Fatty acid and proximate composition of bluefin tuna (*Thunnus thynnus*) muscle with regard to plasma lipids

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Abstract

The composition of tail muscle fatty acids from wild and cultured bluefin tuna reared on a diet based on herring and sardine, along with the plasma lipid profile of the farmed individuals, was determined. The total lipid content of farmed bluefin in this study was 0.922 g/100 g, or 3.49 g of SAFA, 4.48 g of MUFA, 2.58 g PUFA n-3, and 0.37 g of PUFA n-6 fatty acids; for wild specimens it was 0.920 g/100 g, or 2.85 g of SAFA, 4.82 g of MUFA, 2.78 g PUFA n-3, and 0.27 g of PUFA n-6 fatty acids. Major fatty acids in this study were 16:0; 16:1, n-7; 18:1, n-9 and DHA 22:6, n-3 acids. The sum of these major components accounted for more than 57 % and 80 % of the total fatty acids in all samples of farmed and wild tuna, respectively. No significant differences in proximate composition were demonstrated between farmed and wild samples, except for energy value, in favor of the farmed tuna. Statistically, GLU tends to increase together with CHOL and TRIG, since for these pairs it showed positive correlation coefficients and $P > 0.05$. Some measured tuna metabolites demonstrated strong mutual correlations, especially GLU, CHOL and TRIG, which are crucial factors in lipid profile of animals.

Introduction

Tuna farming in the Adriatic Sea (Croatia) implies caging of wild tuna for fattening, mainly for Japanese market. Tuna farming falls in between the definitions of standard fishery involving capture of wild stock, and true aquaculture where fish are bred and reared in captivity. It is considered a post-harvest practice and therefore falls outside the regulations put in place by General Fisheries Commission for the Mediterranean (GFCM) and International Commission for the Conservation of Atlantic Tunas (ICCAT). According to the Croatian Chamber of Economy (2004, 2009), Croatian tuna farming in 2002 reached 4.000 tons, and exceeded sea bream *Sparus aurata* and sea bass *Dicentrarchus labrax* production (2.700 tons), continued to grow and peaked in 2006 with 6.700 tons. With secured export, Atlantic bluefin tuna *Thunnus thynnus* has become one of the most important export products of agriculture and food industry in Croatia. Spain and Croatia are the leading countries in this new tuna farming model. During the past 10 years there has been a very important development of tuna farms in the Mediterranean, reaching at least 20 farms (Basurco & Lovatelli 2003).

Tuna is highly valued as a food fish around the world. It is sold fresh or frozen. Farmed tuna meat is higher in oil content than wild tuna, which makes it desirable for sushi. Such quality fish is especially favored in Japan, where it can fetch a high price in the raw seafood market. Japan and the United States are the largest consumers of tuna, using about 36 percent and 31 percent, respectively, of the world catch (Joseph, Klawe & Murphy 1988). Tuna meat can help lower blood pressure and cholesterol. Research has shown that omega-3 fatty acids, found in abundance in cold water fish like salmon,

herring, bluefish, mackerel, and tuna, can help lower the risk of heart disease, ease the pain of arthritis, reduce asthma complications, and is essential in the growth and development of young children. Other health benefits from intake of marine n-3 polyunsaturated fatty acids (n-3 PUFA) include reduction of atherogenesis and thrombogenesis, beneficial effects in control of blood lipid levels, diabetes mellitus, depression, autoimmune disorders (Soriguer, Serna, Valverde, Hernando, Martin-Reyes, Soriguer, Pareja, Tinahones & Esteva 1997; Moreira, Visentainer, de Souza & Matsushita 2001, Varljen, Sulic, Brmalj, Baticic, Obersnel & Kapovic 2003; Cejas, Almansa, Jerez, Bolanos, Samper & Lorenzo 2004). Containing unsaturated fatty acids makes the lipid in the seafood more easily absorbed in the lower alimentary canal once taken into the body than the foods that contain saturated fatty acids. Thus, the condensation of the lipid in veins is prevented (Otles & Sengor, 2005). Also, in wild bluefin tuna 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 (Nakamura, Ando, Seoka, Kawasaki & Tsukamasa, 2007). Haematological values, especially blood biochemical parameters, are important for clinical pathological diagnoses, identification of bacterial septicaemia and nutritional deficiencies, and many of them are caused by stress factors (Percin & Konyalioglu, 2008).

