Effect of α -Tocopherol Deficiency on Carp—IV. The Composition of Triglycerides

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The triglyceride composition of carp fed an α -tocopherol deficient diet was determined directly by gas chromatography. Rainbow trout fed a vitamin E-free diet were also analysed in a limited comparison study. Carp muscle lipids showed triglyceride distribution from C-43 to C-56 and the dominant components in the control were C-50 to C-54 triglycerides, whereas those of the experimental group were the C-43 and C-45 triglycerides. Furthermore, in the deficient carp, linoleic acid was absent in the muscle lipid. No significant difference between the control and experimental groups was observed in the triglyceride composition of rainbow trout.

In recent years the triglyceride composition of various kinds of lipids from animal and plant origins have been studied by direct analysis of gas chromatography. The authors have also extended the method of direct gas chromatographic analysis of triglycerides to fish lipids in order to investigate the effect of α -tocopherol deficiency on the lipid composition of carp. The analytical method employed has already been discussed in the preceeding paper (III) of this series.

Requirement of fish for vitamin E and its deficiency symptoms have been reported on rainbow trout¹⁾ and carp.²⁻⁴⁾ In these reports the effect of α -tocopherol deficiency on some of the lipids has also been demonstrated concerning fatty acid composition. Succeeding the previous paper⁴⁾ in which protein composition of the dystrophic muscle induced by vitamin E deficiency was demonstrated, the triglyceride composition of lipid from α -tocopherol deficient carp was analysed directly by gas chromatography. Rainbow trout fed a vitamin E-free diet was also analysed in a limited comparison study.

Experimental

Materials Muscle and viscera were obtained from carp and rainbow trout weighing 5 to 8 g which had been kept for 90 days and 152 days, respectively, on an α -tocopherol-free diet. The α -tocopherol deficient carp showed apparent muscular dystrophy, and no apparent deficiency symptom except poor growth was observed in rainbow trout. The

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control fish kept for the same period on a diet enriched with 60 mg per 100 g diet of α -tocopherol were also examined in parallel for both carp and rainbow trout. Details of diet preparation, fish care and feeding method were reported in the previous paper³⁾.

Analytical methods The triglycerides were isolated from each of the fats by the same method described in the preceding paper (III). The fatty acids recovered from triglycerides after preparative thin-layer chromatography were esterified with $\text{BF}_3\text{-MeOH}$ reagent. Removal of non-saponifiable materials from lipids, preparation of methyl esters (by $\text{BF}_3\text{-MeOH}$ esterifications of acids), and all GLC operations were carried out by the procedures described previously^{3,5)}.

Results and Discussion

The triglyceride composition and their component fatty acids of soybean oil used as test oil in diets is shown in Table 1. Soybean oil contains 10.7% C-16 and 89.3% C-18 fatty acids. However, the triglycerides are either C-54, C-52 or C-50. To have such a composition, the major peak, C-54, would correspond to a $\text{C}_{18}\text{C}_{18}\text{C}_{18}$ triglyceride such as trilinolein or oleodilinolein, and the second largest peak, C-52, to a $\text{C}_{16}\text{C}_{18}\text{C}_{18}$ triglyceride such as palmitodilinolein or palmitooleolinolein. The minor component, C-50, could correspond to a $\text{C}_{16}\text{C}_{16}\text{C}_{18}$ triglyceride such as linoleodiplamitin.

Table 1. Triglyceride and fatty acid composition of soybean oil.

	Carbon number	Composition (%)	
Fatty acids	16	16:0	10.7
		18:0	4.5
	18	18:1	24.0
		18:2	52.4
		18:3	8.3
Triglycerides	50	16, 16, 18	3.8
	52	16, 18, 18	31.5
	54	18, 18, 18	64.7

Fig. 1 shows the chromatograms of triglycerides of muscle lipids extracted from carp fed α -tocopherol supplemented and depleted diets. The peak areas in each chromatogram, the carbon number distribution and their fatty acid composition are summarized in Table 2. Muscle lipid of normal carp usually contains C-even triglycerides from C-42 to C-56 and C-odd triglycerides in small amounts⁵⁾. However, the distribution pattern of triglycerides were quite different between control and experimental groups. The dominant components of the control are the C-50 to C-54 triglycerides, whereas those of the experimental are the C-43 and C-45 triglycerides. The C-odd triglycerides, the

Fig. 1. Gas chromatograms of triglycerides in carp muscle lipids.

