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Changes of proximate and fatty acid compositions of the dorsal and ventral ordinary muscles of the full-cycle cultured Pacific bluefin tuna *Thunnus orientalis* with the growth

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Abstract

Using the full-cycle cultured (FC) Pacific bluefin tuna, *Thunnus orientalis* [body weights: 13.1 ± 4.5 (FC1; April in 2004), 20.2 ± 1.8 (FC2; July in 2004), 28.5 ± 6.3 (FC3; November in 2004), 27.0 ± 3.3 (FC4; February in 2005) and 33.5 ± 4.7 kg (FC5; May in 2005), n = 3, respectively] and wild bluefin tuna [33.3 ± 1.5 kg (June in 2005), n = 3], proximate and fatty acid compositions of the cephalal (Ce-) and caudal (Ca-) parts of the dorsal (D) and ventral (V) ordinary muscles (OMs) were investigated. Lipid contents of the Ce-DOM and VOMs of FC1-5 increased with growth. In particular, lipid content of the Ce-DOM (23.0%) and VOMs (55.1%) of FC5 was higher (P < 0.05) than those of wild tuna [D-(2.0%) and VOMs (16.2%)]. However, lipid contents of the Ca-DOM and VOMs of FC1-5 did not change with growth. On the other hand, the fatty acid compositions of the Ce-DOM of FC2-5 resembled each other. However, there was no specific tendency of the changes of each fatty acid composition of the Ce-DOM of FC tuna with growth. On the other hand, total monounsaturated fatty acid content (30.1%) of the Ce-DOM of FC5 was higher (P < 0.05) than that (14.0%) of wild tuna. The ratio of n-3: n-6 (9.4%) of the Ce-VOM of FC5 was lower (P < 0.05) than that (14.0%) of wild tuna. However, the fatty acid compositions of the Ce-DOM and VOMs of sesame mackerel).

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1. Introduction

Fish muscle contains many n-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA: C20:5n-3) and docosahexaenoic acid (DHA: C22:6n-3) (Dyerberg, Bang, & Hjorne, 1975; Ruxton, Reed, Simpson, & Millington, 2004). These fatty acids have various bioactivated functions, such as anti-cancer activity (Takahashi et al., 1993), recovery from heart failure (Albert et al., 2002), attenuation of cerebrovascular disease (Zhang, Sasaki, Amano, & Kesteloot, 1999), and anti-arteriosclerosis action (Saito, Saito, Chang, Tamura, & Yoshida, 1991).

Generally, the fatty acid composition of fish muscle is influenced by various factors. In addition, there are differences of amounts of EPA and DHA among fish species and localities (Owen, Andron, Middleton, & Cowey, 1975; Saito, Ishihara, & Murase, 1997). Furthermore, those of identical fish species are also affected by season, sea area and age (Hearn, Sgoutas, Hearn, & Sgoutas, 1987). In addition, fatty acid compositions in blood and muscle of fish are affected by feed condition and are reflected by the fatty acid composition of feed (Farndale et al., 1999; Toyomizu, Kawasaki, & Tomiyasu, 1963).

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In the muscle of widely migratory fish, such as a tuna, DHA contents of muscle are higher than in non-migratory species (Medina, Auboug, & Martin, 1995; Murase & Saito, 1996). This characteristic of high DHA content of muscle is not affected by the maturity (Ishihara & Saito, 1996; Tanabe, Suzuki, Ogura, & Watanabe, 1999). However, lipid contents of dorsal ordinary muscles differ among species, for example, 0.5% of wild yellowfin tuna, T. albacares (Saito & Ishihara, 1996), 1.88% of wild skipjack tuna, Katsuwonus pelamis (Balogun & Talabi, 1985) and 0.5% of wild Bigeve tuna. T. obesus (Koriyama, Kohata, Watanabe, & Abe, 2000). In wild bluefin tuna, T. thynnus, muscles, lipid contents of the front part of the ventral ordinary muscle and muscles of the skin side are higher than those of the front part of the dorsal ordinary muscle and muscles of the central part, respectively (Fudge, Ballantyne, & Stevens, 2001; Harada, Murata, & Norita, 1983).

In the Fisheries Laboratory of Kinki University, Pacific bluefin tuna, *T. orientalis*, were hatched on June in 2002 from artificially hatched and cultured parent fish. The life cycle of the Pacific bluefin tuna was completed under aquaculture, for the first time in the world. In the previous report (Nakamura, Ando, Seoka, Kawasaki, & Tsukamasa, 2005), we showed that the full-cycle cultured (FC) Pacific bluefin tuna had a high lipid content. In our results, the lipid contents of the front part of the dorsal and ventral ordinary muscles were about 10% and 40%, respectively. However, we did not clarify changes of lipid contents and fatty acid compositions of FC Pacific bluefin tuna muscles with growth.

The purpose of this study is to investigate changes of proximate and fatty acid compositions of dorsal and ventral ordinary muscles of FC Pacific bluefin tuna, *T. orientalis*, with growth, the difference between wild and FC Pacific bluefin tuna muscles and the effect of feed (wild sesame mackerel).

