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Pre-harvest Modulation of N-3 Long-chain Polyunsaturated Fatty Acids in Rainbow Trout Meat for Human Consumption

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Abstract

Since post-harvest improvement of food quality depends on the pre-harvest stage, controlling food quality at the pre-harvest stage is being recognized as an important aspect of food processing technology. n-3 Long-chain polyunsaturated fatty acids (n-3 LCPUFAs) are considered important factors in the suppression of cardiovascular disease and development of the infant brain and visual functions. Fish meat generally contains a larger amount of n-3 LCPUFAs than meat from terrestrial animals, but the amount of n-3 LCPUFAs in fish meat depends on their dietary fatty acid intake. In order to supply n-3 LCPUFAs to consumers, we developed a finishing-up method, through a 4-day lysine-deficient diet feeding for rainbow trout (*Oncorhynchus mykiss*) meat. This 4-day lysine deficiency did not affect fish body weight, while significantly increased the total lipid content in the muscle tissue. In addition, docosahexaenoic acid and total n-3 fatty acid contents increased significantly in the meat throughout the 4-day finishing treatment. We concluded that the finishing-up method using short-term lysine-deficient diet feeding enables us to enrich fish meat with n-3 fatty acids for human consumption.

Keywords: n-3 Long-chain polyunsaturated fatty acids; Docosahexaenoic acid; Fish meat; Lipid content; Fatty acid content

Abbreviations: PUFA: Polyunsaturated Fatty Acid; n-3 LCPUFAs: n-3 Long-Chain Polyunsaturated Fatty Acids; Lys: Lysine; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; DPA: Docosapentaenoic Acid; GC-FID: Gas Chromatography-Flame Ionization Detector; TAG: Triacylglycerol; TLC: Thin-Layer Chromatography; FAMEs: Fatty Acid Methyl Esters; HSI: Hepatosomatic Index; PL: Polar Lipid; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PS: Phosphatidylserine; LPC: Lysophosphatidylcholine; NL: Nonpolar Lipid; SE: Sterol Ester; ST: Sterol; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid

Introduction

Polyunsaturated fatty acids (PUFAs) are recognized as important nutrients for human health. In particular, n-3 long-chain PUFAs (n-3 LCPUFAs), such as docosahexaenoic acid (DHA, 22:6n-3), docosapentaenoic acid (DPA, 22:5n-3), and eicosapentaenoic acid (EPA, 20:5n-3), are gaining attention. DHA is essential for the development of the infant brain and visual functions [1], and supplementation with DHA during pregnancy has been associated with reduced rates of both preterm and underweight births [2]. Previous studies showed that EPA and DHA supplementation improves cognitive function [3,4]. It has also been demonstrated that n-3 LCPUFAs have protective effects on the cardiovascular system: reducing blood pressure, blood viscosity, and the risk of cardiac arrhythmias [5-7].

The intake of fish meat and fish oil is associated with lower rates of cardiovascular disease [8] and hypertriglyceridemia [9], and fish oil consumption in pregnant woman is associated with a reduced risk of allergic disease in their offspring [10,11]. These beneficial effects of fish meat and fish oil are thought to be due to the high n-3 LCPUFA content of these foods. Fish meat contains higher concentrations of n-3 fatty acids than pork or beef [12-14], while n-3 fatty acid content in fish meat is strongly affected by the dietary fatty acid intake of the fish [15].

Since rainbow trout and other teleost fish species cannot desaturate fatty acids at the omega-3 position, the body n-3 fatty acid content in these fish depends on their dietary fat intake. Currently, fish meat enriched with n-3 LCPUFAs requires mass consumption of fish meal and fish oil, increasing the total amount of fish harvested for eventual human consumption. It is, therefore, important to improve the efficiency of aquaculture techniques, and methods for accelerating n-3 LCPUFA synthesis from shorter n-3 fatty acids in fish and/or accumulating n-3 LCPUFAs preferentially in edible tissues.

Lysine (Lys) is an important amino acid for fish and a precursor of carnitine, which is used to transport fatty acids into mitochondria for oxidation. Several amino acids, including Lys, affect hormone secretion [16-18] and intracellular signaling pathways in fish [19]. Thus, dietary Lys levels probably affect energy metabolism including lipid accumulation in fish.

In the present study, we investigated the effects of short-term administration of Lys-deficient (Lys (−)) diets on rainbow trout (*Oncorhynchus mykiss*), focusing particularly on lipid and fatty acid content in the edible part of the fish, i.e., the skeletal muscle.

