Requirement of *Tilapia* for α -Tocopherol

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Feeding experiments were conducted to investigate the requirement of *Tilapia* for vitamin E and to see whether or not the vitamin E requirement of *Tilapia* is increased by elevating dietary lipid levels.

No apparent deficiency symptom was induced in fish receiving diets of different levels of α -tocopherol with 5% of lipid, although fish fed with the tocopherol-free diet showed the lowest hepatosomatic index. The addition of more than 5 mg of α -tocopherol per 100 g diet completely prevented the appearance of this symptom. The concentration of α -tocopherol in the liver of fish receiving the tocopherol-free diet was lowest and increased as dietary tocopherol levels increased.

The fish receiving diets containing 10% and 15% fish oil methyl esters without tocopherol began to show low appetite and reduced growth from the 3rd week of feeding and were low in growth and feed conversion efficiency. The supplement of 50 mg α -tocopherol to these diets effectively improved these conditions. Elevation of the dietary lipid levels from 5 to 15% in the diet containing 50 mg of α -tocopherol reduced the concentration of α -tocopherol in the whole body.

The results obtained in these experiments suggest that 5-10 mg of α -tocopherol per 100 g of diet containing 5% lipid should provide adequate protection in most practical *Tilapia* diets. The elevation of dietary lipid levels increased the tocopherol requirement of *Tilapia*, judging from the growth and mortality.

Vitamin E has been shown to be essential for carp,¹⁻⁵⁾ channel catfish,⁶⁻⁸⁾ rainbow trout,⁹⁻¹⁴⁾ chinook salmon¹⁵⁾ and Atlantic salmon.¹⁶⁾ Dietary vitamin E (DL- α -tocopheryl acetate) required for maximum growth and prevention of gross external deficiency signs was about 10 mg/100 g diet for carp, 3 mg/100 g diet for chinook salmon and channel catfish, and 3 or 10 mg/100 g diet for rainbow trout. The dietary vitamin E requirements of carp and rainbow trout increased when they were fed diets containing higher levels of polyunsaturated fatty acids, although Cowey *et al.*¹⁰⁾ reported that elevation of polyunsaturated fatty acid did not affect the minimum requirement of rainbow trout for α -tocopherol. And they¹⁷⁾ also reported that α -tocopherol requirement may increase as water temperature decreases.

Thus this study was conducted to investigate the requirement of *Tilapia* for vitamin E in a 5% dietary lipid level and to see whether or not the vitamin E requirement of *Tilapia* is increased by elevating dietary lipid levels. For this purpose two experiments were designed. In the first experiment (Experiment I) *Tilapia* were fed diets containing a 5% fatty acid mixture of methyl

esters of 18:1 ω 9, 18:2 ω 6 and 18:3 ω 3 which satisfies the essential fatty acid requirement of *Tilapia* and different levels of α -tocopherol, in order to determine their vitamin E requirement. The second experiment (Experiment II) was conducted to investigate whether there is an increased requirement for dietary tocopherol as the unsaturation of fat in the diet is increased.

This paper deals with these results.

Materials and Methods

The compositions of experimental diets are given in Tables 1 and 2. The commercial vitamin free casein, α -starch and dextrin were defatted three times with boiling ethanol to remove vitamins as completely as possible. The compositions of the vitamin and the mineral mixtures were the same as described previously.¹⁸⁾ The dietary lipid used for Expt. I was a mixture of fatty acid methyl esters (18:1 ω 9: 18:2 ω 6: 18:3 ω 3=2.5: 1.5: 1.0). Pollock liver oil obtained from Ricken Vitamin Co., Ltd. was derived into the methyl esters totally free of vitamin E by the same method described in the preceding paper.¹²⁾

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Table 1. Composition of the experimental diets for *Tilapia nilotica* in Experiment I (%)

Ingredient	Diet no.				
	1	2	3	4	5
Casein* ¹	40	40	40	40	40
α -Starch* ¹	20	20	20	20	20
Dextrin* ¹	15	15	15	15	15
Cellulose	13	13	13	13	13
Mineral mix.	5	5	5	5	5
Vitamin mix.* ²	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamin E (mg)	0	5	10	30	50
Lipid* ³	5	5	5	5	5

*¹ Defatted three times by boiling with methyl alcohol.*² α -Tocopherol free vitamin mixture.*³ 2.5% 18:1 ω 9 + 1.5% 18:2 ω 6 + 1.0% 18:3 ω 3.**Table 2.** Composition of the experimental diets for *Tilapia nilotica* in Experiment II (%)

Ingredient	Diet no.				
	6	7	8	9	10
Casein* ¹	40	40	40	40	40
α -Starch* ¹	20	20	20	20	20
Dextrin* ¹	15	15	15	15	15
Cellulose	13	13	8	3	3
Mineral mix.	5	5	5	5	5
Vitamin mix.* ¹	1.5	1.5	1.5	1.5	1.5
Vitamin E (mg)	0	50	0	0	50
Fish oil-ME* ²	5	5	10	15	15

*¹ See the footnote of Table 1.*² Methyl esters of pollock liver oil.

