Bull. Fac. Bioresources, Mie Univ.
No.16 : 17 − 24
March 1, 1996

Study on Vitamin E Deficiency in Amago Salmon

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Abstract

In order to investigate the symptoms and histopathological changes of vitamin E deficiency in amago salmon, *Oncorhynchus rhodurus*, two purified diets with or without supplement of 400 mg vitamin E (*α*-tocopherol) per kg of diet were administered to fish for 22 weeks in flowing-water aquaria. A recovery experiment was then performed, feeding the deficient fish with a complete diet for the following 4 weeks. Deficient fish showed lower appetite, reduced growth rate and mild anaemia. However, the survival rate was not significantly different from that of controls. The liver of most fish displayed focal atrophic hepatocytes with dilated sinusoids, multifocal necrosis, perivasculitis and also pericholangitis. In fish with these hepatic lesions, periarteritis was occasionally observed in the submucosa layer of intestine. These changes were clearly reversed at the end of recovery period. No sign of myopathy and ceroidosis was found.

Key wards : Amago salmon, Vitamin E deficiency, Histopathology

Introduction

Vitamin E, a lipophilic antioxidant, functions together with selenium and ascorbic acid in the enzyme glutathione peroxidase or superoxide dismustase to inhibit the chain reactions of polyunsaturated fatty acid peroxidation. It was found that vitamin E localized in biological membranes inhibits peroxidation of membrane-bound lipids[1, 2]. Thus, a dietary requirement for vitamin E has been demonstrated in chinook salmon, *Oncorhynchus tschawytscha*[3], carp, *Cyprinus carpio*[4, 5], channel catfish, *Ictalurus punctatus*[6-8], Atlantic salmon, *Salmo salar*[9], rainbow trout *Oncorhynchus mykiss*[10, 11], tilapia, *Oreochromis niloticus*[12] and blue tilapia, *0. aureus*[13]. The reported requirements of vitamin E for maximum growth and prevention of gross deficiency signs vary from 20−300 mg/kg of diet depending largely on the fish species. This requirement increased when fish were fed with diets containing higher levels of polyunsaturated fatty acids[5, 10, 14, 15] and peroxidized lipids[6, 16, 17]. Pathological changes

Accepted : Oct. 30, 1995
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associated with vitamin E deficiency of animals were mainly characterized by myopathy (=muscular dystrophy) of striated muscle, yellow fat disease and erythrocyte fragility[1, 18, 19]. Pathological changes usually developed in the cases of feeding on diets added with peroxidized lipids resulting in the occurrence of severe myopathy, ceroidosis and haemolysis[10, 20-22]. However, there are also reports showing severe myopathy in fish fed with vitamin E deficient diets containing non-oxidized lipids at low water temperatures[16, 23].

Amago salmon is one of the important culturing fish spread widely in the western regions of Japan, and the study on vitamin E deficiency in this species has never been reported although other salmonids fish were studied. In this study, growth, haematology, and clinical and histopathological signs were examined in vitamin E deficient amago salmon. The taken data should be useful for the development of cultural management.

Materials and Methods

1. *Fish, diets, growth, haematology and histopathology*

The amago salmon used in this study were hatched in a laboratory of the National Research Institute of Aquaculture (Tamaki-cho, Mie) under controlled conditions. At first feeding the fish were fed with a commercial diet. Then the fish (mean body weight 2.9 g) were divided into two dietary groups (control and deficient) with two replicates, each of 45 fish in aquaria (0.2 × 0.5 × 0.38 m) with a flow-through system (16.5−17.0 ℃). The control groups were fed a nutritionally complete casein diet (Table 1) containing 400 mg vitamin E (α-tocopherol) kg^{-1} of diet while the deficient groups were fed the same diet without supplement of vitamin E. The dietary oils, used in this study were linolenic acid methyl ester and linolic acid methyl ester, and the both were free of peroxides and α-tocopherol. Fish were fed to satiation twice a day. External signs and the number of dead fish were monitored daily through the experimental period. To evaluate growth, all fish from vitamin E deficient groups and control groups were collected at two week intervals, anesthetized in a 0.01 % aqueous solution of tricaine and individually weighed. After 22 weeks, the feeding test was concluded and five fish from one control group and from each replication of deficient groups were randomly selected for haematological test and histopathological study. In addition, one of the deficient groups was given the nutritionally complete diet (Table 1) for the following 4 weeks to investigate recovery as well as the control. At the end of recovery test, five fish from each recovery and control group were sampled again for histopathological study. The methodology for haematological and histological study used in this experiment was