Materials and Methods

Diet

Control and Lys (−) diets were designed as shown in Table 1. Fish meal and corn gluten meal were used as the main protein source in control and Lys (−) diets, respectively. These diets were freeze-dried and stored at 4°C until use. The amino acid composition of each diet was analyzed using an amino acid analyzer L-8900 (Hitachi, Tokyo,

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Japan), and the fill rates of nutritionally important amino acids were calculated based on the results and the amino acid requirements of rainbow trout [20] (Table 2). The fatty acid composition of each diet

Ingredients (%)	Control	Lys (−)
Anchovy meal	45	5
Defatted soybean meal	-	10
Blood meal	-	4
Corn gluten meal	5	33
Wheat flour	17	17
Pre-gelatinized starch	5	5
Fish oil	5	7.4
Soy bean oil	10	10
Vitamin mixture[a]	3	3
Choline chloride	0.5	0.5
Vitamin E	0.1	0.1
Mineral mixture[b]	1	1
Mono calcium phosphate	1	1
Cellulose	7.4	3
Total	100	100
Moisture (%)	7.07	7.14
Crude protein (%)	32.2	33.0
Crude lipid (%)	22.0	22.7

Control: Control diet; Lys (−): Lysine-deficient diet
[a]Vitamin mixture composition (amounts in kg^{-1}): vitamin A acetate, 2,420,000 IU; vitamin D3, 2,420,000 IU; vitamin K3, 6.05 g; thiamin hydrochloride, 3.025 g; riboflavin, 3.63 g; pyridoxine hydrochloride, 2.42 g; cyanocobalamin, 0.006 g; ascorbic acid, 368.902 g: niacin, 24.2 g; calcium pantothenate, 6.05 g; inositol, 121 g; biotin, 0.363 g; folic acid, 0.908 g; *p*-aminobenzoic acid, 3.025 g.
[b]Mineral mixture composition (amounts in kg^{-1}): sodium chloride, 50 g; magnesium sulfate, 745 g; iron (III) citrate n-hydrate, 125 g; trace element mixture, 50 g; cellulose, 30 g. (The trace element mixture contained (amounts in kg^{-1}): zinc sulfate heptahydrate, 353 g; manganese sulfate, 162 g, copper (II) sulfate pentahydrate, 31 g; aluminum chloride hexahydrate, 10 g; cobalt chloride, 3 g; potassium iodate, 1 g; cellulose, 440 g).

Table 1: Diet formulae in Lys-deficient feeding.

Nutritionally important amino acid (%)	Control	Lys (−)
Threonine	1.3	1.3
Valine	1.7	1.6
Cysteine	0.2	0.2
Methionine	0.9	0.7
Isoleucine	1.4	1.3
Leucine	2.7	4.0
Tyrosine	0.9	1.2
Phenylalanine	1.4	1.7
Lysine	2.3	1.3
Histidine	0.9	0.7
Arginine	1.8	1.4
Fill-rate of amino acids (%)	**Control**	**Lys (−)**
Threonine	92.0	98.7
Valine	137.6	134.0
Cysteine	121.2	140.6
Methionine	136.3	100.2
Isoleucine	151.7	143.6
Leucine	157.6	238.8
Tyrosine	114.1	145.7
Phenylalanine	112.9	146.1
Lysine	112.6	61.7
Histidine	144.6	119.2
Arginine	133.3	103.1

Amino acid contents (% in diet) of control and lysine-deficient (Lys (−)) diets were obtained by amino acid analyzer, and fill-rates of amino acids were calculated by the amino acid contents and amino acid requirement in rainbow trout.

Table 2: Nutritionally important amino acid compositions of diets and adequacy for requirement of amino acids in rainbow trout.

Fatty acids (%)	Control	Lys (−)
14:0	2.71	2.08
15:0	0.12	0.16
16:0	14.23	12.96
16:1n-9	0.05	0.11
16:1n-7	2.89	2.57
17:0	0.39	0.21
18:0	3.37	3.14
18:1n-9	18.49	19.58
18:1n-7	1.78	1.63
18:2n-6	34.85	36.35
18:3n-3	3.65	3.53
18:3n-9	0.87	0.90
20:0	0.25	0.23
20:1n-9	1.94	2.88
20:1n-7	1.27	1.41
20:2n-6	0.09	0.08
20:3n-6	N.D.	N.D.
20:4n-6	0.33	0.17
20:4n-3	0.24	0.27
20:5n-3	4.37	3.47
22:0	0.18	0.19
22:1n-11	3.02	4.14
22:1n-9	0.42	0.51
22:4n-3	0.06	0.04
22:5n-6	0.11	0.05
22:5n-3	0.59	0.45
22:6n-3	3.74	2.90
24:1n-9	N.D.	N.D.
∑SFA	21.24	18.97
∑MUFA	29.86	32.83
∑PUFA	48.90	48.20
∑n-3	12.65	10.66
∑n-6	35.38	36.65

The fatty acid composition of each diet was measured with a gas chromatography-flame ionization detector. Control, control diet; Lys (−), lysine-deficient diet; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-3, n-3 fatty acid; n-6, n-6 fatty acid; N.D., Not Detected.