Fingerlings of *Tilapia nilotica* weighing around 0.5 g were kept on a tocopherol-free diet for 2 weeks before the initiation of the feeding experiments. They were then divided into five lots of 30 fish each in both Expts. I and II. A group of fish was held in a 30 liter glass tank supplied with city water, dechlorinated with activated charcoal and adjusted to 20°C in both experiments. Food was offered three times daily and each time as long as the fish continued to feed. The feeding periods were 26 weeks for Expt. I and 9 weeks for Expt. II. Preparation of diet, fish care and feeding were all carried out as reported previously.¹⁸⁾

In Expt. I, the dietary tocopherol levels in diets 1-5 were varied from 0 to 50 mg per 100 g diet and the supplement of mixed methyl ester of 18:1 ω 9, 18:2 ω 6 and 18:3 ω 3 was 5% of the diet. In Expt II, the methyl esters of pollock liver oil was added at levels of 5, 10 and 15% of the diet and DL- α -tocopheryl acetate was supplemented at a level of 50 mg per 100 g for diets 7 and 10. Diets

6, 8 and 9 were arranged to contain no tocopherol.

At the end of feeding experiment in Expt. I, all the fish were taken from each lot and used for the determination of α -tocopherol, lipid content and thiobarbituric acid (TBA) values in the liver and muscle. Three to five fish from each lot were pooled for determination of moisture, protein, lipid, α -tocopherol and TBA value in whole body of fish in Expt. II.

The analytical procedures such as lipid extraction, separation of polar and nonpolar lipids, preparation of methyl esters, GLC operating conditions, quantitative determination of α -tocopherol and TBA value were those described in the previous papers.^{4,12)}

Results and Discussion

Experiment I

Mean weight gain, feed conversion efficiency, and hepatosomatic index for 26 weeks of feeding are summarized in Table 3. During the feeding experiment of 26 weeks no apparent deficiency symptom was observed in fish receiving diets with different levels of α -tocopherol. There was also no marked difference in weight gain and feed efficiency among the fish receiving diets containing different levels of α -tocopherol, although feed conversion efficiency in fish fed on diet 3 containing 10 mg of α -tocopherol was slightly lower. The fish receiving the diet without supplement of tocopherol showed the lowest hepatosomatic index among the experimental groups, and swollen pale livers. The addition of 5 mg of α -tocopherol per 100 g diet completely prevented the appearance of these symptoms. Elevation of the tocopherol level from 5 to 50 mg did not result in any further increase in growth rate and feed conversion.

Thus, any apparent deficiency symptom was not observed in *Tilapia* fed on the diet containing 5% lipid without α -tocopherol supplement for 28 weeks including 2 weeks of preliminary feeding with the same diet. The same result was obtained with rainbow trout fed on tocopherol-free diets containing 5% lipid, although apparent deficiency symptoms were induced by elevating the lipid level to 15% with pollock liver oil methyl ester. Woodall *et al.*¹⁵⁾ also reported a low value for vitamin E requirement of chinook salmon, less than 3 mg of α -tocopherol per 100 g dry diet. Requirement of salmonid fishes for vitamin E may be lower than that of other fish species, such as carp, channel catfish and eel. The results ob-

Table 3. Results of the 26 weeks of feeding in Experiment I

Diet no.	DL- α -Tocopheryl acetate (mg/100 g diet)	Av. body wt. (g)		Growth rate (%)	Feed efficiency	Hepatosomatic index
		Initial	Final			
1	0	0.50	6.89	1090	0.97	0.80
2	5	0.49	7.83	1330	1.02	1.29
3	10	0.55	7.40	1246	0.89	1.58
4	30	0.55	8.22	1216	0.98	1.17
5	50	0.48	7.34	1172	0.90	1.91

Table 4. Effect of dietary levels of α -tocopherol on the α -tocopherol contents and lipid class of livers in Experimental I

Diet no.	DL- α -Toc. (mg/100 g diet)	α -Toc. (μ g/g liver)	% in Liver							
			TL	PL	CE	TG	FFA	FS	DG	MG
1	0	—	9.0	1.9	0.1	4.3	2.0	tr	0.2	0.1
2	5	2.3	11.2	2.2	0.1	6.9	1.7	—	0.2	0.1
3	10	55.7	14.2	2.0	0.1	9.2	2.0	0.1	0.3	0.1
4	30	341.3	11.1	1.8	0.1	5.8	2.6	0.1	0.3	0.1
5	50	817.6	15.5	1.6	tr	11.9	1.5	—	0.4	0.2