Table 1 Composition of the complete (control) test diet for amago salmon

Ingredient	Percentage of diet
Vitamin-free casein	41.00
Cellulose flour	15.22
Dextrin	18.00
Palmitic acid methyl ester	6.00
Linolic acid methyl ester[1]	0.50
Linolenic acid methyl ester[1]	0.50
Amino acid mixture[2]	5.28
Carboxymethyl cellulose	5.00
Vitamin mixture[2]	4.50
Mineral mixture[2]	4.00

[1] Free of vitamin E
[2] Composition as described by Taveekijakarn *et al.*[20]

the same as described by Taveekijakarn *et al.*[20].

2. *Statistical procedures*

To evaluate statistical differences ($P<0.05$) among treatment means for growth, feed conversion ratio, and haematological data, Student's *t*-test was used.

Results

1. *Growth and mortality*

After 4 weeks, the fish fed on vitamin E free diet began to lose their appetite resulting in a significant decrease in average body weight at the end of the experimental period (Fig. 1). These average were 22.2 and 26.5 g in the deficient groups and 34.2, 36.7 g in the control groups. The deficient groups had no significant difference in cumulative mortality compared to the controls. Deficiency symptoms such as convulsion or muscular atrophy were not observed. During the recovery period, fish displayed an increase in appetite and no mortalities.

2. *Haematology*

The mean haematological values for each blood parameter are shown in Table 2. After 22 weeks, haemoglobin and haematocrit content were significantly lower in the deficient groups. These results are concurrent with the decrease in RBC value although no statistical difference was noted. This may indicate a tendency to anaemia if deficiency period was prolonged.

3. *Histopathology*

Histological changes were only found in liver of the fish fed with a vitamin E free diet. The changes most frequently observed were focal to diffuse necrotic lesions of hepatocytes (Fig. 2a), distributed among atrophic or normal hepatic parenchyma. Within the necrotic foci there were hepatocytes with vacuolized cytoplasm or karyopyknosis, infiltrated macrophages, newly formed collagen fibers and fibrinous exudate. These necrotic lesions were always scattered throughout the parenchyma and more widely distributed in liver with signs of periarteritis, perivaculitis and per icholangitis, which showed disorientation of surrounding connective tissue and infiltrated by inflammatory cells (Fig. 2b). However, early necrotic

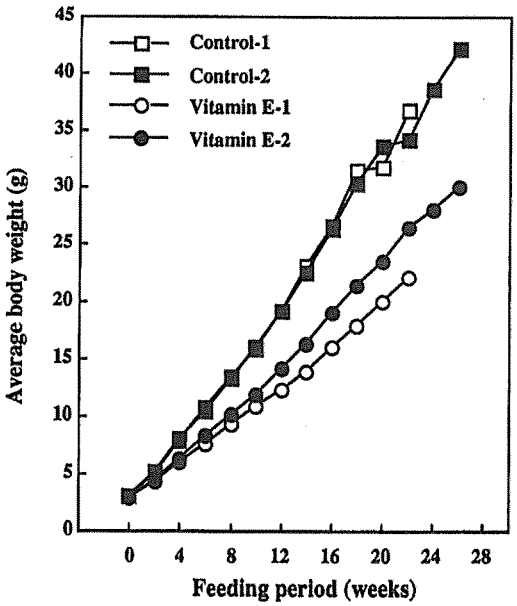

Fig. 1 Growth curves for amago salmon fed a vitamin E deficient diet (open and closed circles). Control groups of fish were fed the same diet supplemented with 400 mg vitamin E kg^{-1} of diet (open and closed squared) and the recovery group (closed circle) was also fed the same complete diet from week 22 to week 26.

Table 2 Haematological paramenters (mean±SE) of amago salmon fed a complete test diet (CTD) or a vitamin E deficient diet (EDD) after 22 weeks

Haematological parameters	Feeding examination		
	CTD	EDD-1	EDD-2
RBC ($10^4 mm^{-3}$)	116.0±5.2	106.6±5.8	110.0±7.4
Hb (g dl^{-1})	10.3±0.4	8.4±0.3*	9.1±0.2*
Ht (%)	40.5±1.2	33.3±2.5*	33.7±2.2*
MCH (pg)	89.4±2.7	79.8±4.5	84.3±6.3
MCV (μm^3)	352.1±11.6	312.4±14.4	310.2±11.3
MCHC (%)	25.5±1.1	25.7±1.9	27.4±1.6

Mean that are significantly different ($p<0.05$) from those of control are indicated by asterisk.

lesions were seen near or adjacent to focal perivascular necrosis. Atrophic hepatocytes with compact cytoplasm were also observed. Sinusoid between atrophied hepatocytes were dilated and partly infiltrated by loose fibrous tissue (Fig. 2c). These severe changes were found in five of ten fish while the other fish only showed small focal perivascular inflammation accompanied by slight focal necrotic and atrophic hepatocytes.