Table 3: Fatty acid compositions in control and Lys-deficient diets.

was measured with a gas chromatography-flame ionization detector (GC-FID, GC-2014, Shimadzu, Kyoto, Japan), under conditions described later. We confirmed that combinations of soybean oil and fish oil provided similar fatty acid compositions in both diets (Table 3).

Fish and experimental conditions

Rainbow trout (n=15) were maintained under a 12:12 h light-dark photoperiod at approximately 10°C, in a tank equipped with a recirculating system, and fed the control diet for approximately one month. In the experimental stage, fish were fed the Lys (−) diet up to four days (experimental feeding). The experimental period of four days was decided because a slight loss in body weight was observed after six days in the preliminary trials. Fish were fed until satiation during both periods. Five individuals each were collected without treatment on Day 0, Day 2, and Day 4 of the experimental feeding period.

All animal care and use guidelines were followed according to the institutional protocol and approved by the University of Tokyo (P17-093, approved on Aug 16, 2017).

Total lipid and triacylglycerol contents

Total lipid fractions were extracted from the liver and muscle

of rainbow trout according to the method of Bligh and Dyer [21]. The total lipid content of each extracted solution was determined gravimetrically. Triacylglycerol (TAG) content was measured using a Triglyceride E-test Wako kit (Wako Pure Chemical Industries, Osaka, Japan).

Lipid class composition

Lipid class compositions in the muscle and liver tissues were determined through thin-layer chromatography (TLC). The extracted total lipids were spotted on glass TLC plates coated with silica gel 60 (Merck, Tokyo, Japan). A developing solvent mixture of chloroform/methanol/water (65:30:5, v/v/v) was used to separate polar lipids, and then a mixture of hexane/diethyl ether/acetic acid (80:20:1.5, v/v/v) was used to separate nonpolar lipids [22]. The plate was sprayed with 40% sulfuric acid and heated at about 100°C. Images obtained by scanning plates were analyzed using ImageJ (Version 1.51 for Windows, National Institutes of Health, Bethesda, MD, USA).

Fatty acid content

Methanolic NaOH was added to tissue total lipid extracts and incubated for 10 min at 90°C for saponification. The saponification products were converted to methyl esters using boron trifluoride/methanol and the resulting fatty acid methyl esters (FAMEs) were extracted with isooctane. The obtained FAMEs were analyzed in a gas chromatograph system GC-2014 with FID (Shimadzu, Kyoto, Japan) equipped with a Supelcowax-10 capillary GC column (0.25 mm i.d. × 30 m, Supelco, Tokyo, Japan). Helium was used as a carrier gas at a column inlet pressure of 120 kPa. Column temperature was programmed to increase from 140°C to 180°C at a rate of 5°C/min, after initially holding at 140°C for 5 min. Column temperature was then raised from 180°C to 240°C at a rate of 1.2°C/min, before being kept at 240°C for 10 min. Methyl tricosanoate (23:0) was used as an internal standard, and methyl palmitate (16:0), methyl stearate (18:0), methyl oleate (18:1n-9), methyl arachidonate (20:4n-6), methyl eicosapentaenoate (20:5n-3), and methyl docosahexaenoate (22:6n-3) were used as standards. Fatty acids were identified based on the peaks of these standards and equivalent chain length values, together with our laboratory retention time databases confirmed by GC-mass spectrometry analyses.

Phosphoric acid content

To quantify phospholipids, phosphoric acid contents in total lipid extracts were measured according to the Fiske and Subbarow [23], Bartlett [24] methods. Potassium dihydrogen phosphate solution was used as a standard. A 5 M solution of sulfuric acid was added to the aliquoted total lipid extracts. The mixtures were incubated at 150°C for 3 h in a wet ashing procedure. After cooling, 30% hydrogen peroxide was added to the mixtures, followed by incubation at 150°C for 1.5 h. A 0.22% solution of aqueous ammonium molybdate was then added, and the mixtures were vortexed. A Fiske-Subbarow reagent was immediately poured into the mixtures, followed by incubation in boiling water for 7 min. Absorbances of the mixtures were measured at 830 nm.