Although there is a number of data on various fish fatty acid composition, available published information about the composition of fatty acids and chemical composition of Atlantic bluefin tuna muscle tissue is meager. Objective of this study thus was to determine the fatty acid and proximate composition of ventral ordinary tail muscle

of Atlantic bluefin tuna, both farmed and wild, compare those findings with plasma parameters of farmed bluefin and analyze the lipid profile of the species.

Materials and Methods

Sixty-five farmed Atlantic bluefin tuna *T. thynnus* (average weight 280 kg, average length 180 cm, of unrecorded sex) were randomly sampled from a Croatian floating-cage farm located in the mid-Adriatic Sea. Tuna were originally captured by seine net in the waters off Malta and Tunisia, towed, and transferred to the farm in the Adriatic Sea, where they were kept 6 months prior to sampling. They were fed sardine *Sardina pilchardus* and defrosted herring *Clupea harengus harengus* to satiation. The age of specimens examined in this study was over 20 years as estimated by standard length and body weight. Fish were not administered any medications. One day prior to sampling fish were not fed. They were randomly selected, confined in a net of smaller radius and depth, in series of small groups for faster and easier handling, rapidly sacrificed and immediately bled. Long syringes were used for blood withdrawal from the caudal artery and vein. Muscle samples were taken from the ventral portion of the tail region. Muscle samples of wild adult bluefin tuna (n = 30) were obtained at the fish market from a certified trader.

One hundred grams of ventral ordinary tail muscle of every fish was cut out. The flesh was stored at -20°C until analyzed. The muscle of every fish was separately homogenized using a warring blender. Moisture content of 5 g of homogenized sample was determined by drying the sample in an oven at 105°C until a constant mass was obtained (AOAC 1990). Total protein was determined by the Kjeldahl method measuring the nitrogen concentration. A conversion factor of 6.25 was used to convert total nitrogen

111 to crude protein (Ritzman & Daniels 1975). Crude fat content was measured by the
112 Soxhlet extraction system.

113 Lipids were extracted according to Ankorion & Moav (1967) and butirometrically
114 according to Meester-Krol, a modified Gerber method (Rede & Rahelic, 1969), based on
115 protein destruction with a strong acid. After centrifugation, extracted lipid level was
116 determined with a butirometer. The fatty acid composition of tuna muscle was
117 determined by gas chromatograpy. Samples were homogenized in 20 volumes of
118 chlorophorm/methanol (2:1 v/v) and total lipid extracted by the method of Folch, Lees &
119 Stanly (1957). Fatty acid methyl esters were produced from aliquots of total lipids. HCl-
120 methanol reagent was added to the extracted lipid samples and they were then heated at
121 100 °C for 3 hours. Fatty acid methyl esters were extracted in hexane, and preserved at -
122 30 °C prior to chemical analyses. Fatty acid methyl esters were analyzed by gas-liquid
123 chromatograpy with a Shimadzu GC-14B instrument (Shimadzu Seisakusho Co, Japan)
124 equipped with a flame ionization detector and a Supelcowax capillary column. The
125 column temperature was 220 °C. Helium served as the carrier gas at 150 kPa (P1) and 25
126 kPa (P2) (ratio of split 60:1). Individual methyl esters were identified against the
127 retention time of a standard mixture of methyl esters. All the analyses were performed at
128 least three times.

