

魚類の脂肪酸組成に及ぼすストレスの影響

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On the Fatty Acid Composition of Fish Affected by Excessive Stress

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The flesh lipids of gilled and impounded sardine, *Sardinops melanosticta*, were compared. In gilled fish, the ratios of neutral lipids to phospholipids increased with a decrease of the latter. The acid values of the neutral lipids were significantly high in dead gilled fish. There was little difference between gilled and impounded fish in the fatty acid compositions (%) of either triglycerides or neutral lipids. The phospholipid fatty acids of gilled fish showed a remarkable decrease of 20:5 and 22:6 acids and an increase of 16:0 acid.

The obvious decrease of polyenoic acids in the phospholipids of the gilled fish might be due mainly to a metabolic peculiarity induced under excessive stress.

The variations in the fatty acid composition of fish based on the external causes would be attributed to environmental changes¹⁾, starvation²⁻⁴⁾ or excessive exercise⁵⁾. In the case of starvation or excessive exercise in fish, the diminution of neutral lipids as energy sources was observed⁶⁻⁹⁾. Further, the phospholipids in the tissues were also observed to be depleted.

The utilization of the energy released by oxidation of fatty acids in fish is most evident.¹⁰⁾ However, the selectivity in utilizing fatty acids of lipids by fish is not yet well understood.

This report is concerned with the comparison of fatty acid compositions of neutral lipids, triglycerides and phospholipids in the flesh of sardine, *Sardinops melanosticta*, subjected to various degrees of stress, *i. e.*, gilled and impounded fish.

Being caught in the mesh of the net, the fish is subjected to excessive exercise resulting to the weakening of the animal. This excessive exercise may possibly affect the fatty acid metabolism in the fish.

Materials and Methods

Materials A gilled live fish (GL) and a gilled dead fish (GD) used in this study were those which were caught in the mesh of the bag, while an impounded fish (IF) was swimming in the same bag, of a set net located near Hakodate, Japan. The impounding time spent either in a gilled state or swimming state could not be known as the samples were taken at the same time early morning. Although, it was assumed that GD had been subjected to a longer period of stress than GL.

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Ten samples were randomly taken for each of the GL, GD and IF, and their body length and weight (mean value \pm standard deviation) were 18.1 ± 0.7 cm and 76 ± 10 g, 18.4 ± 0.4 cm and 87 ± 5 g, and 18.2 ± 0.6 cm and 76 ± 9 g, respectively.

Analytical methods Whole lipids in the flesh of one fish of each sample were extracted by the method of BLIGH and DYER¹¹). Neutral lipids were separated from phospholipids by chromatography on silicic acid (Mallinckrodt Co.)-Celite (2:1) columns. Neutral lipids were eluted with chloroform by passing through a column and phospholipids were done with methanol. Acid values and unsaponifiable material contents of neutral lipids were determined in an usual way. Triglycerides in neutral lipids were separated by chromatography on silicic acid columns, using 10% diethyl ether-hexane after 4% diethyl ether-hexane as solvent for development.

The fatty acid compositions of neutral lipids, triglycerides and phospholipids were determined with a Yanagimoto gas chromatograph (model G8) equipped with a dual hydrogen flame detector. The column used was 1.5 m \times 3 mm i. d., U shaped stainless steel and was packed with 10% diethyleneglycol-succinate on Chromosorb W. Column temperature was 190°C and inlet pressure of nitrogen 0.7 kg/cm². The fatty acid methyl esters prepared with boron trifluoride-methanol¹²) were identified by comparison with commercial standard reagents (14:0, 16:0, 18:0, 16:1, 18:1, 18:2 and 18:3 acids). Identification of other fatty acids was accomplished by the log-plot of the retention time against the number of carbons in the chain and the comparison of ECL (equivalent chain length) values with those in literatures. The percentage of each fatty acid was derived from the area of the peaks. Statistical significance was based on Student's *t* test and probability ($P < 0.05$) on the fatty acids ($> 1.0\%$) of either member of the IF-GD pair.

Results and Discussion

Characteristics of lipids Both gilled and impounded fish examined were nearly of the same size, however, comparing the whole lipid contents ($4.8 \pm 1.3\%$ in GD, $5.0 \pm 1.5\%$ in GL and $4.6 \pm 0.7\%$ in IF), there was a little difference between gilled and impounded fish. The ratios of neutral lipids to phospholipids gave remarkable differences in gilled and impounded fish, *i. e.*, 5.5 ± 1.9 in GD, 4.6 ± 1.2 in GL and 3.3 ± 0.8 in IF, and were significance ($P < 0.001$) between IF and GD. These results showed that phospholipids decreased noteworthy in gilled fish. The decreasing tendency of phospholipids in whole lipids was similar to the results reported on the lipids of spring-spawning herring, *Clupea harengus* L., maintained in captivity without food for a period of four months⁷), and on those of serum⁸) and liver⁹) for post-spawning pink salmon, *Onchorhynchus gorbuscha*. The same was also true for the flesh of king salmon, *O. tshawitscha*⁶). These results imply that phospholipids depleted unusually in addition to the diminution of neutral lipids as energy sources in the case of excessive exercise or starvation.