Statistical analysis

In all data, outliers in each group were excluded by Smirnov-Grubbs test ($p<0.05$). Dunnett's test was used for multiple comparisons of all variables between the initial group (Day 0) and groups fed the Lys (−) diet (Day 2 and Day 4). Values with $p<0.05$ were considered statistically significant. Statistical analyses were performed using R software (Version 3.3.2).

Results

Condition factor, hepatosomatic index and total lipid content

No significant difference was noted in condition factor among the initial (Day 0) and Lys (−) groups (Day 2 and Day 4), while hepatosomatic index (HSI) on Day 4 was significantly higher than that on Day 0 (Table 4). Total lipid content in the muscle was significantly elevated on Day 4 (3.26% ± 0.41), about 2-fold the level on Day 0 (1.72% ± 0.27). The liver total lipid content on Day 4 (4.31% ± 0.25) was also significantly higher than that on Day 0 (3.33% ± 0.11).

Lipid class compositions in muscle and liver

No significant difference was noted in lipid class compositions in the muscle among the initial and Lys (−) groups (Table 5). Phospholipid content and proportions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and lysophosphatidylcholine (LPC) in the muscle did not change during the feeding trials. There was no significant difference in the proportions of sterol ester (SE), triacylglycerol (TAG),

Variables	Control	Lys (−)	
	Day 0	Day 2	Day 4
Standard length (cm)	25.60 ± 0.55	26.06 ± 0.58	24.40 ± 0.90
Body weight (g)	257.28 ± 14.95	290.06 ± 15.59	236.76 ± 28.31
Condition factor[a]	1.53 ± 0.08	1.65 ± 0.09	1.60 ± 0.04
HSI[b]	1.27 ± 0.14	1.60 ± 0.14	1.70 ± 0.09*
Total lipid content (% wet tissue)			
Muscle	1.72 ± 0.27	1.93 ± 0.38	3.26 ± 0.41*
Liver	3.33 ± 0.11	3.59 ± 0.12	4.31 ± 0.25*

Values are means ± SEM (n=5, *p<0.05)
Control: Control diet; Lys (−): Lysine-deficient diet
[a]Condition factor=[body weight × 100] / standard length
[b]HSI: Hepatosomatic index=liver weight / body weight × 100

Table 4: Standard length (cm), body weight (g), hepatosomatic index (HSI), condition factor, and total lipid content in tissue (% wet tissue) in Lys-deficient trial.

Variables	Control	Lys (−)	
	Day 0	Day 2	Day 4
Phospholipid content (mol/g wet tissue)			
Phospholipid	13.90 ± 0.41	17.08 ± 0.85	18.18 ± 2.11
PL class composition (%)			
PE	24.69 ± 1.18	22.77 ± 0.37	23.50 ± 0.06
PI	4.89 ± 0.56	4.74 ± 0.09	4.34 ± 0.19
PC	59.19 ± 0.92	59.23 ± 0.79	62.32 ± 1.41
PS	7.94 ± 1.15	9.20 ± 1.16	6.95 ± 1.57
LPC	3.28 ± 0.35	4.76 ± 0.95	3.19 ± 0.59
NL class composition (%)			
SE	12.15 ± 2.29	11.89 ± 1.39	10.75 ± 1.51
TAG	74.75 ± 2.97	71.89 ± 4.40	78.99 ± 3.61
ST	15.20 ± 2.32	16.22 ± 3.08	10.26 ± 2.19
TAG content (% wet tissue)			
TAG	1.13 ± 0.15	1.68 ± 0.17*	2.67 ± 0.14*

Lipid class composition of muscle was determined through thin-layer chromatography, and TAG contents were measured by using a Triglyceride E-test Wako kit (Wako Pure Chemical Industries, Osaka, Japan).
Phospholipid contents in muscle were measured according to Fiske/Subbarow and Bartlett methods [23,24].
Values are means ± SEM (n=5, *p<0.05).
Control: Control diet; Lys (−): Lysine-deficient diet; PL: Polar lipid; NL: Non-polar lipid; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PC: Phosphatidylcholine; PS: Phosphatidylserine; LPC: Lysophosphatidylcholine; SE: Sterol ester; TAG: Triacylglycerol; ST: Sterol

Table 5: Lipid class composition (%), phospholipid content (mol/g wet tissue), and triacylglycerol content (% wet tissue) in muscle.

and sterol (ST) among the initial and Lys (−) groups. On the other hand, TAG content in the muscle gradually increased through the experimental feeding period ($p<0.05$).