2. Materials and methods

2.1. Materials

Full-cycle cultured (FC) Pacific bluefin tuna *T. orientalis* was cultured in the Fisheries Laboratory of Kinki University (Oshima experimental station, Wakayama, Japan). All specimens were spawned on August 2002 and killed on 30

April (FC1, n = 3), 27 July (FC2, n = 3), 2 November (FC3, n=3) in 2004, 2 February (FC4, n=3) and 4 May (FC5, n = 3) in 2005 at E 135°. N 50°, respectively (Table 1). The cultured net cage size was 25 m in diameter and 10 m in depth, and culture density was about 3000 fish for a 30 kg (body weight: BW) commercial-size culture net cage (Sawada, Okada, Miyashita, Murata, & Kumai, 2005), and wild sesame mackerel was used to feed the FC Pacific bluefin tuna to satiation. Usually, cultured bluefin tuna are sold at around 30 kg in Japan. After fishing from a cultured net cage, the brain and spinal cord of the bled slaughtered fish were destroyed immediately (a hole was opened in the forehead with an awl, and a wire was run through the spinal cord), the gills and internal organs were removed and the carcasses were stored in ice water. After each of the tuna bodies were divided into individual parts at low temperature (4 °C), the cephalal (Ce-) and caudal (Ca-) parts of the dorsal (D) and ventral (V) ordinary muscles (OMs) (Fig. 1) were used for chemical analyses within 9 h of slaughter.

Three specimens of wild Pacific bluefin tuna (killed at about E 137°, N 37° on 11–25 June in 2004) that had been caught in a fixed shore net (off Notoshima, Ishikawa, Japan) were used (Table 1). After the fish were slaughtered and bled, the internal organs were removed and carcasses were stored in ice water. After each of the tuna bodies were divided into individual parts at low temperature (4 °C), the Ce-DOM and VOMs (Fig. 1) were used for chemical analyses within 9 h of slaughter.

For investigate the lipid content and fatty acid compositions of feedstuff (wild sesame mackerel) for FC Pacific bluefin tuna, wild sesame mackerel (SM) was bought in the market (Table 2) and used for chemical analyses. SM bodies (with internal organs and gonads) were mixed by a food processor and stored at -60 °C prior to chemical analyses.

2.2. Measurement of proximate compositions

Moisture content was measured by the conventional method. Samples (1-2 g) were dried at 105 °C overnight. Crude protein content was measured by the Kjeldahl method (Kjeldahl, 1883). The coefficient of conversion from the amount of nitrogen to crude protein content was 6.25, based on the Standard Tables of Food Composition in Japan (STFCJ, 2001). Crude fat content was

Table 1 Data of full-cycle cultured (FC) and wild Pacific bluefin tuna

	FC1	FC2	FC3	FC4	FC5	Wild		
Sampling date	29 April 2004	27 July 2004	2 November 2004	2 February 2005	4 May 2005	11-25 June 2004		
п	3	3	3	3	3	3		
Body weight (kg)	$13.1 \pm 4.5a$	$20.2 \pm 1.8 ab$	$28.5\pm6.3bc$	$27.0 \pm 3.3c$	$33.5\pm4.7bc$	33.3 ± 1.5		
Fork length (cm)	$79.4\pm9.1a$	$94.6\pm2.7b$	$105.6\pm6.6c$	$104.8\pm3.8bc$	$110.5\pm4.4c$	_		

Values are means and standard deviation of three specimens.

Different lower case letters indicate significant differences among FCI-5 ($P \le 0.05$, LSD test).

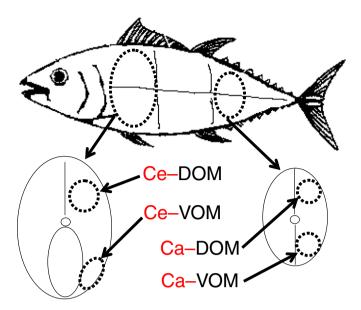


Fig. 1. The samples used in the cephalal (Ce-) and caudal (Ca-) parts of the dorsal (D) and ventral (V) ordinary muscles (OMs) of the wild and full-cycle cultured Pacific bluefin tuna.

measured by the Soxhlet extraction system. Crude ash content was measured by heating at 600 °C overnight. Each sample was assayed intriplicate.

2.3. Measurement of fatty acid compositions

Samples (5 g) were homogenized in 20 volumes of chloroform/methanol (2:1 v/v) and total lipid extracted by the method of Folch, Lees, and Stanly (1957).

Fatty acid methyl esters were produced from aliquots of total lipids. In detail, HCl-methanol reagent (3 ml) was added to the extracted lipid samples (50 mg) and they were then heated at 100 °C for 3 h. Fatty acid methyl esters were extracted in hexane, and preserved at -30 °C prior to chemical analysis.

Fatty acid methyl esters were analyzed by gas–liquid chromatography (GLC) with a Shimadzu GC-14B instrument (Shimadzu Seisakusho Co., Kyoto, Japan) equipped with a flame ionization detector and a Supelcowax capillary column (OmegawaxTM 250, Fused Silica Capillary Column, 30 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco Inc., Bellefonte, PA). The column temperature was 220 °C (from initial to final). Helium served as the carrier gas at 150 kPa (P1) and 25 kPa (P2) (ratio of split, 60:1) in a flow-controller. Peak area percentages were obtained with a chromatopac (C-R8A, Shimadzu). Individual methyl esters were identified against the retention time of a standard mixture of methyl esters (SupelcoTM 37 Component FAME Mix, No. 47885-U, Supelco).