The contents of unsaponifiable materials in the neutral lipids gave a little difference between $6.9 \pm 2.0\%$ in GD and $7.7 \pm 3.2\%$ in IF. Whereas, acid values of the neutral lipids showed a significant high level of 10.3 ± 7.3 in GD comparing with 3.7 ± 1.1 in IF. Triglyceride contents in the neutral lipids were $82.3 \pm 9.0\%$ in GD and $91.7 \pm 3.3\%$ in IF, but their contents in whole lipids were closely the same; $68.3 \pm 11.6\%$ in GD and $70.0 \pm 4.6\%$ in IF. This would correspond to a relative decrease in triglycerides against an increase of free fatty acids in the neutral lipids.

Fatty acid composition The fatty acid compositions of neutral lipids, triglycerides and phospholipids in the flesh of sardine examined were compared using only fatty acids of more than 1.0% of either member of impounded and gilled fish pair, and also using saturated, monoenoic and polyenoic acids, to find the significance between gilled and impounded fish. A little difference in triglycerides showed a decrease of saturated acids and an increase of monoenoic acids in GD, whereas the difference in each fatty acid content listed was not significant between IF and GD (Table 1). In neutral lipids of GD, 16:0 and saturated acids slightly increased, and 20:5, 22:6 and polyenoic acids slightly decreased (Table 2). However, the difference between IF and GD, was not significant in fatty acids except for 17:0 and 18:3 acids. On the other hand in phospholipids, the contents of 16:0,

Table 1. Main fatty acid composition of flesh triglycerides of impounded and gilled dead sardine (% wt.)

Fatty acid ^a	Impounded fish	Gilled dead fish	Statistical significance ^c
14:0	12.9 ± 5.3^b	11.6 ± 2.7^b	NS
16:0	22.1 ± 3.4	21.2 ± 2.2	NS
17:0	1.8 ± 0.3	1.7 ± 0.3	NS
18:0	3.9 ± 0.7	4.1 ± 0.5	NS
19:0	1.1 ± 0.2	1.3 ± 0.2	NS
16:1	10.8 ± 1.8	10.8 ± 2.0	NS
17:1	1.2 ± 0.1	1.2 ± 0.2	NS
18:1	11.5 ± 1.2	12.2 ± 1.7	NS
20:1	2.8 ± 1.0	3.6 ± 2.5	NS
22:1	2.7 ± 1.2	3.1 ± 2.2	NS
18:2	1.4 ± 0.3	1.4 ± 0.3	NS
18:4	1.9 ± 0.3	1.8 ± 0.7	NS
20:4	1.8 ± 0.5	1.7 ± 0.7	NS
20:5	10.4 ± 3.1	11.1 ± 2.2	NS
22:5	0.9 ± 0.7	1.2 ± 0.4	NS
22:6	6.7 ± 2.7	6.3 ± 2.8	NS
Saturates	44.1 ± 8.4	41.8 ± 4.7	NS
Monoenes	31.1 ± 3.0	32.8 ± 3.2	NS
Polyenes	24.9 ± 6.9	25.4 ± 3.9	NS

^a Minor components (12:0, 13:0, 15:0, 12:1, 13:1, 14:1, 15:1, 19:1, 21:1, 18:3 and 21:5 acids) were also detected to be less than 1.0% in both impounded and gilled dead fish.

^b Values given were mean \pm standard deviation of 10 fish.

^c Statistical significance showed probability ($P < 0.05$) by Student's *t* test (impounded fish vs gilled dead fish). NS: not significant.

Table 2. Main fatty acid composition of flesh neutral lipids of impounded and gilled dead sardine (% wt.)

Fatty acid ^a	Impounded fish	Gilled dead fish	Statistical significance
14:0	11.5±3.0	11.6±2.8	NS
16:0	21.5±1.4	23.1±4.3	NS
17:0	1.7±0.2	1.1±0.3	P<0.001
18:0	4.1±0.6	4.1±0.6	NS
19:0	1.0±0.3	1.1±0.3	NS
16:1	11.1±2.1	11.0±2.5	NS
17:1	1.3±0.2	1.2±0.2	NS
18:1	11.8±0.5	11.4±1.8	NS
20:1	3.0±0.8	3.5±2.9	NS
22:1	2.7±0.5	2.5±3.1	NS
18:2	1.3±0.3	1.5±0.2	NS
18:3	0.8±0.4	1.2±0.3	P<0.05
18:4	1.9±0.4	1.7±0.6	NS
20:4	2.0±0.2	2.2±0.6	NS
20:5	11.0±1.9	10.3±2.3	NS
22:5	1.3±0.5	1.2±0.5	NS
22:6	7.0±3.4	5.9±2.6	NS
Saturates	41.7±3.4	43.1±7.2	NS
Monoenes	32.0±2.3	31.4±4.3	NS
Polyenes	26.4±5.0	25.4±5.5	NS

^a Minor components (12:0, 13:0, 15:0, 21:0, 13:1, 14:1, 15:1, 19:1, 21:1, 24:1, 21:5 and 22:2 acids) were also detected to be less than 1.0% in both impounded and gilled dead fish. Other abbreviations were same as Table 1.