Additionally, in fish showing severe changes in liver, the connective tissue surrounding blood vessels in the submucosa layer of intestine or pyloric caeca was always damaged and infiltrated by inflammatory cells (Fig. 2d), whereas, mucosal epithelium was still normal. Only slight necrosis of red muscle fibers with infiltrated macrophages was found in some fish while no myopathy change was observed in the white musculature. Moreover, neither haemosiderin nor ceroid deposition were noticed in liver, spleen or kidney as well as no pathological sign indicating yellow fat in the adipose tissues of all deficient samples.

Above changes were obviously reversed after 4 weeks of feeding a nutritionally complete diet excepting the liver that showed only mild atrophic hepatocytes associated with small granulomatous lesions in the parenchyma and within the connective tissue surrounding intrahepatic bile duct, arteries and veins.

Discussion

In this study on amago salmon, there was no visual dorsal atrophy associating with myopathy (=muscular dystrophy) changes of white musculature although the red musculature had slight necrosis. This finding was different from Atlantic salmon, rainbow trout, common carp, channel catfish, and mammals fed vitamin E-free diets. Among them the occurrence of myopathy was an indication of vitamin E deficiency[4, 8, 9, 19, 21, 23]. Cowey *et al.*[16] and McLoughlin *et al.*[23] reported severe myopathy in rainbow trout fed with vitamin E deficient diets containing fresh oil at water temperatures from 6 to 12 ℃. They concluded that low water temperature might be an important factor in producing the vitamin E responsive myopathy. Furthermore, supported work by Hazel[25] showed that polyunsaturated fatty