2.4. Statistical analysis

The data were expressed as mean \pm standard deviation of three (FC Pacific bluefin tuna) and five (SM) specimens. Significance of difference (P < 0.05) was tested by one-way ANOVA (LSD test) of variance among FC Pacific bluefin tuna and among SM. Significance of difference (P < 0.05) was tested by *t*-test between FC 5 and wild Pacific bluefin tuna.

3. Results and discussion

3.1. Changes of proximate and fatty acid compositions of FC Pacific bluefin tuna muscles with growth

Proximate compositions of the Ce- and Ca- of D- and VOMs of FC Pacific bluefin tuna, *T. orientalis*, are shown in Table 3. In the Ce-DOM and VOMs of FC Pacific bluefin tuna, moisture, protein and ash contents decreased with growth. However, lipid content increased, and lipid content of FC5 (BW: about 30 kg) was higher (Ce-DOM: about 2 times, Ce-VOM: 1.5 times) than that of FC1 (BW: about 10 kg). On the other hand, lipid contents of the Ca-DOM and VOMs of FC Pacific bluefin tuna did not change with growth. These results indicate that lipid content of FC tuna muscle increase with the growth, however, there is a difference of degree of increase by position. In particular, the proximate compositions of the cephalal part of ordinary muscle of FC Pacific bluefin tuna changes easily with the growth.

Generally, the lipid content of cultured fish muscle is higher than that of wild fish (sea bass *Dicentrarchus labrax*, white seabream *Diplodus sargus* and black seabream *Spondyliosoma cantharus*) (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002; Cejas et al., 2004; Rodríguez et al., 2004). The high lipid content of cultured fish is caused by a lack of exercise, overfeeding and high energy

Table 2

Data of feedstuff (wild sesame mackerel: SM) for full-cycle cultured Pacific bluefin tuna

	SM1	SM2	SM3	SM4
Sampling date	July 2004	July Nov	2005 February	2005 May
Sampling region (the coast of pref. in Japan)	Nagasaki	Shizuoka	Kagoshima	Wakayama
n	5	5	5	5
Body weight (g)	$493.4\pm24.0a$	$601.4\pm34.2b$	$617.5\pm42.0b$	$611.8\pm72.9b$

Values are means and standard deviation of five specimens.

Different lower case letters indicate significant differences among SM 1–4 ($P \le 0.05$, LSD test).

Table 3 Proximate compositions of cephalal (ce-) and caudal (ca-) parts of dorsal (D) and ventral (V) ordinary muscles (OMs) of full-cycle cultured (FC) and wild Pacific bluefin tuna

		FC1	FC2	FC3	FC4	FC5	Wild
Ce-DOM (%)	Moisture	$67.9 \pm 4.4a$	$68.0 \pm 3.0a$	$64.7\pm4.0a$	$63.4 \pm 2.1a$	$55.3 \pm 4.9 \mathrm{b}$	$72.8\pm0.2^*$
	Protein	$23.3 \pm 0.9 \mathrm{ac}$	$24.5 \pm 1.3 ab$	$25.8\pm0.1\text{b}$	$22.6 \pm 0.5c$	$21.8 \pm 1.4c$	$26.1\pm0.1^*$
	Lipid	$11.0 \pm 6.2a$	$9.7\pm2.8a$	$11.5\pm5.6a$	$14.9 \pm 3.7 ab$	$23.0\pm8.0b$	$2.0\pm0.4^{*}$
	Ash	$1.6 \pm 0.2a$	$1.6 \pm 0.1a$	$1.6 \pm 0.1a$	$1.2\pm0.5ab$	$0.9\pm0.2b$	$1.6\pm0.1^{*}$
Ca-DOM (%)	Moisture	$67.8 \pm 3.4a$	$71.3 \pm 1.9a$	$69.6 \pm 2.6a$	$67.7 \pm 2.6a$	_	_
	Protein	$24.2 \pm 1.2a$	$24.4\pm0.0a$	$22.1 \pm 0.8b$,	$26.1 \pm 0.3c$	_	_
	Lipid	$8.9\pm7.5a$	$5.8 \pm 3.2a$	$7.6\pm 6.0a$	$10.8\pm2.4a$	_	_
	Ash	$2.1 \pm 1.1a$	$1.8 \pm 0.3a$	$1.4 \pm 0.2a$	$1.2 \pm 0.1a$	_	_
Ce-VOM (%)	Moisture	$46.7 \pm 9.2a$	$51.0 \pm 13.5a$	$49.5 \pm 4.0a$	$45.0 \pm 9.3a$	$37.7 \pm 7.5a$	$63.8\pm1.7^*$
	Protein	$16.2 \pm 3.8a$	$18.1 \pm 5.7a$	$15.8\pm0.3a$	$15.4 \pm 3.8a$	$14.6 \pm 3.0a$	$24.0 \pm 1.2^*$
	Lipid	$39.2 \pm 12.5a$	$38.2 \pm 15.6a$	$39.6 \pm 1.7a$	$45.7 \pm 10.8a$	$55.1 \pm 11.2a$	$16.2\pm2.7^*$
	Ash	$1.2\pm0.4a$	$1.1 \pm 0.3a$	$1.2\pm0.3a$	$0.6\pm0.2b$	$0.3 \pm 0.1 \mathrm{b}$	$1.4\pm0.1^*$
Ca-VOM (%)	Moisture	$69.2 \pm 4.8a$	$69.1 \pm 1.2a$	$69.1 \pm 3.7a$	$67.3 \pm 2.9a$	_	_
	Protein	$24.4 \pm 1.7a$	$23.9 \pm 1.4a$	$24.0 \pm 1.1a$	$25.3\pm0.7a$	_	_
	Lipid	$8.0\pm5.6a$	$10.3 \pm 2.0a$	$7.8\pm3.5a$	$7.7 \pm 1.1a$	_	_
	Ash	$1.8\pm0.3a$	$1.6 \pm 0.5 a$	$1.2\pm0.2a$	$1.3 \pm 0.4a$	_	_