There was no significant difference in the liver phospholipid content among the three groups (Table 6). Significant differences were not observed in lipid class levels in the liver during the feeding trials. No significant difference was noted in the proportions of nonpolar lipids, SE, TAG, and ST levels among the initial and Lys (−) groups, while TAG content in the liver on Day 4 was significantly higher than that on Day 0.

Variables	Control	Lys (−)	
	Day 0	Day 2	Day 4
Phospholipid content (mol/g wet tissue)			
Phospholipid	50.75 ± 2.83	46.45 ± 3.56	55.11 ± 6.27
PL class composition (%)			
PE	22.84 ± 0.36	21.13 ± 0.71	25.26 ± 1.12
PI	5.12 ± 0.20	4.47 ± 0.35	4.99 ± 0.25
PC	59.10 ± 1.61	63.36 ± 1.02	59.70 ± 1.45
PS	6.41 ± 0.87	6.01 ± 0.50	7.22 ± 1.80
LPC	5.69 ± 0.25	5.04 ± 0.28	4.02 ± 0.24*
NL class composition (%)			
SE	36.81 ± 1.98	33.59 ± 1.98	36.47 ± 5.48
TAG	33.77 ± 3.07	39.71 ± 4.40	42.61 ± 5.25
ST	29.42 ± 3.00	26.70 ± 2.94	20.92 ± 2.33
TAG content (% wet tissue)			
TAG	2.01 ± 0.16	1.87 ± 0.16	3.23 ± 0.29*

Lipid class composition of liver was determined through thin-layer chromatography, and TAG contents were measured by using a Triglyceride E-test Wako kit (Wako Pure Chemical Industries, Osaka, Japan). Phospholipid contents in muscle were measured according to Fiske/Subbarow and Bartlett methods [23,24].
Values are means ± SEM (n=5, * $p<0.05$).
Control: Control diet; Lys (−): Lysine-deficient diet; PL: Polar lipid; NL: Non-polar lipid; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PC: Phosphatidylcholine; PS: Phosphatidylserine; LPC: Lysophosphatidylcholine; SE: Sterol ester; TAG: Triacylglycerol; ST: Sterol

Table 6: Lipid class composition (%), phospholipid content (mol/g wet tissue), and triacylglycerol content (% wet tissue) in liver.

Variables	Control	Lys (−)	
	Day 0	Day 2	Day 4
14:0	0.216 ± 0.048	0.282 ± 0.072	0.302 ± 0.037
15:0	0.022 ± 0.004	0.035 ± 0.003	0.052 ± 0.013*
16:0	2.089 ± 0.294	2.519 ± 0.374	2.835 ± 0.341
16:1n-9	0.015 ± 0.005	0.022 ± 0.007	0.040 ± 0.012
16:1n-7	0.308 ± 0.071	0.418 ± 0.116	0.498 ± 0.082
17:0	0.019 ± 0.003	0.029 ± 0.003	0.034 ± 0.007
18:0	0.566 ± 0.089	0.699 ± 0.114	0.754 ± 0.055
18:1n-9	2.068 ± 0.503	2.750 ± 0.706	3.015 ± 0.301
18:1n-7	0.183 ± 0.062	0.337 ± 0.072	0.363 ± 0.040
18:2n-6	1.801 ± 0.422	2.711 ± 0.721	2.452 ± 0.495
18:3n-3	0.158 ± 0.032	0.248 ± 0.064	0.243 ± 0.028
18:3n-9	0.041 ± 0.011	0.061 ± 0.020	0.065 ± 0.010
20:0	0.021 ± 0.005	0.029 ± 0.009	0.029 ± 0.003
20:1n-9	0.307 ± 0.093	0.416 ± 0.123	0.373 ± 0.070
20:1n-7	0.232 ± 0.059	0.325 ± 0.082	0.311 ± 0.028
20:2n-6	0.150 ± 0.036	0.179 ± 0.046	0.193 ± 0.020
20:3n-6	0.088 ± 0.022	0.103 ± 0.016	0.117 ± 0.010
20:4n-6	0.138 ± 0.001	0.178 ± 0.011	0.208 ± 0.034
20:4n-3	0.065 ± 0.010	0.089 ± 0.019	0.092 ± 0.014
20:5n-3	0.421 ± 0.032	0.520 ± 0.043	0.533 ± 0.051
22:0	0.009 ± 0.002	0.016 ± 0.003	0.031 ± 0.013
22:1n-11	0.345 ± 0.106	0.465 ± 0.132	0.427 ± 0.066
22:1n-9	0.053 ± 0.014	0.071 ± 0.018	0.066 ± 0.006
22:4n-3	0.009 ± 0.003	0.020 ± 0.002*	0.033 ± 0.003*
22:5n-6	0.053 ± 0.007	0.084 ± 0.012	0.102 ± 0.018*
22:5n-3	0.154 ± 0.017	0.228 ± 0.033	0.225 ± 0.020
22:6n-3	2.984 ± 0.111	3.515 ± 0.169	3.698 ± 0.276*
24:1n-9	0.012 ± 0.005	0.013 ± 0.004	0.019 ± 0.008
∑SFA	2.942 ± 0.442	3.615 ± 0.577	4.013 ± 0.430
∑MUFA	3.524 ± 0.861	4.818 ± 1.246	5.094 ± 0.541
∑PUFA	6.114 ± 0.596	7.933 ± 1.062	8.353 ± 0.683
∑n-3	3.827 ± 0.150	4.562 ± 0.268	4.824 ± 0.382*
∑n-6	2.246 ± 0.485	3.256 ± 0.774	3.264 ± 0.321

