} g^{-1} tissue when calculated by the authors from μmol AA μg AA synthesised h^{-1} g^{-1} tissue [10,26]. The result obtained in the rat liver in the present experiment was within the range, where mean GLO activity at 37°C was approximately 900 μg AA synthesised h^{-1} g^{-1} tissue.

Oxygen gas was not supplied to the reaction mixture in the present experiment. Although continuous aeration was necessary in experiments described by other authors [23,27], the results of the present study were reproducible without oxygen. The GLO activity decreased when air was bubbled to the mixture instead of oxygen [27]. However, in an experiment with rat liver microsome fraction the decrease in oxygen saturation level was 10–20% during 30 min incubation and conclusion was that no supply for oxygen is necessary during an assay run for 3 h [4]. The enzyme activity in the present study was higher at 37 than 22°C, but the linearity and r^2 of the curves were similar. The linear curves indicated that oxygen was not a limiting factor in the enzyme assay. Duration of the AA synthesis for 3 h was chosen to include detection of very low GLO activities.

The reduction in the slope of the GLO activity curve in the rat liver after 60 min (Fig. 3) was likely also to appear in the enzyme assay of kidney tissue of dogfish and sterlet. As a consequence, some lower enzyme activities per hour were calculated for the kidneys of sterlet and dogfish and rat liver in this study due to termination of the enzyme assay after 3 h. The linearity of the enzyme activity curve in various experiments varies from 20 min in carp hepatopancreas [27], 40 min in goldfish kidney [23] and up to 120 min in rat and pigeon liver [4]. This study demonstrated that the enzyme reaction with rat liver was almost linear for 180 min.

GLO activity measurements in fish tissues in other studies were performed at temperatures between 25 and 37°C [4,23,27]. 22°C was chosen due to the higher GLO activity in the sterlet kidney tissue at 22°C compared to activity at 37°C. Increased GLO activity at 22°C compared to 37°C in the kidneys of sterlet indicates different temperature optima between mammalia and fish species. When AA synthesis occurs in tissue of poikilotherms the AA synthesis depends on the ambient temperature, concomitant with activity of general metabolism [21]. The rate of AA formation in sterlet and dogfish would be dependent, among several factors, on the availability of substrate and temperature of the surroundings as in mullet [23].

In summary herring, eel, Atlantic salmon, cod, mackerel, halibut and turbot do not have the ability to synthesise AA. These results verify that dietary supplementation of AA is required in aquaculture of these teleosts.

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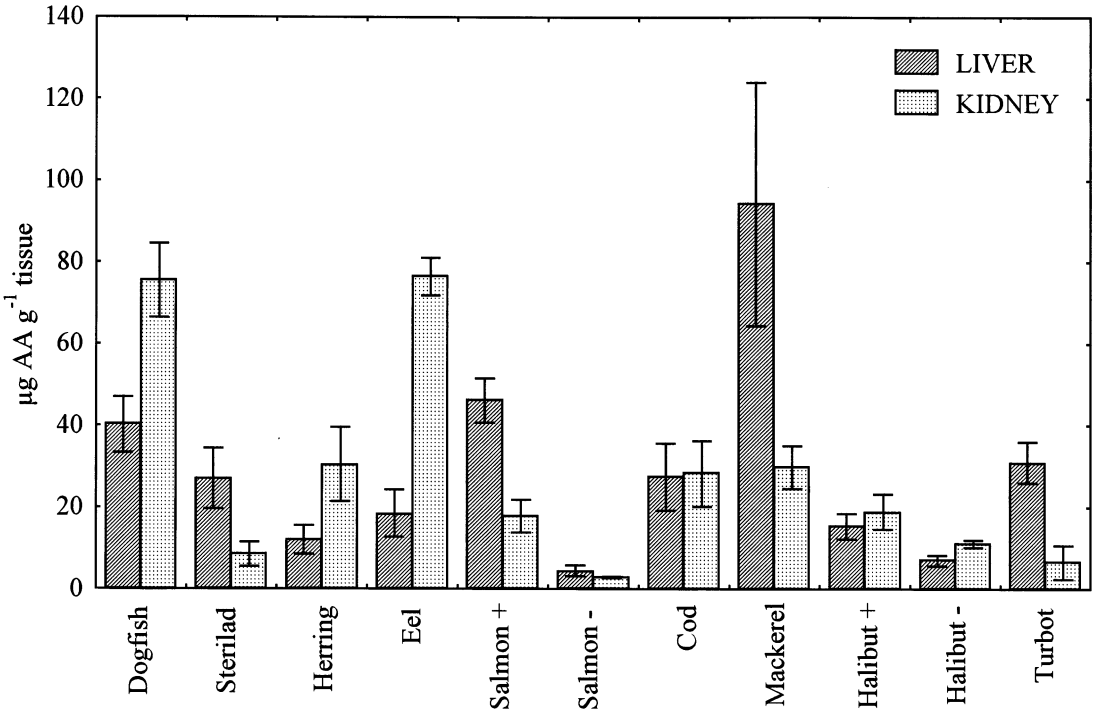

Fig. 3. AA content in livers and kidneys of the species studied in the present experiment. The + and − signs indicate that the fish were fed a diet with or without AA supplementation, respectively.

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References

[1] Albrektsen S, Lie Ø, Sandnes K. Ascorbyl palmitate as a dietary vitamin C source for rainbow trout (*Salmo gairdneri*). Aquaculture 1988;71:359–68.

[2] Chatterjee IB. Evolution and the biosynthesis of ascorbic acid. Science 1973;182:1271–2.