Values are means and standard deviation of three specimens.

Different lower case letters indicate significant differences among PC 1–5 ($P \le 0.05$, LSD test).

Asterisks denote significant differences between FC5 and wild Pacific bluefin tuna (P < 0.05, t-test).

diets. The remarkably high lipid content of FC Pacific bluefin tuna in this study may be due to culture conditions. In short, the cultured net cage size was 25 m in diameter and 10 m in depth, and culture density was about 3000 fish for a 30 kg commercial-size culture net cage (Sawada et al., 2005), and wild sesame mackerel were fed to satiation. Therefore, it seemed that these conditions produced a high lipid content in the ordinary muscle of FC Pacific bluefin tuna.

We have not investigated the fatty acid compositions of FC Pacific bluefin tuna and other tuna species muscles until now. Fatty acid compositions of the Ce-DOM and VOMs of FC Pacific bluefin tuna are shown in Tables 4 and 5. The fatty acid compositions of the Ce-DOM and VOMs of FC2-5 resembled each other. In detail, high concentrations of C16:0 (Ce-DOM: 15.2–17.8% and VOM: 15.7–16.6%), C18:1 (20.6-23.0 and 20.8-23.9%) and DHA (19.4-21.5 and 19.1-23.1%) were detected in FC Pacific bluefin tuna in this study. However, in the Ce-DOM and VOMs, there was no fixed tendency of changes of composition of each fatty acid or changes of total saturated, mono- and polyunsaturated fatty acid contents (SFA, MUFA and PUFA, respectively), and a fixed ratio of n-3: n-6 of FC Pacific bluefin tuna with the growth. In other reports of tuna muscle, for example, wild Bigeye tuna T. obesus had C16:0 (DOM: 21.8% and VOM: 15.8%), C18:1 (15.1 and 27.2%) and DHA (28.0 and 21.7%) (Koriyama et al., 2000). In wild Albacore tuna T. alalunga (BW: 5.1-20.8 kg), there were high concentrations of C16:0 (DOM: 18.4-19.0% and VOM: 16.6-18.6%), C18:1 (15.5-15.6 and 15.8-16.4) and DHA (24.3-25.9 and 24.2-25.1%) (Murase & Saito, 1996). These results indicate that fatty acid compositions of FC Pacific bluefin tuna muscles differ from those of other tuna species.

3.2. Comparison of proximate and fatty acid compositions between wild and FC Pacific bluefin tuna muscles

Lipid content (23.0%) of the Ce-DOM of FC5 (BW: about 30 kg) was higher (P < 0.05, about 11.5 times) than that (2.0%) of wild tuna (BW: about 30 kg), and that (55.1%) of the Ce-VOM of FC5 was higher (P < 0.05, about 3.5 times) than that (16.2%) of wild fish (Table 3). In addition, there was a difference of fatty acid compositions between FC5 and wild Pacific bluefin tuna muscles (Tables 4 and 5). In the Ce-DOM, C17:1 (0.6%), C18:1 (21.2%), C20:4n-6 (1.9) and SFA (30.1%) of FC5 were higher ($P \le 0.05$) than those of wild tuna (0.4, 15.5, 1.3) and 25.5%, respectively). On the other hand, in the Ce-VOM, C15:0 (0.7%), C16:1 (5.7%), C17:0 (1.5%), C17:1 (0.7%), C18:0 (6.1%), C18:1 (22.2%), C20:4*n*-6 (1.7%), C22:4n-6 (0.9%) and C22:5n-3 (2.1%) of FC5 were higher (P < 0.05) than those of wild tuna (0.4, 4.8, 1.2, 0.5, 4.6, 14.5, 0.9, 0.4 and 1.5%, respectively). However, C18:3n-3 (0.9%) and a ratio of n-3: n-6 (9.4) of FC5 were lower (P < 0.05) than those of wild tuna (2.1% and 14.0, respectively) (Tables 4 and 5). Generally, fatty acid compositions of wild and cultured fish muscles differ (red porgy Pagrus pagrus, sea bream Sparus aurata and white seabream Diplodus sargus) (Cejas et al., 2004; Grigorakis, Alexis, Taylor, & Hole, 2002; Rueda, López, Martínez, & Zamora, 1997); however, there are no specific tendencies of fatty acid compositions and contents among wild and cultured fish groups. On the other hand, wild bluefin tuna had C16:0 (19.1% of DOM and 15.5% of VOM), C18:0 (9.3 and 4.9%), C18:1 (24.7 and 20.7%), C20:4n-6 (2.1 and 0.8%), EPA (3.6 and 6.4%) and DHA (15.6 and 14.3%) (Kagawa, 2001). Therefore, the fatty acid compositions of FC Pacific bluefin tuna muscles in this study differed from those of Table 4