Fatty acid contents of muscle were measured with a gas chromatography-flame ionization detector.
Values are means ± SEM (n=5, * $p<0.05$).
Control: Control diet; Lys (−): Lysine-deficient diet; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; n-3: n-3 fatty acid; n-6: n-6 fatty acid.

Table 7: Fatty acid content (mg/g) in muscle of rainbow trout from Lys-deficient trial.

Muscle fatty acid content

Some fatty acids in muscle significantly increased in groups of Lys (−) diet-fed fish, compared to the initial group (Table 7). Contents of 22:6n-3 and 22:4n-3 on Day 4 were higher than those on Day 0 ($p<0.05$). Total amount of n-3 fatty acids on Day 4 was also significantly higher than that on Day 0 in the muscle.

Liver fatty acid content

Contents of some fatty acids in liver of rainbow trout changed during the course of the Lys (−) feeding period (Table 8). Total saturated fatty acid (SFA) and total monounsaturated fatty acid (MUFA) contents in the liver on Day 4 were significantly higher than those on Day 0, respectively. Level of 20:0 was significantly higher on Day 4 than on Day 0. Levels of 16:1n-7, 18:1n-9, 18:2n-6, and 18:3n-6 were higher on Day 4 than on Day 0. No significant change was noted in the liver fatty acid content between Day 2 and Day 0, except that 20:2n-6 level was higher on Day 2 than on Day 0.

Discussion

In this study, the effects of short-term Lys (−) diet feeding on lipid compositions in rainbow trout tissues were investigated to develop a method for enriching fish meat with n-3 LCPUFAs. Lys-deficient feeding for four days successfully increased total n-3 fatty acid and DHA contents in fish meat.

Under short-term Lys-deficiency, no significant failure in condition factor was observed. In long-term feeding, a diet based mainly on plant protein leads to inadequate intake of nutritionally important amino acids and loss of body weight [25,26]. The experimental period of this study was too short to cause such a body weight loss. Total lipid content in muscle on Day 4 was significantly higher than that on Day 0, suggesting that short-term Lys-deficient feeding had increased lipid content in rainbow trout muscle without body weight loss. We have also found that liver lipid content and HSI also increased throughout the experimental feeding period, and that the increase in lipid content probably contributed to the HSI increment.

Previous studies have shown that longer Lys-deficient feeding periods of 14 and 25 days increased lipid content in the livers of rats [27,28]. In Atlantic salmon, 3-month Lys-deficiency also caused an increase in the liver lipid content [29]. Katsumata et al. [30] have