Operating conditions: Shimadzu Seisakusho Model GC-5APF gas chromatograph with a hydrogen flame ionization detector; 50 cm $\times$ 3 mm i.d. glass column packed with 2% OV-17 (phenyl methyl silicone) on 80/100 mesh silanized Shimalite W; column programmed 200–340°C at 4°C/min with 80 ml/min nitrogen carrier gas; detector and injection port temperatures at 350°C.

main components in the deficient group, must contain one C-odd fatty acid per triglyceride molecule, however C-odd fatty acids (such as C₁₃, C₁₅, C₁₇, or C₁₉) are excluded from the Tables since they are in small amounts. The result for triglyceride distribution seems to be in agreement with their fatty acid composition. The control lipid contains 49.56% C₁₈ fatty acids, reflecting higher amounts of C-50, C-52 and C-54 triglycerides. On the other hand, in vitamin E deficient carp lower levels of the C₁₈ acid family reflect a lower proportion of C-50 to C-54 triglycerides. The most striking feature of fatty acid composition of muscle lipids between the two groups is the disappearance of linoleic acid in the

Table 2. Triglyceride and fatty acid composition of carp muscle lipids.

Carbon number	Triglycerides (%)		Fatty acids	Fatty acids (%)	
	Control	Experimental		Control	Experimental
C 43	12.42	30.61	14:0	3.41	6.51
C 44	0.80	3.13	16:0	14.29	20.08
C 45	13.37	28.91	16:1	7.77	7.19
C 48	6.44	8.44	18:0	6.00	13.57
C 50	17.09	9.60	18:1	28.07	17.23
C 51	2.56	—	18:2	15.49	—
C 52	26.18	10.98	18:3	—	—
C 54	17.74	8.33	20:0	23.26	35.69
C 56	3.40	—	20:1	1.71	—

Table 3. Triglyceride and fatty acid composition of carp visceral lipids.

Carbon number	Triglycerides (%)		Fatty acids	Fatty acids (%)	
	Control	Experimental		Control	Experimental
C 43	0.74	0.78	14:0	1.01	2.02
C 46	1.90	1.56	16:0	12.93	23.06
C 48	2.87	5.32	16:1	4.60	7.08
C 50	12.99	20.50	18:0	4.38	5.73
C 52	36.00	37.41	18:1	38.15	49.76
C 54	38.11	27.74	18:2	31.82	7.08
C 56	7.38	6.60	18:3	2.37	—
			20:0	1.29	1.55
			20:1	3.45	3.71

Table 4. Triglyceride composition of rainbow trout.

Carbon number	Muscle (%)		Viscera (%)	
	Control	Experimental	Control	Experimental
C 35	0.23	0.68	0.59	2.05
C 37	0.80	2.26	8.12	6.62
C 39	0.74	1.92	6.27	4.35
C 42	0.11	0.26	0.97	0.82
C 44	0.27	0.47	1.01	0.82
C 46	0.65	0.64	0.85	1.04
C 48	4.06	3.64	4.49	5.77
C 50	15.67	14.84	18.59	20.76
C 52	39.29	34.13	31.61	32.77
C 54	29.90	31.23	22.51	21.80
C 56	8.27	9.98	3.19	3.20

deficient group (15.49% C_{18:2} acid was detected in the control). This fact may also support the results of triglyceride distribution in this group which showed low levels of longer chain triglycerides.

The visceral oil chromatogram of carp resembles slightly that of soybean oil. The visceral lipids showed triglyceride distribution from C-43 to C-56 and C-50, C-52 and

Fig. 2. Gas chromatograms of triglycerides in carp visceral lipids. Operating conditions as for Fig. 1.