Fatty acid compositions	of cephalal (Ce-) part of dorsal (D) ordinary muscles (OMs) of full-cycle	e cultured (FC) and wild Pacific bluefin tuna
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	FC2	FC3	FC4	FC5	Wild
Ce-DOM					
Fatty acid compositions (%)					
C14:0	$3.2 \pm 0.7a$	2.5 ± 0.1 ab	$2.2\pm0.2\mathrm{b}$	$2.8 \pm 0.3 ab$	2.9 ± 0.9
C15:0	$0.8 \pm 0.1a$	$0.7\pm0.0 { m ab}$	$0.5 \pm 0.1 \mathrm{bc}$	$0.5\pm0.1c$	0.4 ± 0.1
C16:0	$16.6 \pm 2.6a$	$17.8 \pm 2.0a$	$15.2 \pm 0.8a$	$15.2 \pm 0.3a$	17.8 ± 5.0
C16:1	$4.9 \pm 0.8a$	$5.0 \pm 0.2a$	$4.5 \pm 0.5a$	$4.8 \pm 0.2a$	3.7 ± 0.8
C17:0	$1.3 \pm 0.4 ab$	$1.7 \pm 0.1a$	$1.0\pm0.1\mathrm{b}$	$1.4 \pm 0.1a$	1.1 ± 0.2
C17:1	$0.6\pm0.0a$	$0.6\pm0.0a$	$0.6\pm0.0a$	$0.6\pm0.0a$	0.4 ± 0.0
C18:0	$7.3 \pm 0.2a$	$8.0\pm0.2b$	$6.9\pm0.3a$	$6.9 \pm 0.2a$	6.2 ± 1.2
C18:1	$20.6\pm0.5a$	$23.0 \pm 2.2a$	$22.8\pm0.4a$	$21.2\pm~1.8a$	15.5 ± 1.5
C18:2 <i>n</i> –6	$1.1 \pm 0.0a$	$1.0 \pm 0.1a$	$1.1 \pm 0.1a$	$1.1 \pm 0.1a$	1.0 ± 0.1
C18:3 <i>n</i> –3	$0.7\pm0.1a$	$0.5\pm0.1b$	$0.6\pm0.0 { m ab}$	$0.9 \pm 0.1 c$	1.2 ± 0.2
C20:0	$2.9\pm0.5a$	$2.4\pm0.2a$	$2.9\pm0.5a$	$4.2\pm0.4\mathrm{b}$	6.3 ± 1.3
C20:1	$0.8 \pm 0.2a$	$0.6\pm0.0a$	$0.7\pm0.0a$	$0.7 \pm 0.1a$	0.3 ± 0.1
C20:2 <i>n</i> –6	$0.2 \pm 0.1a$	$0.2 \pm 0.2a$	$0.3\pm0.0a$	$0.2\pm0.0a$	0.1 ± 0.1
C20:3n-6	$0.1\pm0.1a$	$0.1 \pm 0.1a$	0.2 ± 0.0 a	$0.1\pm0.0a$	0.0 ± 0.0
C20:4 <i>n</i> -6	$2.4 \pm 0.1a$	$2.2 \pm 0.1a$	$2.0\pm0.1b$	$1.9\pm0.1b$	1.3 ± 0.1
C20:5n-3 (EPA)	$6.4 \pm 0.3a$	$5.3\pm0.6b$	$6.9 \pm 0.3 \mathrm{ac}$	$7.3\pm0.4c$	6.9 ± 1.0
C22:1	$1.3 \pm 0.7a$	$1.1 \pm 0.4a$	$1.6 \pm 0.5a$	$3.0\pm0.3b$	5.7 ± 2.3
C22:4 <i>n</i> –6	$1.3 \pm 0.4a$	$1.3 \pm 0.2a$	$1.2\pm0.1a$	$1.0 \pm 0.1a$	0.4 ± 0.3
C22:5n-3	$2.2\pm0.2a$	$2.1 \pm 0.2a$	$2.4 \pm 0.1a$	$2.1 \pm 0.2a$	0.8 ± 0.7
C22:6n-3 (DHA)	$21.5 \pm 1.0a$	$20.1 \pm 1.6 ab$	$21.0\pm0.9ab$	$19.4 \pm 0.1b$	23.3 ± 3.9
Total saturated (%)	$32.0\pm2.5ab$	$33.0 \pm 2.0a$	$28.7 \pm 1.2 \mathrm{b}$	$30.9 \pm 1.1 ab$	34.8 ± 6.2
Total monounsaturated (%)	$28.1\pm0.4a$	$30.4 \pm 2.1a$	$30.2\pm0.5a$	$30.1 \pm 1.2a$	25.5 ± 1.1
Total polyunsaturated (%)	$35.8 \pm 1.4a$	$32.9\pm3.0a$	$35.6\ \pm 1.0a$	$34.0\pm0.5a$	34.8 ± 6.0
A ratio of <i>n</i> –3: <i>n</i> –6	$8.3\pm0.8ab$	$7.9\pm0.5a$	$8.8\pm0.4ab$	$9.1 \pm 0.2 \mathrm{b}$	13.3 ± 2.2

Values are means and standard deviation of three specimens.