Table 5. Fatty acid compositions of liver polar lipids in Exp. I

Fatty acid	VE (mg/100 g diet)				
	0	5	10	30	50
16:0	17.3	18.4	17.3	17.8	19.1
16:1	2.3	2.6	2.3	2.2	2.3
18:0	14.6	13.6	12.6	13.2	12.8
18:1 ω 9	14.0	14.4	14.0	13.4	13.7
18:2 ω 6	5.4	5.6	5.3	5.5	5.2
18:3 ω 3	1.1	1.9	1.1	1.1	0.9
20:3 ω 9	1.8	1.7	1.8	1.8	2.0
20:4 ω 6	9.0	7.6	9.1	8.1	9.3
22:5 ω 6	5.7	5.8	6.0	6.0	4.7
22:6 ω 3	12.4	14.7	15.3	17.2	15.5

tained in this experiment with *Tilapia* also suggest a lower vitamin E requirement of *Tilapia* like salmonid fishes.

The α -tocopherol contents and lipid class of livers are summarized in Table 4. The concentration of α -tocopherol in the liver of fish fed on the α -tocopherol-free diet was lowest among the experimental groups and increased as dietary tocopherol levels increased, as observed in carp and rainbow trout. The lipid content of the livers also increased in proportion to dietary tocopherol levels due to increase of triglyceride as described later. This result was different from the previous data with carp,⁴⁾ in which dietary α -tocopherol levels exerted on appreciable effect on lipid content and its composition. The triglyceride content increased as the elevation of dietary α -tocopherol.

The fatty acid composition of polar lipid fractions in livers of *Tilapia* fed on diets containing different levels of α -tocopherol for 26 weeks is shown in Table 5. The fatty acid profiles were clearly reflected by those of dietary lipids. The percentages of 20:4 ω 6, 22:5 ω 6 and 22:6 ω 3 were high in each experimental group, indicating that dietary 18:2 ω 6 and 18:3 ω 3 were elongated and desaturated to highly unsaturated fatty acids, as already demonstrated in the previous experiments on essential fatty acids for *Tilapia*.^{19,20)} In the liver polar lipid of *Tilapia* fed the tocopherol deficient diet, the proportion of 16:0 and 22:6 ω 3 was low, and that of 18:0 and 18:1 ω 9 was high. With elevation of the dietary α -tocopherol levels, the proportion of former fatty acids increased and that of the latter fatty acids decreased. These results are in good agreement with those obtained in the previous study with carp.⁴⁾ In the fish receiving α -tocopherol at more than 10 mg (diet 3) their fatty acid distribution was not much different at all the tocopherol levels, suggesting that a tocopherol deficiency exerts some effects on fatty acid composition, but tocopherol supplements exceeding a level required by fish to carry out basic biochemical functions have little effect on fatty acids profiles.

The proximate compositions, the α -tocopherol content and TBA value of muscles are shown in Table 6. The results obtained for the muscle are found to be very similar to those obtained in carp. A high water content reflected a lower protein content in the muscle of the fish receiving the

Table 6. Proximate compositions, α -tocopherol contents and TBA values of muscle in Experiment I

Diet no.	DL- α -Toc. (mg/100 g diet)	Muscle					
		α -Toc. (μ g/g)	TBA value*	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
1	0	tr	2.08	78.3	18.8	1.5	1.4
2	5	0.7	1.29	78.2	19.5	1.8	1.2
3	10	6.6	1.04	77.5	19.7	1.9	1.3
4	30	15.8	0.78	77.8	19.9	1.9	1.3
5	50	17.0	1.00	77.6	20.0	1.9	1.3

* TBA concentration, read as absorbance at 540 nm per 100 g of sample, was expressed as relative values, with fish fed diet 5 constituting the reference group.