129 Blood of only farmed bluefin was collected in tubes coated with anticoagulant
130 lithium heparin, centrifuged at 12 000 x *g* for 90 seconds and resultant plasma was frozen
131 at -20 ºC until analysis two weeks later. Cholesterol (CHOL), plasma triglyceride
132 (TRIG), glucose (GLU) and total protein (TP) levels were determined by the Olympus
133 AU2700 high-volume chemistry-immuno analyzer (Olympus America Inc., USA). The

analyzer, originally designed for clinical tests of high volume laboratories, is an open system using multiwavelength diffraction grating spectrophotometric method, monochromatic or bichromatic mode with light path of 6 mm. The total sample volume was up to 120 µl of plasma per test, using 1-25 µl of sample volume increments. Samples were prediluted at dilution ratio 5-100 by 1-fold increments. Flexible Microsoft Windows NT® software was used for operating. Software controlled test order and enhanced washing cycles to avoid cuvette contamination or carryover.

Statistical analyses were performed using Sigma Statistical Software, Version 1.0. Mean, median, standard deviation, standard error of mean, 25th percentile, 75th percentile and range difference of minimum and maximum values were calculated for each variable measured.

Results

In the analyzed muscle tissue samples, 19 fatty acids in the rear part of the bluefin tuna ventral ordinary muscle samples were identified, as follows: myristic (14:0), palmitic (16:0), palmitoleic (16:1, n-7), C16:2 (by extrapolation), C16:4 (by extrapolation), stearic (18:0), oleic (18:1, n-9), linoleic (18:2, n-6), α-linolenic (18:3, n-3), C18:4 (by extrapolation), arachidic (20:0), C20:1, n-7 (by extrapolation), C20:2 (by extrapolation), arachidonic (20:4, n-6), C20 polyunsaturated (by extrapolation), eicosapentaenoic (EPA) (20:5 n-3), erusic (22:1 n-7), docosapentaenoic (22:5, n-6), and docosahexaenoic (DHA) (22:6, n-3), as shown in Table 1.

The total lipid content in the wet tissue of the rear ventral ordinary muscle of farmed bluefin in this study was 0.922 g/100 g, or 3.49 g of saturated fatty acids (SAFA), 4.48 g of monounsaturated fatty acida (MUFA), 2.58 g PUFA n-3, and 0.37 g of PUFA n-6 fatty acids; for wild specimens it was 0.920 g/100 g, or 2.85 g of SAFA, 4.82 g of MUFA, 2.78 g PUFA n-3, and 0.27 g of PUFA n-6 fatty acids (Fig. 1.)

Major fatty acids in this study were 16:0; 16:1, n-7; 18:1, n-9 and DHA 22:6, n-3 acids. The sum of these major components accounted for more than 57 % and 80 % of the total fatty acids in all samples of farmed and wild tuna, respectively. The major SAFA was 16:0 in both groups. Oleic acid was identified as the major MUFA, significantly higher in wild specimens. MUFA accounted for approximately one third of fatty acids in both farmed and wild tuna. With regard to PUFA, bluefin tuna is considered as a good source of the n-3 fatty acids, particularly of DHA, showing the highest levels in wild specimens. DHA occurred in greater proportion than EPA in both farmed and wild tuna.

The sum of both highly unsaturated fatty acids (HUFA), namely DHA and EPA, reached approximately 19 % and 22 % for farmed and wild specimens, respectively. Arachidonic acid was 5-fold higher in wild tuna samples, reaching 1.05 %.

Proximate composition of the farmed and wild bluefin tuna ventral ordinary tail muscle is shown in Table 2. No significant differences were demonstrated between farmed and wild samples, except for energy value, in favor of the farmed tuna.