Different lower case letters indicate significant differences among FC2-5 ($P \le 0.05$, LSD test). Asterisks denote significant differences between FC5 and wild Pacific bluefin tuna ($P \le 0.05$, *t*-test).

Table 5

Fatty acid compositions o	f cephalal (Ce-)	part of ventral (V)	ordinary muscles (OMs) of full-cycle cultured	(FC) and wild Pacific bluefin tuna

	FC2	FC3	FC4	FC5	Wild
Ce-VOM					
Fatty acid compositions (%)					
C14:0	$3.2 \pm 0.3a$	$2.6 \pm 0.8a$	$2.6 \pm 0.4a$	$3.4 \pm 0.4a$	3.7 ± 1.1
C15:0	$0.7 \pm 0.1a$	$0.7 \pm 0.2a$	$0.6 \pm 0.1a$	$0.7\pm0.1a$	$0.4\pm0.0^{*}$
C16:0	$15.9 \pm 1.4a$	$16.6 \pm 3.2a$	$15.7 \pm 1.1a$	$16.5 \pm 0.5a$	14.5 ± 1.6
C16:1	$5.5\pm0.4a$	$5.2 \pm 0.8a$	$5.3 \pm 0.5a$	$5.7\pm0.6a$	$4.8\pm0.7^{*}$
C17:0	$1.4 \pm 0.5a$	$1.6 \pm 0.2a$	$1.3 \pm 0.4a$	$1.5 \pm 0.1a$	$1.2\pm0.0^{*}$
C17:1	$0.6\pm0.0a$	$0.6 \pm 0.1a$	$0.6 \pm 0.0a$	$0.7\pm0.0a$	$0.5\pm0.0^{*}$
C18:0	$6.4 \pm 0.5a$	$6.7 \pm 0.3a$	$6.7 \pm 0.3a$	$6.1 \pm 0.3a$	$4.6\pm0.1^*$
C18:1	$20.8\pm0.5a$	$23.2 \pm 1.1a$	$23.9 \pm 2.1a$	$22.2\pm2.5a$	$14.5\pm0.6^*$
C18:2 <i>n</i> –6	$1.1 \pm 0.1a$	$1.0 \pm 0.1a$	$1.0 \pm 0.1a$	1.1 ± 0.1 a	1.2 ± 0.0
C18:3 <i>n</i> -3	$0.8\pm0.0{ m ab}$	$0.7 \pm 0.2a$	$0.7\pm0.0a$	$0.9~\pm 0.0 \mathrm{b}$	$2.1\pm0.2^{*}$
C20:0	$2.7\pm0.3a$	$2.5\pm0.3a$	$2.7\pm0.2a$	$3.3\pm0.3b$	7.1 ± 2.1
C20:1	$0.6\pm0.0a$	$0.7\pm0.0a$	$0.6 \pm 0.2a$	$0.6\pm0.0a$	0.4 ± 0.1
C20:2 <i>n</i> –6	$0.2 \pm 0.1a$	$0.2 \pm 0.2a$	$0.2\pm0.0a$	$0.2\pm0.0a$	0.2 ± 0.0
C20:3 <i>n</i> –6	$0.1 \pm 0.1a$	$0.1 \pm 0.1a$	$0.1 \pm 0.1a$	$0.1\pm0.1a$	0.1 ± 0.1
C20:4 <i>n</i> -6	$2.3 \pm 0.1a$	$2.1 \pm 0.1b$	$1.9 \pm 0.1c$	1.7 ± 0.1 d	$0.9\pm0.1^{*}$
C20:5n-3 (EPA)	$7.5\pm0.2a$	$6.7\pm0.4b$	$7.2\pm0.3ab$	$7.1 \pm 0.3 ab$	9.8 ± 1.5
C22:1	$1.0 \pm 0.3a$	$1.0 \pm 0.9a$	$1.5 \pm 0.2ab$	$2.2\pm0.3b$	6.3 ± 3.0
C22:4 <i>n</i> –6	$0.8\pm0.7a$	$1.1 \pm 0.3a$	$1.0 \pm 0.2a$	$0.9\pm0.1a$	$0.4\pm0.0^{*}$
C22:5n-3	$2.2\pm0.4a$	$2.2 \pm 0.4a$	$2.3 \pm 0.2a$	$2.1 \pm 0.1a$	$1.5\pm0.1^{*}$
C22:6n-3 (DHA)	$23.1 \pm 0.5a$	$20.9 \pm 1.8 \mathrm{ab}$	$19.9\pm2.0b$	$19.1 \pm 2.0 \mathrm{b}$	20.2 ± 0.4
Total saturated (%)	$30.3\pm2.7a$	$30.7\pm4.0a$	$29.6\pm1.6a$	$31.4 \pm 1.0a$	31.5 ± 0.7
Total monounsaturated (%)	$28.5\pm0.3a$	$30.6 \pm 1.1a$	$31.9\pm2.3a$	$31.5\pm2.9a$	26.6 ± 1.9
Total polyunsaturated (%)	$37.9 \pm 1.6a$	$35.0 \pm 2.8a$	$34.3\pm2.9a$	$33.1\pm2.6a$	36.3 ± 1.7
A ratio of <i>n</i> –3: <i>n</i> –6	$9.4 \pm 0.3a$	$9.0 \pm 0.2a$	$9.1 \pm 0.2a$	$9.4 \pm 0.3a$	$14.0\pm0.8^*$

Values are means and standard deviation of three specimens.

