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Effect of α-Tocopherol Deficiency on Carp—V The Compositions of Triglycerides and Cholesteryl Esters in Lipids of Young Carp

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A feeding experiment was conducted to investigate the effects of α-tocopherol deficiency on the compositions of triglycerides and cholesteryl esters in lipids of young carp. Feeding the α-tocopherol deficient diet resulted in reduced growth and, in muscle tissue, a high water content reflecting a lower protein content as observed in the case of carp fingerlings. In the lipids of all tissues of the tocopherol deficient group, the proportion of linoleic acid decreased. On the other hand, in general, there were no marked differences in the compositions of triglycerides and cholesteryl esters due to the tocopherol deficiency, although the percentages of C-54 and C-56 triglycerides were lower in the muscle lipids of fish fed the α-tocopherol-depleted diet and the lipid of the hepatopancreas from the deficient fish showed a lower proportion of the C-18 esters, mostly replaced by higher percentages of the C-16 and the C-20 esters.

In the previous study[1] in which the triglyceride composition of carp fingerlings fed an α-tocopherol deficient diet was determined, carp muscle lipids showed the C-43 and C-45 triglycerides to be the dominant components in the experimental group, whereas in the control group the C-50 to C-54 triglycerides were dominant. This occurence of C-odd triglycerides as the dominant components in the deficient group seemed however to be an unusual pattern even though for abnormal fish. This report concerns an examination of the tissue lipids of young carp given a diet deficient in vitamin E to discover if similar changes occur in carp older than fingerlings. This could then be interpreted as a general symptom of α-tocopherol deficiency.

Materials and Methods

The composition of the basal diet, preparation of diet, fish care and feeding were all the same as described previously[2,3]. Carp weighing about 30 g were divided into two lots of 30 fish each and were kept on either the control diet enriched with 70 mg of DL-α-tocopheryl acetate(α-tocopherol) per 100 g diet or an α-tocopherol deficient diet for 90

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days. To eliminate all possible vitamin E from the dietary test oils methyl esters of fatty acids were prepared from soybean oil and pollock liver oil (3: 2) after removal of unsaponifiable material and were used for both the control and the experimental groups.

At the end of the 90-day feeding trial, 30 fish were taken from each lot for determination of moisture, protein and lipids. Lipids were extracted from the fish by the method of FOLCH *et al.*[4]

The triglycerides and the cholesteryl esters were isolated from each of the lipid lots by the same method described in the previous papers[5] and were analysed directly by gas-liquid chromatography (GLC) after preliminary separation by thin layer chromatography (TLC)[6]. Fatty acids recovered from the triglycerides after TLC were esterified with BF_3–MeOH reagent. Removal of nonsaponifiables, preparation of methyl esters, separation of polar and nonpolar lipids by gel column chromatography, and all GLC operations were carried out by the procedures described previously[5].

Results and Discussion

The results of the feeding experiment, and the analytical data on the whole bodies, muscle and hepatopancreas of fish at the end of feeding trial, are summarized in Table 1. There was no mortality of the experimental fish throughout the feeding trial period of 90 days, a result quite different from the case of carp fingerlings on the α-tocopherol deficient diet which showed lordosis accompanied by a high mortality. The growth rate of the deficient group was considerably lower as observed previously.

Table 1. Results of feeding and proximate compositions of the experimental fish (%)

No. of Fish	Av body wt (g)		Percent gain	Whole body			Muscle			Hepato-pancreas
	Initial	Final		Moisture	Protein	Lipid	Moisture	Protein	Lipid	Lipid
Control 30	29.8	98.1	230	78.5	18.1	2.5	79.4	18.3	1.0	4.6
Experimental 30	30.4	61.8	105	81.5	14.2	2.8	86.1	12.4	1.3	4.2

As observed in our previous studies[2,7], a high water content reflected a low protein content in every part of the deficient fish. This was one of the characteristics of carp dystrophic muscle arising from an α-tocopherol deficiency[2], or oxidized lipids[8,9], but there were no differences in the lipid contents of whole bodies, muscle and hepatopancreas for the two groups.