20:5 and 22:6 acids varied widely in gilled and impounded fish (Table 3). In gilled fish, it showed a decreasing tendency of 20:5 and 22:6 acids, and in contrast, an increase of 16:0 acid. The values of 16:0, 20:5 and 22:6 acids in GL were between those of IF and GD. Saturated, monoenoic and polyenoic acids showed each mean value as 54.9, 18.9, 26.3% in IF, 69.1, 18.4, 12.4% in GL, and 83.2, 13.5, 3.4% in GD, respectively. This result therefore indicates an increasing tendency in saturated acids and a decrease in polyenoic acids. Except for 14:0, 17:1, 20:1 and 18:2 acids, the differences in all other fatty acids tabulated showed a significance of P<0.05, P<0.01 or P<0.001 (Table 3). It is implied that fatty acid metabolism in flesh phospholipids is obviously different between gilled and impounded fish.

SADDLER *et al.*¹⁾ reported that chum salmon, *O. keta*, artificially introduced into sea water from freshwater incurred substantial diminution in 20:5 and 22:6 of polyenoic acids with an increase of 16:0, 18:0 and 18:1 acids in the fatty acid composition. SADDLER *et al.* indicated that this response might be due to the stress of capture, handling, anesthesia, partial starvation and confinement to which the artificially reared fish were exposed. KRUEGER *et al.*⁵⁾ reported that juvenile coho salmon, *O. kisutch*, when forced to swim at a water velocity (59 cm/sec) greater than that which could be sustained, were found to be depleted of 22:6, 18:2 and 18:4 acids, while at a velocity of 52 cm/sec, 18:1,

Table 3. Main fatty acid composition of flesh phospholipids of impounded, gilled live and gilled dead sardine (% wt.)

Fatty acid ^a	Impounded fish	Gilled live fish	Gilled dead fish	Statistical significance ^c
14:0	7.2±2.6 ^b	3.5±0.5 ^b	5.5±1.4 ^b	NS
15:0	1.0±0.4	0.9±0.2	2.0±0.3	P<0.001
16:0	37.3±3.2	59.2±4.0	62.1±2.8	P<0.001
17:0	1.9±0.2	2.2±0.2	3.1±0.2	P<0.001
18:0	5.1±0.3	8.6±1.1	7.3±1.3	P<0.001
19:0	1.4±0.6	0.5±0.3	2.1±0.3	P<0.01
16:1	7.7±1.3	5.7±0.8	4.2±0.6	P<0.001
17:1	1.0±0.1	0.5±0.1	1.1±0.2	NS
18:1	7.5±0.8	9.9±1.0	5.7±0.9	P<0.001
20:1	0.6±0.3	1.1±0.7	0.4±0.3	NS
18:2	1.4±0.2	1.2±0.2	1.5±0.3	NS
18:4	0.8±0.2	0.6±0.3	1.1±0.3	P<0.05
20:4	1.8±0.4	0.8±0.3	None	P<0.001
20:5	8.0±1.3	2.4±0.9	0.5±0.4	P<0.001
22:6	12.6±4.4	7.2±3.9	None	P<0.001
Saturates	54.9±3.3	69.1±4.4	83.2±1.4	P<0.001
Monoenes	18.9±2.1	18.4±1.4	13.5±1.4	P<0.001
Polyenes	26.3±4.9	12.4±5.2	3.4±1.1	P<0.001

^a Minor components (12:0, 13:0, 21:0, 12:1, 13:1, 14:1, 15:1, 19:1, 21:1, 22:1, 18:3, 20:2, 21:5 and 22:5 acids) were also detected to be less than 1.0% in impounded, gilled live and gilled dead fish.

^b Values given were mean ± standard deviation of 10 fish.

^c Statistical significance showed probability (P<0.05) by Student's *t* test (impounded fish vs gilled dead fish). NS: not significant.

16:0 and 16:1 acids were the major fatty acids utilized. Obviously, the excessively high velocities of water acted in this instance as a stress which disrupted fatty acid metabolism. It was also reported that long term starvation in fish usually resulted in a marked depletion of polyenoic acids, whereas short term starvation was characterized by their conservation²⁻⁴). In mammals, NEPTUNE *et al.*¹³) reported that after 48-hour fast, the phospholipid fatty acids of rat diaphragm were reduced much more than the triglyceride fatty acids.

In view of these facts on literatures, and our data on a decrease of 20:5 and 22:6 acids in flesh phospholipids of gilled fish under excessive stress, it is suggested that polyenoic acids might have a peculiar metabolism in fish. It is well known that large amounts of polyenoic acids such as 20:5 and 22:6 acids in phospholipids are in the components of cell membranes or those of mitochondria in the flesh of fish. In addition, it is probable that these acids are playing a possible role in energy sources by an unusual metabolic pathway in fish under excessive stress.

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