Different lower case letters indicate significant differences among FC2-5 ($P \le 0.05$, LSD test).

Asterisks denote significant differences between FC5 and wild Pacific bluefin tuna ($P \le 0.05$, t-test).

wild fish (data in this study and other reports). We consider that the difference of fatty acid compositions between FC and wild Pacific bluefin tuna muscles in this study is caused by a lack of exercise and/or feedstuff. However, further study is necessary.

3.3. Relationship of fatty acid compositions between FC Pacific bluefin tuna muscle and sesame mackerel as a feed for cultured bluefin tuna

In this study, FC Pacific bluefin tuna were saturated with wild sesame mackerel (SM) in all seasons. We investigated the changes of lipid content and fatty acid compositions of SM with the season in this study. The lipid content (whole fish body with internal organs and gonad) of SM changed 6.6-8.2% with the season (Table 6). In particular, lipid content (8.2%) in February, 2005 was higher (P < 0.05) than in other months (Julv and November. 2004; 6.8 and 6.6%). There is a seasonal variation in the lipid level of SM in this study. In other reports, lipid contents of dorsal and ventral muscles of wild yellowtail increased in the winter season (from October to February) in comparison with the summer season (from May to September) (Shimizu, Tada, & Endo, 1973). On the other hand, lipid content of the whole body of wild sardine Sardinops melanosticta from January to February was higher

(about 2-4 times) than from March to April (Kurokawa, 1983). In our results, lipid content of SM was highest in the winter season (in February, 2005) (Table 6). This result indicates that SM caught in the sea near Japan has a high lipid content in the winter season. However, in this study, SM samples contained internal organs and gonad. The decrease of lipid content of fish body in the summer season is caused by the consumption of stored lipid within muscle because of the development of the gonads (sexual maturation) in the breeding season (Shimizu et al., 1973; Zama & Igarashi, 1954). Therefore, there is a possibility that the changes of lipid contents of SM in this study are affected by the development of gonads. However, the lipid contents of FC5 muscles (Ce-DOM and VOMs) were higher than those of FC1-4 (Table 3). These results indicate that the lipid content of FC Pacific bluefin tuna muscle is not affected by the lipid content of feedstuff, in spite of overfeeding.

The fatty acid compositions of SM are shown in Table 6. In addition, the comparison of fatty acid compositions between SM1-4 (whole fish body) and FC2-5 muscles (Ce-DOM and VOMs) are shown in Fig. 2. Generally, the lipid content and fatty acid composition of fish blood and muscle (rainbow trout, sea bass, salmon, saithe) are influenced by the composition of feed (Farndale et al., 1999; Hemre & Sandnes, 1999; Skog, Hylland, Torstensen, & Berntssen, 2003; Toyomizu et al., 1963). On the other

Table 6 Total lipid content and fatty acid compositions of wild sesame mackerel (SM)