Variables	Control	Lys (−)	
	Day 0	Day 2	Day 4
14:0	0.232 ± 0.012	0.237 ± 0.005	0.275 ± 0.018
15:0	0.032 ± 0.004	0.031 ± 0.002	0.024 ± 0.002
16:0	3.867 ± 0.353	3.142 ± 0.164	4.162 ± 0.434
16:1n-9	0.052 ± 0.004	0.075 ± 0.011	0.074 ± 0.006
16:1n-7	0.365 ± 0.038	0.263 ± 0.019	0.774 ± 0.101*
17:0	0.030 ± 0.001	0.030 ± 0.003	0.033 ± 0.003
18:0	1.569 ± 0.110	2.045 ± 0.239	2.057 ± 0.274
18:1n-9	2.586 ± 0.248	1.970 ± 0.134	4.416 ± 0.556*
18:1n-7	0.396 ± 0.051	0.532 ± 0.018	0.433 ± 0.025
18:2n-6	1.922 ± 0.150	2.138 ± 0.264	2.705 ± 0.181*
18:3n-6	0.049 ± 0.005	0.052 ± 0.004	0.080 ± 0.007*
18:3n-3	0.096 ± 0.016	0.106 ± 0.009	0.119 ± 0.013
18:3n-9	0.024 ± 0.004	0.019 ± 0.003	0.029 ± 0.003
20:0	0.044 ± 0.005	0.053 ± 0.002	0.071 ± 0.006*
20:1n-9	0.123 ± 0.008	0.152 ± 0.029	0.204 ± 0.021
20:1n-7	0.471 ± 0.073	0.674 ± 0.066	0.583 ± 0.013
20:2n-9	0.044 ± 0.015	0.031 ± 0.004	0.070 ± 0.008
20:2n-6	0.566 ± 0.033	1.035 ± 0.150*	0.614 ± 0.044
20:3n-9	0.035 ± 0.009	0.054 ± 0.010	0.051 ± 0.009
20:3n-6	0.540 ± 0.123	0.735 ± 0.114	0.817 ± 0.050
20:4n-6	0.909 ± 0.126	1.303 ± 0.280	0.543 ± 0.228
20:4n-3	0.094 ± 0.011	0.083 ± 0.010	0.080 ± 0.004
20:5n-3	0.630 ± 0.065	0.587 ± 0.042	0.441 ± 0.065
22:0	0.010 ± 0.001	0.006 ± 0.002	0.019 ± 0.007
22:1n-11	0.095 ± 0.007	0.120 ± 0.029	0.187 ± 0.029
22:1n-9	0.039 ± 0.005	0.032 ± 0.011	0.075 ± 0.014
22:3n-6	0.019 ± 0.001	0.019 ± 0.002	0.022 ± 0.001
22:4n-3	0.158 ± 0.007	0.176 ± 0.015	0.172 ± 0.012
22:5n-6	0.283 ± 0.027	0.238 ± 0.033	0.254 ± 0.013
22:5n-3	0.310 ± 0.030	0.271 ± 0.028	0.290 ± 0.010
22:6n-3	8.311 ± 0.745	8.735 ± 0.727	5.945 ± 1.724
24:1n-9	0.017 ± 0.002	0.012 ± 0.002	0.020 ± 0.002
∑SFA	5.792 ± 0.453	5.560 ± 0.107	7.246 ± 0.137*
∑MUFA	4.200 ± 0.441	3.742 ± 0.097	7.859 ± 0.212*
∑PUFA	14.011 ± 1.096	15.621 ± 0.922	12.743 ± 1.812
∑n-3	9.599 ± 0.836	9.959 ± 0.701	7.004 ± 1.833
∑n-6	4.309 ± 0.399	5.540 ± 0.367	5.570 ± 0.399

Fatty acid contents of liver were measured with a gas chromatography-flame ionization detector.
Values are means ± SEM (n=5, * p<0.05).
Control: Control diet; Lys (−): Lysine-deficient diet; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; n-3: n-3 fatty acid; n-6: n-6 fatty acid.

Table 8: Fatty acid content (mg/g) in liver of rainbow trout from Lys-deficient trial.

shown that Lys-deficient feeding for about two months induced lipid level increment in the porcine skeletal muscle. Lipid increases in the liver and muscle caused by the Lys (−) diet in the present study agree with the results of these previous studies at the first glance. The present feeding period was, however, markedly different from those studies, and then we have considered that the underlying mechanisms responding to Lys-deficiency were probably different from each other. Although the mechanism underlying lipid increases during long-term Lys-deficiency have not been clarified, it is thought that an increase in the number and hypertrophy of adipocytes would lead to an increase in the intramuscular fat in Lys-deficiency in porcine [31], and that impaired lipid oxidation would be involved in fatty livers in Lys-deficient rats [32]. In this study, the increase in lipid content was probably due to more rapid response mechanisms, such as immediate changes in intracellular and/or intercellular signal transduction networks. In addition, the Lys (−) diet contained a larger amount of leucine than the control diet. Leucine is known to be an important amino acid for the activation of intracellular signaling pathways in mammals [33,34]. Previous study reported that leucine affects signaling pathways involved in energy metabolism as well as in fish [19]. The effects of other amino acid contents, such as leucine, will be investigated and discussed in our future physiological reports. Since Lys is a precursor of carnitine, Lys-deficiency possibly reduces carnitine content in fish body. A decrease in fatty acid oxidation in the muscle would partly contribute to the increase in lipid and fatty acid contents observed in this study. It is possible that various regulatory systems for lipid metabolism were affected by the Lys (−) diet in this study.