[3] Coustans MF, Guillaume J, Metailler R, Dugornay O, Messager J. Effect of ascorbic acid deficiency on tyrosinemia and renal granulomatous disease in turbot (*Scophthalmus maximus*) interaction with a slight polyhypovitaminosis. Comp Biochem Physiol 1990;97A:145–52.

[4] Dabrowski K. Gulonolactone oxidase is missing in teleost fish. The direct spectrophotometric assay. Biol Chem Hoppe-Seyler 1990;371:207–14.

[5] Dabrowski K. Administration of gulonolactone does not evoke ascorbic acid synthesis in teleost fish. Fish Physiol Biochem 1991;9:215–21.

[6] Dabrowski K. Primitive actinopterigian fishes can synthesize ascorbic acid. Experientia 1994;50:745–8.

[7] Dabrowski K, Hinterleitner S, Sturmbauer C, El-Fiky N, Wieser W. Do carp larvae require vitamin C? Aquaculture 1988;72:295–306.

[8] Hamre K, Waagbø R, Lie Ø. Vitamin C and E interacts in juvenile Atlantic salmon (*Salmo salar*, L.). Free Radic Biol Med 1997;22:137–49.

[9] Hardie LJ, Fletcher TC, Secombes CJ. The effect of dietary vitamin C on the immune respons of the Atlantic salmon (*Salmo salar* L.). Aquaculture 1991;95:201–14.

[10] Jenness R, Birney EC, Ayaz KL. Variation of L-gulonolactone oxidase activity in placental mammals. Comp Biochem Physiol 1980;67B:195–204.

[11] Koshio S, Sakakura Y, Iida Y, Tsukamoto K, Kida T, Dabrowski K. The effect of vitamin C intake on schooling behaviour of amphidromous fish, ayu *Plecoglossus altivelis*. Fisheries Sci 1997;63:619–24.

[12] Moreau R, Dabrowski K. The primary localization of ascorbate and its synthesis in the kidneys of acipenserid (*Chondrostei*) and teleost (*Teleostei*) fishes. J Comp Physiol B 1996;166:178–83.

[13] Moreau R, Kaushik SJ, Dabrowski K. Ascorbic acid status as affected by dietary treatment in the Siberian sturgeon (*Acipenser baeri* Brandt): tissue concentration, mobilisation and L-gulonolactone oxidase activity. Fish Physiol Biochem 1996;15:431–8.

[14] Nandi A, Mukhopadhyay CK, Ghosh MK, Chattopadhyay DJ, Chatterjee IB. Evolutionary significance of vitamin C biosynthesis in terrestrial vertebrates. Free Radic Biol Med 1997;22:1047–54.

[15] Nishikimi M, Yamauchi N, Kiuchi K, Yagi Y. Homology of L-gulonolactone oxidase of species belonging to mammalia, aves and amphibia. Experientia 1981;37:479–80.

[16] Rosenlund G, Jørgensen L, Waagbø R, Sandnes K. Effects of different dietary levels of ascorbic acid in plaice (*Pleuronectes Platessa* L.). Comp Biochem Physiol 1990;96A:395–8.

[17] Sandnes K, Hansen T, Killie JEA, Waagbø R. Ascorbate-2-sulfate as a dietary vitamin C source for Atlantic salmon (*Salmo salar*): 1. Growth, bioactivity, haematology and immune response. Fish Physiol Biochem 1990;8:419–27.

[18] Sandnes K, Torrissen O, Waagbø R. The minimum dietary requirement of vitamin C in Atlantic salmon (*Salmo salar*) fry using Ca ascorbate-2-monophosphate as dietary source. Fish Physiol Biochem 1992;10:315–9.

[19] Sandnes K, Utne F. Processing loss and storage stability of ascorbic acid in dry fish feed. Fisk Dir Skr Ser Ernæring 1982;11:39–44.

[20] Soliman AK, Jauncey K, Roberts RJ. Qualitative and quantitative identification of L-gulonolactone oxidase activity in some teleosts. Aquacult Fish Manag 1985;16:249–56.

[21] Shikata T, Iwanaga S, Shimeno S. Regulation of carbohydrate-metabolism in fish. 25. Metabolic response to acclimation temperature in carp. Fisheries Sci 1995;61:512–6.

[22] Tacon AGJ. Nutritional Fish Pathology. United Nations Development Programme, Food and Agriculture Organization of the United Nations, Rome, Italy, 1992:1–33.

[23] Thomas P, Bally MB, Neff JM. Influence of some environmental variables on the ascorbic acid status of mullet, *Mugil cephalus* L. tissues. II. Seasonal fluctuations and biosynthetic ability. J Fish Biol 1985;27:47–57.

[24] Touhata K, Toyohara H, Mitani T, Kinoshita M, Satou M, Sakaguchi M. Distribution of L-gulono-1,4-lactone oxidase among fishes. Fisheries Sci 1995;61:729–30.

[25] Toyohara H, Nakata T, Touhata K, Hashimoto H, Kinoshita M, Sakaguchi M, Nishikimi M, Yagi K, Wakamatsu Y, Ozato K. Transgenic express of L-gulono-γ-lactone oxidase in medaka (*Oryzias latipes*), a teleost fish that lacks this enzyme necessary for L-ascorbic acid biosynthesis. Biochim Biophys Res Commun 1996;223:650–3.

[26] Tsao CS, Young M. Enzymatic formation of ascorbic acid in liver homogenate of mice fed dietary ascorbic acid. In Vivo 1990;4:167–70.

[27] Yamamoto Y, Sato M, Ikeda S. Existence of L-gulonolactone oxidase in some teleosts. Bull Jpn Soc Sci Fish 1978;44:775–9.