	SM1	SM2	SM3	SM4
Lipid content (%)	$6.8\pm0.4a$	$6.6\pm0.7a$	$8.2\pm0.9\mathrm{b}$	7.3 ± 0.2ab
Fatty acid compositions (%)				
C14:0	$4.8\pm0.9a$	$5.4 \pm 1.2a$	$3.9 \pm 1.6a$	$3.3 \pm 1.0a$
C15:0	1.2 ± 0.1 a	1.0 ± 0.3 ac	0.5 ± 0.1 bc	$0.8\pm0.2c$
C16:0	$21.5 \pm 1.7a$	$19.2 \pm 3.0a$	$17.9 \pm 2.4a$	$20.9\pm2.8a$
C16:1	5.3 ± 0.4 ab	5.1 ± 0.9 ab	$6.7 \pm 1.1a$	$5.0\pm0.8b$
C17:0	$1.7 \pm 0.5a$	$2.0\pm0.2a$	$1.6 \pm 0.2a$	$1.8 \pm 0.1a$
C17:1	$0.6\pm0.0\mathrm{a}$	$0.5\pm0.0\mathrm{b}$	$0.6 \pm 0.1 \mathrm{ab}$	$0.6\pm0.1 \mathrm{ab}$
C18:0	$5.4 \pm 0.2a$	$6.4 \pm 0.5 \mathrm{b}$	$6.4 \pm 0.1 \mathrm{b}$	$7.0 \pm 0.2c$
C18:1	$18.4 \pm 1.3a$	$13.7 \pm 2.2b$	$15.5 \pm 0.2b$	$21.5 \pm 0.4c$
C18:2 <i>n</i> –6	1.2 ± 0.1 a	$1.2 \pm 0.1a$	1.0 ± 0.1 ab	1.0 ± 0.1 ab
C18:3 <i>n</i> -3	$0.9\pm0.0{ m ac}$	$0.7\pm0.1b$	$0.7\pm0.0\mathrm{bc}$	$0.4\pm0.0d$
C20:0	$2.4 \pm 0.3a$	$3.7\pm0.8b$	$3.3 \pm 0.3 ab$	$3.0\pm0.3ab$
C20:1	$0.8\pm0.1a$	$0.8 \pm 0.1a$	0.4 ± 0.0 b,	$0.6\pm0.1c$
C20:2 <i>n</i> –6	0.2 ± 0.0 a	$0.3\pm0.0\mathrm{b}$	$0.2\pm0.0a$	$0.3\pm0.0\mathrm{b}$
C20:3 <i>n</i> -6	0.2 ± 0.0 a	$0.1 \pm 0.1a$	0.2 ± 0.0 a	$0.2\pm0.0a$
C20:4 <i>n</i> -6	$1.9\pm0.3a$	$2.5\pm0.2a$	2.0 ± 0.5 a	$2.2\pm0.3a$
C20:5n-3 (EPA)	$5.7\pm0.8 \mathrm{ac}$	$6.9\pm0.6 \mathrm{ab}$	$7.3 \pm 1.2 \mathrm{b}$	$4.6 \pm 0.3c$
C22:1	$2.0\pm0.4a$	$3.4\pm0.8ab$	$5.3 \pm 1.2c$	$1.9\pm0.5a$
C22:4 <i>n</i> -6	$0.9\pm0.2 \mathrm{ab}$	$0.8\pm0.1a$	$0.4 \pm 0.4a$	$1.1\pm0.2b$
C22:5n-3	$1.6 \pm 0.3a$	$1.6 \pm 0.2a$	$2.6 \pm 0.5 \mathrm{b}$	$1.6 \pm 0.3a$
C22:6n-3 (DHA)	$17.6 \pm 1.9a$	$19.8 \pm 2.4a$	$18.2 \pm 3.2a$	$17.0 \pm 2.3a$
Total saturated (%)	$39.1 \pm 2.7a$	$40.9 \pm 2.5a$	$38.9\pm3.0a$	$38.7\pm3.2a$
Total monounsaturated (%)	$25.1 \pm 1.6 \mathrm{ac}$	$19.9\pm3.0b$	23.2 ± 1.3 ab	$27.7\pm0.7\mathrm{c}$
Total polyunsaturated (%)	$30.1\pm3.5a$	$33.9 \pm 3.2a$	$32.6 \pm 4.8a$	$28.3\pm3.4a$
A ratio of <i>n</i> –3: <i>n</i> –6	$7.3\pm0.4ab$	7.2 ± 0.4 ab	$8.7 \pm 2.2a$	$6.5\pm0.3\text{b}$

Values are means and standard deviation of five specimens.

Different lower case letters indicate significant differences among SM 1–4 ($P \le 0.05$, LSD test).

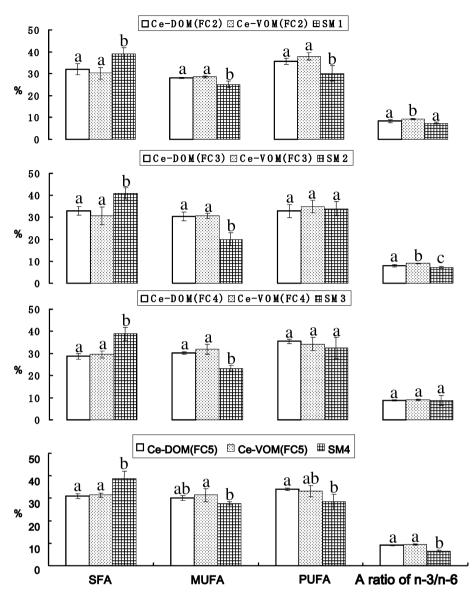


Fig. 2. The comparison of fatty acid composition between feedstuff (wild sesame mackerel: SM1-4) and FC Pacific bluefin tuna (FC2-5) muscles. SFA, MUFA and PUFA: total saturated, mono- and polyunsaturated fatty acid contents, respectively. Values are means \pm standard deviation of three (FC2-5) and five (SM1-4) specimens. Different sets of letters on each value of the same group show a significant difference ($P \le 0.05$) by one-way ANOVA.

hand, in the dorsal muscle of horse mackerel, the MUFA content tends to increase in the summer season (from June. to September) (Osako et al., 2003). However, the muscle lipid of Atlantic salmon Salmo salar had much MUFA from January to March (Nordgarden, Torstensen, Frøyland, Hansen, & Hemre, 2003). These results indicate that the fatty acid compositions in fish muscles are influenced by the season and fish species. However, in this study, there was no specific tendency of changes of each fatty acid composition of SM according to season. In addition, the fatty acid compositions of the Ce-DOM and VOMs of FC Pacific bluefin tuna were not reflected by those of feed. These results indicate that there is a lipid metabolism mechanism affecting bluefin tuna muscles. Therefore, we consider that it is difficult to control the fatty acid compositions of cultured tuna muscles by feedstuff (in particular, raw fish).

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