The percentages of certain fatty acids of the triglyceride and polar lipid fractions are listed in Table 2. The effect of the α-tocopherol deficiency on these fatty acid compositions is not as significant as that observed in the previous experiment with fingerlings of carp, although basically similar results were again obtained in this experiment. In fish fed on the tocopherol deficient diet, the percentages of linoleic acid were low in both

triglycerides and polar lipids in every tissue, but the percentages of oleic acid were higher only in whole bodies and muscle. The deficient fish also showed a high percentage of the abnormal polyunsaturated 20: 3ω9, usually associated with essential fatty acid deficiencies in mammals. There were lower percentages of 20: 5ω3 in the polar lipids, but no differences in the concentrations of 22: 6ω3. The decrease of linoleic acid is therefore also one of the characteristics of α-tocopherol deficiency in carp, together with the higher water content of the tissues. The same phenomenon is also recognized in rats[10] and human plasma[11] deficient in α-tocopherol.

Table 2. Effects of α-tocopherol deficiency on fatty acid compositions of polar lipid and triglyceride fractions in young carp (area %)

Fatty acid	Polar lipid						Triglyceride					
	Whole body		Hepato-pancreas		Muscle		Whole body		Hepato-pancreas		Muscle	
	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp
14: 0	4.1	3.2	3.1	1.7	2.4	2.3	6.6	8.7	4.4	6.7	4.5	7.1
16: 0	22.7	22.6	21.5	20.2	20.5	20.1	17.8	18.6	17.9	18.5	18.5	17.3
16: 1ω7*	16.6	11.8	12.5	10.3	12.4	11.4	19.7	16.8	24.0	24.8	19.3	16.5
18: 0	4.8	7.4	4.7	7.3	6.4	9.3	3.5	1.9	3.2	1.3	3.1	2.1
18: 1ω9*	24.8	30.7	24.5	24.3	25.0	28.8	36.5	43.5	41.4	39.3	41.0	43.5
18: 2ω6	5.2	2.1	2.3	1.6	5.3	1.5	3.6	0.8	3.1	0.6	2.8	1.6
18: 3ω3	1.3	0.8	0.4	0.5	1.1	0.7	3.3	3.1	1.4	1.4	2.1	2.9
20: 1ω9*	0.8	2.0	1.2	2.7	3.0	2.0	3.1	2.5	2.1	2.1	2.6	2.8
20: 2ω9	tr	1.1	tr	0.6	0.5	0.7	0.3	0.7	0.3	0.4	0.6	0.5
20: 3ω9	1.7	5.8	8.4	9.6	3.8	7.5	0.3	0.7	0.3	0.4	0.5	0.6
20: 3ω6	0.5	0.8	1.2	1.5	1.3	0.6	—	—	—	—	—	—
20: 3ω3 } 20: 4ω6 }	2.0	1.6	2.9	2.7	2.5	2.6	0.2	—	tr	tr	0.1	0.2
20: 4ω3 } 22: 1 }	—	—	0.4	0.3	0.7	0.1	0.5	—	0.2	0.3	2.0	1.6
20: 5ω3	3.9	0.4	2.4	0.6	5.5	0.8	tr	tr	tr	tr	tr	tr
22: 5ω6	—	—	tr	1.3	0.4	0.9	0.3	tr	tr	tr	tr	tr
22: 5ω3	—	—	1.0	1.1	1.5	0.9	tr	tr	tr	tr	tr	tr
22: 6ω3	6.9	5.7	11.9	12.0	6.8	6.2	0.3	tr	tr	tr	tr	tr

* Small amounts of the other monoenes were inculded in the figures for each chain length.