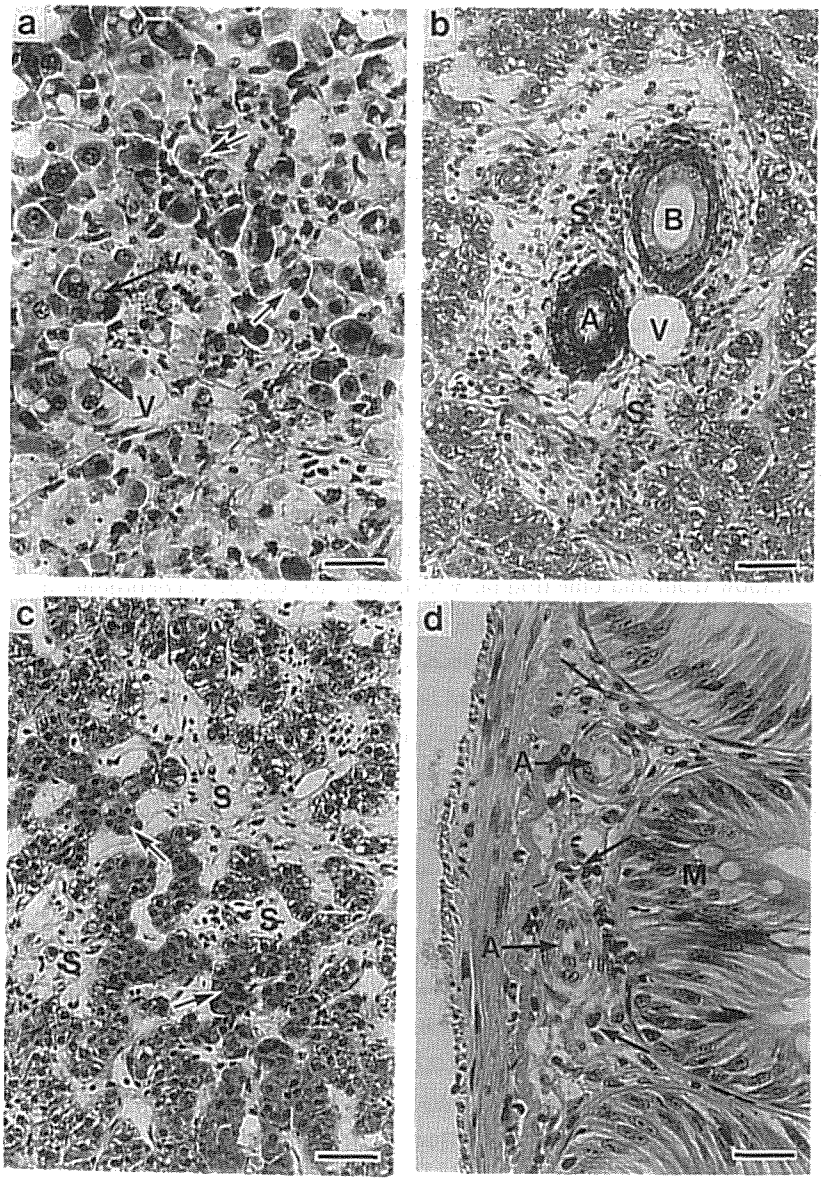

Fig. 2 Histological changes of vitamin E deficient fish after 22 weeks. a : Liver showed focal necrosis containing necrotic hepatocytes with vacuoles in cytoplasm (V) or pyknotic nuclei (arrows) (AZ, bar=20 μm). b : Hepatic triad displayed disorientation of surrounding connective tissue which were infiltrated by inflammatory cells (S) (AZ, bar=40 μm). B=bile duct ; A=artery ; V=portal vein. c : In affected liver, atrophic hepatocytes (arrows) accompanied with dilated sinusoid (S) were usually found and partly infiltarted by loose fibrous tissue (AZ, bar=40 μm). d : The fish with severe lesions in liver, the artery (A) within submucosa layer of intestine showed periarteritis which were infiltrated by macrophages (arrows) (H & E, bar=20 μm). M=mucosa layer.

acid content of the cell membranes of rainbow trout significantly increased at 5 ℃ compared to at 20 ℃. An increased content of polyunsaturated fatty acids in the cell membranes was likely to be associated with an increased requirement for vitamin E to maintain the integrity of the membranes. In other reports, rainbow trout fry held at 15 ℃ and fed with vitamin E free diet added with or without oxidized oil did not develop myopathic lesions although they showed an increase in erythrocyte fragility and a decrease in haematocrit[14, 26, 27]. So it might be considered possible that no evidence of severe myopathy in amago salmon was due to rearing in a high water temperature.

On the other hand, amago salmon fed the vitamin E-free diet displayed hepatocytic changes such as atrophy, necrosis, and perivaculitis in liver. The same hepatocytic necrosis were only known in pig with vitamin E and selenium deficiency[18], however, this is the first finding of elevated hepatic necrosis in fish fed a diet without supplement of oxidized oil and vitamin E. Moreover, damaged cell membranes of hepatocytes were also found in tiger puffer *Takifugu rubripes*, fed on moist pellets containing high levels of peroxidized lipids and low level of vitamin E [28]. These facts would suggest that hepatocytes were significantly damaged by auto-oxidation of lipids contained in the membrane and cytoplasm under the condition of vitamin E dificiency. Additionally, there are reports showing occurrence of hepatocytic ceroidosis in rainbow trout and blue tilapia, which were fed with diets containing high level of lipid or rancid brown meal [13, 20-22]. The rainbow trout which took in rancid lipids showed ceroidosis of the visceral organs including cardiac muscle, spleen, gill and digestive tracts as well as hepatocytes. As mentioned above, visceral ceroidosis would not be caused by simple vitamin E deficiency, but high level of dietary lipid peroxides should contribute to the occurrence of this lesion as mentioned by Miyazaki [20]in the studies on Nutritional Myopathy Syndrome associated with vitamin E deficiency and intake of rancid oils in cultured fish.

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アマゴのビタミンE欠乏症に関する研究

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アマゴのビタミンE欠乏症の病徴および病理組織像を解明するために，ビタミンE欠乏症カゼイン飼料を作成し，アマゴ稚魚の飼育試験を行った。対照区には完全カゼイン飼料を投与した。22週間の飼育の結果，欠乏区の魚は食欲減退，成長不良，軽度な貧血を起こしたが顕著な斃死はなかった。病理組織学的には肝臓に肝細胞の萎縮と巣状壊死，血管周囲炎と胆管周囲炎が観察され，これら肝臓に障害を持つ魚では腸管粘膜下織にも血管周囲炎が見られた。23週以降4週間完全カゼイン飼料の投与で回復試験を行なった結果，以上の欠乏魚の異常は消失していた。