C-54 as main components (Fig. 2 and Table 3). A higher level of C-50 triglyceride along with a lower amount of C-54 in comparison with the control was observed in α -tocopherol deficient carp. This triglyceride pattern is also in agreement with the results of fatty acid analysis, indicating that higher amounts of C_{18} acids along with lower levels of C_{16} acids were determined in the control, while in the experimental group a lower proportion of C_{18} acid members reflects higher levels of C_{16} fatty acids. Generally triglycerides which have the C_{18} acid as a fatty acid moiety are C-50, C-52 and C-54 triglycerides. Thus the decrease of C_{18} acids could be related with the lower amount of C-54 triglyceride. In particular the low linoleic acid level in vitamin E depleted carp seems to directly reflect

Fig. 3. Gas chromatograms of triglycerides in rainbow trout muscle lipids. Operating conditions as for Fig. 1.

the low amount of C-54 triglyceride. The decrease of linoleic series (ω 6*) was also observed in rats deficient in vitamin E⁶⁾.

Carp oil usually contains $C_{22:6\omega3}$ acid, however, the polyunsaturated acid was not detected through our experiments³⁾. The absence of $C_{22:6\omega3}$ acid in both control and experimental groups seems to reflect incorporation of a low level of $C_{18:3\omega3}$ acid from experimental oil, which contains linolenic acid in small amount. The same result was reported by CASTELL *et al.*⁷⁾ in the case of rainbow trout. The diet without linolenate decreased $C_{22:6\omega3}$ tissue level in rainbow trout. The pathway of ω 3 fatty acids (linolenate series) to polyunsaturated fatty acids was also demonstrated by KELLY *et al.*⁸⁾ and KAYAMA⁹⁾, indicating that fish metabolize fatty acids in essentially the same manner as terrestrial animal.

* Position of ultimate double bond relative to the terminal methyl group.

Fig. 4. Gas chromatograms of triglycerides in rainbow trout visceral lipids. Operating conditions as for Fig. 1.

On the other hand, the lipids of rainbow trout showed a wider triglyceride distribution from C-35 to C-54 than those of carp, and major components are C-48 to C-54 triglycerides in both muscle and viscera. As shown in Figs. 3-4 and Table 4, the triglyceride pattern of muscle lipids was similar to those of visceral lipids with slight emphasis on the amount of the C-54 triglyceride. Furthermore, no significant difference in the composition of triglycerides was observed between control and experimental groups. It is of interest that there is no effect of α -tocopherol deficiency on the triglyceride composition of rainbow trout, which is quite different from the case of carp. The same kind of difference between carp and rainbow trout has been demonstrated in the previous work^{2-4,10)}, in which carp fed α -tocopherol deficient diet showed muscular dystrophy, but the symptom was not observed in rainbow trout. These differences are probably due to species-specificity as mentioned in the previous paper¹⁰⁾.

References

- 1) A. N. WOODALL, L. M. ASHLEY, J. E. HALVER, H. S. OLCOTT, and J. VAN DER VEEN: *J. Nutrition*, **84**, 125–135 (1964).
- 2) T. WATANABE, F. TAKASHIMA, C. OGINO, and T. HIBIYA: *This Bull.*, **36**, 623–630 (1970).
- 3) T. WATANABE, F. TAKASHIMA, C. OGINO, and T. HIBIYA: *ibid.*, **36**, 972–976 (1970).
- 4) T. WATANABE, F. TAKASHIMA, C. OGINO, and T. HIBIYA: *ibid.*, **36**, 1231–1234 (1970).
- 5) N. IKEKAWA, M. MATSUI, T. YOSHIDA, and T. WATANABE: *ibid.*, **38**, 1267–1274 (1972).
- 6) D. J. W. LEE and M. McC. BARNES: *Br. J. Nutrition*, **23**, 289–295 (1969).
- 7) J. D. CASTELL, D. J. LEE, and R. O. SHINNHUBER: *J. Nutrition*, **102**, 93–100 (1972).
- 8) P. B. KELLY, R. REISER, and D. W. HOOD: *J. Am. Oil Chem. Soc.*, **35**, 120–189, 503–505 (1958).
- 9) M. KAYAMA: *This Bull.*, **30**, 647–657 (1964).
- 10) Y. HASHIMOTO, T. OKAICHI, T. WATANABE, A. FURUKAWA, and T. UMEZU: *ibid.*, **32**, 64–69 (1966).