Lipid and n-3 LCPUFA contents of fish meat are important in determining its commercial value, and change depending upon factors such as water temperature, habitat environment, diet, and maturity. Previous studies on several species reported that the lipid content in fish muscles changes seasonally [35,36], and that cultured fish meat tends to contain more lipids [37-39]. A high-fat diet causes an increase in the lipid content of fish tissues [40-42], and short-term fasting causes an increase of lipid content in the muscle [43]. However, feeds with higher lipid content are more expensive. In addition, long-term feeding of high-fat diets negatively influences fish health (e.g. hepatic failure), and short-term fasting causes a slight loss in body weight, resulting in an economic loss. Thus, methods for enriching fish meat with lipids and n-3 LCPUFAs, without economic or fish health problems, are in demand. The present study has demonstrated that short-term Lys-deficiency using a low-cost substitute for fish meal, corn gluten meal, successfully enriched fish meat with lipid and n-3 LCPUFAs.

Lipid and fatty acid contents in fish tissues are determined by lipid metabolism dynamics. The increase in lipid and n-3 LCPUFA contents in this study are likely to result from the following two systems: biosynthesis of fatty acids and uptake of dietary lipids.

First, the changes in liver fatty acid levels in this study imply that biosynthesis of fatty acids was enhanced in the liver of rainbow trout fed with Lys (−) diet. The liver is one of the main organs responsible for lipid biosynthesis in fish [44], and SFAs are produced through *de novo* synthesis from malonyl CoA and acetyl CoA with subsequent elongation. A portion of synthesized SFAs are desaturated to MUFAs. Previous studies have shown that 16:0, 18:0, 16:1, and 18:1 are mainly synthesized from acetate in *Tilapia zillii* [45], and that biosynthesis of fatty acid in fish is affected by dietary composition [46] and water temperature [47]. In this study, the total amounts of SFAs and MUFAs in the liver were significantly higher on Day 4 than on Day 0. This result suggests that the Lys (−) diet should enhance *de novo* synthesis and desaturation of fatty acids in the liver.

Secondly, it is likely that lipid accumulation derived from dietary lipids through Lys (−) feeding also contributes to the increase in lipid and n-3 LCPUFA contents. As stated above, fatty acid composition in fish tissues strongly reflects fatty acid composition in a fish diet [15]. In the liver tissues, a significant increase in 18:2n-6 content on Day 4 suggested that liver fatty acid content might reflect dietary fatty acid composition. Liver 20:2n-6 content on Day 2 was higher than that on Day 0. This 20:2n-6 might derive from dietary intake or fatty acid conversion from other n-6 fatty acids, such as 18:2n-6. Since 18:2n-6 is a major fatty acid in both of the diets used in this study, the uptake of dietary n-6 fatty acids possibly relate to the increase in 20:2n-6 on Day 2. These results suggest that lipids and fatty acids from dietary lipids would be, at least in part, accumulated in the muscle tissues in the Lys (−) group.

Some n-3 LCPUFA and total n-3 fatty acid levels in muscle were higher on Day 4 than on Day 0. Since rainbow trout are not able to desaturate fatty acids at the omega-3 position, n-3 fatty acid content in the muscle depends on the dietary intake. The accumulation of n-3 fatty acids was enhanced by short-term Lys deficiency, though there was no difference between fatty acid compositions in the control and Lys (−) diets. Therefore, it is likely that actively enhancing uptake of dietary n-3 LCPUFAs and the transport of lipids from other tissues to the muscle, contributes to an increase in n-3 LCPUFA content in fish meat during Lys-deficient feeding.

Conclusion

It was found that short-term Lys-deficient feeding induces an increase in lipid content and enrichment of n-3 fatty acids in rainbow trout muscle. These results are promising for the development of novel methods for producing fish meat containing a large amount of n-3 fatty acids. Moreover, our results imply that a short-term Lys-deficient feeding regimen might induce dynamic changes in the biosynthesis of fatty acids, and uptake of dietary lipids. In addition, there is also a possibility that the Lys-deficient feeding in this study affected the transport of fatty acids between organs. The underlying physiological and biomolecular mechanisms for these systems require further investigation. Further research is needed to determine optimal amino acid compositions of feed and/or feeding-terms to establish practical enrichment methods for various fish species.

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