The fatty acid chain-length compositions of triglycerides and cholesteryl esters are shown in Table 3. Carp lipids extracted from whole bodies, hepatopancreas and muscle contained triglycerides ranging from C-38 to C-58, with the principal components being C-50 to C-54 triglycerides as observed previously[1]. In the earlier study conducted with carp fingerlings, the lipid from the deficient fish contained C-odd triglycerides, C-43 and C-45, as principal components, whereas in this experiment with young carp they were barely detectable. In general, there were no marked differences in triglyceride compositions, although the percentages of C-54 and C-56 triglycerides were lower in the muscle lipid of fish fed the α-tocopherol-depleted diet.

Table 3. Effects of α-tocopherol deficiency on the distribution of triglycerides and cholesteryl esters in young carp (area %)

Carbon number*	Triglyceride						Carbon number	Cholesteryl ester			
	Whole body		Hepato-pancreas		Muscle			Whole body		Hepato-pancreas	
	Cont	Exp	Cont	Exp	Cont	Exp		Cont	Exp	Cont	Exp
C-38	1.3	1.6	0.5	1.5	0.7	0.1	C-10	2.6	1.8	—	—
C-40	1.5	1.7	1.5	1.5	1.0	1.4	C-12	3.5	4.1	2.4	4.1
C-42	3.4	3.5	3.0	2.8	2.4	3.0	C-13	3.1	2.9	—	—
C-44	6.0	7.4	4.5	5.1	4.5	6.2	C-14	4.6	5.9	6.5	5.3
C-46	7.9	8.7	7.2	6.6	6.5	7.7	C-16	23.2	26.4	17.0	21.7
C-48	13.4	14.2	12.7	11.1	12.1	13.3	C-18	44.1	45.3	66.9	56.7
C-50	17.1	17.6	19.9	18.7	16.2	17.9	C-20	14.5	12.4	7.2	12.2
C-52	20.0	18.9	27.8	25.4	21.7	21.8	C-22	4.5	0.9	—	—
C-54	19.7	17.9	19.6	20.6	22.7	18.8					
C-56	8.3	8.4	3.4	6.4	12.2	9.1					
C-58	1.0	—	—	—	—	—					

* Trace amounts of C-odd numbered triglycerides were detected but were not calculated.

The fatty acid distribution of cholesteryl esters was almost the same as that reported previously[6]. Although the cholesteryl C-18 esters dominated in the carp, the lipids of the hepatopancreas from the deficient fish showed a lower proportion of the C-18 esters, mostly replaced by higher percentages of the C-16 and the C-20 esters. There was however no significant difference in the composition of cholesteryl esters of the whole bodies due to the tocopherol deficiency.

Thus, the results of an α-tocopherol deficiency in young carp are essentially the same as those in carp fingerlings, with the exception of the non-appearance of C-odd triglycerides. Feeding the α-tocopherol deficient diet resulted in reduced growth and in muscle tissue a high water content reflecting a lower protein content. In the lipids of all tissues of the tocopherol deficient group the proportions of linoleic acid decreased but that of oleic acid increased only in the muscle lipids. However, the influence of the tocopherol deficiency on the triglyceride composition was very different between fingerlings and young carp. In the case of fingerlings weighing about 5 g, C-odd numbered triglycerides dominated in fish kept on the tocopherol deficient diet, whereas in young carp weighing about 30 g they were negligible. However in both sizes of fish the body(muscle) C-54 triglyceride decreased in the absence of tocopherol. The influence of the tocopherol deficiency on triglyceride distribution may differ according to the size of fish, and/or it is possible to think that there was sufficient α-tocopherol stored in the young carp to offset some of the effects of the deficiency induced by the tocopherol deficient diet, since in this experiment the tocopherol level in the experimental carp was not determined before and after the 90 days. However, the appearance of C-odd numbered triglycerides unlikely to be attributable to the tocopherol deficiency, and might involve other factors. This is

also supported by the results obtained in another experiment conducted using adult carp (Part-VI) in which carp weighing about 100 g were kept on an α-tocopherol deficient diet for 17 months. C-odd numbered triglycerides were again virtually absent in the experimental group. Further experiments would be necessary to clarify this point.

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