Table 7. Results of the 9 weeks of feeding in Experiment II

Diet no.	DL- α -Toc. (mg/100 g diet)	Fish oil-ME*	Av. body wt. (g)		Growth rate (%)	Feed efficiency	Mortality (%)
			Initial	Final			
6	0	5	0.52	1.64	215	0.80	3
7	50	5	0.56	1.80	221	0.84	7
8	0	10	0.64	0.81	27	0.18	7
9	0	15	0.57	0.83	46	0.26	20
10	50	15	0.67	1.86	178	0.90	3

* Methylster of pollock liver oil.

vitamin E deficient diet. The lipid content of the liver was also low in these fish. The concentration of α -tocopherol in the muscle increased as the dietary tocopherol level increased, although the rate of accumulation per g tissue was significantly lower than the liver. This result suggests that α -tocopherol absorbed accumulated mainly in the livers and scarcely at all in the muscle, as already observed in the previous work.³⁾

TBA concentration, read as absorbance at 540 nm per 100 g of sample, was expressed as relative values with fish fed diet 5 constituting the reference group. It was the highest in the tocopherol deficient group and the lowest in fish fed on the diet containing 30 mg of α -tocopherol per 100 g diet, as observed in carp muscle from the tocopherol deficient group.⁴⁾

Experiment II

Mean weight gain, feed conversion efficiency, and mortality for 9 weeks of feeding are summarized in Table 7, and the changes of average body weight during the experimental period are shown in Fig. 1. The fish receiving the 10% and 15% fish oil methyl ester diets without tocopherol began to show low appetite and reduced growth rate from the 3rd week of feeding and were lowest in weight gain and feed conversion efficiency. The fish fed on the 15% fish oil methyl ester diet without tocopherol showed highest

Fig. 1. The changes of the average body weight during the experimental period of *Tilapia* in Experiment II.

mortality of 20% at the termination of the experiment. These symptoms were not observed in the fish fed on the 5% lipid diet without α -tocopherol, agreeing with the results obtained in Expt. I and rainbow trout.¹²⁾

The α -tocopherol contents, TBA values and proximate compositions in whole body of *Tilapia* receiving the experimental diets are shown in Table 8. The results obtained for the whole

Table 8. Proximate compositions, α -tocopherol content and TBA values of whole bodies in Experiment II

Diet no.	Diet		Whole body					
	DL- α -Toc. (mg/100 g diet)	Pollock liver oil-ME	α -Toc. (μ g/g)	TBA value*	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Initial			7.8					
6	0	5	1.3	1.63	73.4	15.7	6.9	3.9
7	50	5	47.3	1.00	73.1	15.4	8.2	3.2
8	0	10	0.8	1.73	77.9	14.4	4.0	3.8
9	0	15	0.7	2.64	78.0	14.3	4.2	3.9
10	50	15	11.8	1.14	74.3	15.1	7.3	3.4

* See the footnote of Table 6.

bodies are very similar to those obtained for muscles in carp by Watanabe *et al.*,⁵⁾ namely, a high water content reflected a lowered protein content in the whole bodies of fish fed on the α -tocopherol-free diet with 10% and 15% fish oil methyl esters.

The lipid content of whole bodies was low in the fish fed on diets 8 and 9, both without supplement of α -tocopherol.

The concentration of α -tocopherol in the whole body of fish fed on the diet containing 5% fish oil methyl esters with 50 mg of α -tocopherol was high, whereas that of the fish fed on the α -tocopherol-free diet was very low. Elevation of the dietary lipid level from 5 to 15% in the diet containing 50 mg of α -tocopherol reduced the whole body α -tocopherol content from 47.3 to 11.8 μ g/g, indicating a direct relationship between the PUFA intake and the vitamin intake as already demonstrated previously.^{4,5,12)} These results are also supported by the results of TBA determination. Addition of the methyl esters of fish oil to the α -tocopherol-free diet at more than 5% resulted in exhaustion of tissue tocopherol, indicating the same relationship as suggested by Horwitt²¹⁾ and Watanabe *et al.*^{4,5,12)} Watanabe *et al.*¹²⁾ demonstrated that at the same dietary lipid level of 15% the requirement of rainbow trout for α -tocopherol increased as the unsaturation of the lipid in the diet increased.

The TBA values are indicated by the relative value to that obtained in the fish fed on diet 7 containing 5% lipid and 50 mg of α -tocopherol. The TBA value in whole bodies of the fish fed on the α -tocopherol-free diet increased as the dietary level of fish oil methyl esters increased, but was effectively kept at a low value by addition of α -tocopherol to the diets.

The present study was conducted to investigate

the α -tocopherol requirement of *Tilapia* and whether there is an increased requirement for dietary tocopherol as the unsaturation of the fat in the diet is increased. The results obtained in these experiments suggest that 5–10 mg of α -tocopherol per 100 g of diet containing 5% lipid should provide adequate protection in most practical *Tilapia* diets. Data from this study also show that an elevation of dietary lipid levels increases the tocopherol requirement of *Tilapia*, judging from the appearance of apparent deficiency symptoms such as reduced appetite and growth rate, and a high mortality together with the reduced concentration of tocopherol in the livers and whole bodies due to elevation of unsaturation in diet.

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