Plasma GLU, CHOL, TRIG and TP values are presented in Table 3. For tuna under examination, stress was minimized in duration, due to small groups of fish that were selected for sampling at the time period, and rapid handling. However, every confinement in restricted areas and severe exercise is highly stressful for fish, especially for fast swimmers, and in all likelihood is to affect measured plasma parameters. All blood biochemistry parameters, except TRIG failed a Kolmogorov-Smirnov normality test, indicating that the data varied significantly from the pattern expected if drawn from a population with a normal distribution. Normal distribution around the mean, close to 0 (skewness) was drawn for CHOL and TRIG. Therefore, all values for every blood parameter in Table 2. are presented as the mean, median, standard deviation (S.D.), standard error of mean (S.M.), 25th percentile, 75th percentile and range difference of minimum and maximum values. Spearman Rank Order Correlation revealed that GLU tends to increase together with CHOL and TRIG, since for these pairs it showed positive correlation coefficients and $P > 0.05$. Some measured tuna metabolites demonstrated strong mutual correlations, especially GLU, CHOL and TRIG, which are crucial factors in lipid profile of animals.

Discussion

In cultured fish, compared to wild fish, there is a chronic motion insufficiency, which, according to the Japanese market, improves meat characteristics of cultured fish (Nakamura, Ando, Seoka, Kawasaki & Tsukamasa, 2005). Such a tendency seems to appear remarkably within the bluefin tuna, which is a large migratory fish. In this study the sum of major fatty acids amounted to more than 57 % and 80 % of the total fatty acids in all samples of farmed and wild tuna, respectively. A similar relationship of major obtained fatty acids was also reported by Soriguer *et al*. (1997) for wild tuna *Euthynnus alletteratus* captured in the Mediterranean. Oleic acid was identified as the major MUFA in both groups, significantly higher in wild specimens. In farmed sea bass the higher amount of oleic acid was attributed to its dominance in commercial feed (Fuentes, Fernandez-Segovia, Serra & Barat 2010). The most abundant saturated fatty acid (SAFA) in this study was 16:0, which is noted for being a predominant source of potential metabolic energy in fish during growth (Huynh, Kitts, Hu & Trites 2007), and it is also the predominate SAFA in sardine and herring, the main feed source of the farmed tuna in this work (Huynh *et al.* 2007; Zlatanos & Laskaridis 2007).

According to Cejas *et al*. (2004) the fatty acid composition of lipids from fish tissue reflects the fatty acid content of the lipid supplied in the diet, therefore, the fatty acid composition of tissue lipids in farmed fish can differ from that of wild fish. It is believed that the accumulation of fatty acids in bluefin tuna specimens under highly saturated diet correlates with the general fatty acid patterns from the dietary source. The high level of C20:4n-6 in wild tuna samples in this survey, reaching 1.05 %, could be

correlated with the limited feed variations of farmed tuna (herring and sardine) where namely herring has very low C20:4n-6 content. It was assumed (Huynh *et al.* 2007) that wild tuna, just as sea lions and dolphins, must obtain this fatty acid from other dietary sources, or through active biosynthesis pathways for conversion of C18:2n-6 to C20:4n-6. Since seawater fish are largely unable to synthesize DHA by themselves, and they require it as an essential fatty acid, they may selectively accumulate it through predatory feeding. Indeed, wild tuna in this study had higher DHA contents, which correlates with its high levels in one of its principal food source, sardine, where it averages 20.83 % through the year (Zlatanos & Laskaridis 2007). Also, in the muscle of widely migratory fish, such as tuna, DHA contents of muscle are higher than in non-migratory species. This characteristic of high DHA content of muscle is not affected by the maturity (Nakamura *et al*. 2007), and it further explains significantly higher levels in wild as opposed to farmed tuna in this assay.

It was demonstrated (Parisi, Mecatti, Lupi, Giorgi, Michelotti, Galigani & Poli 2007) that fattening of bluefin did not influence flesh color or total lipid content, producing small differences in its chemical composition: greater C18:0, C18:1n9 and PUFA. Such comparison between wild and fattened bluefin in our study could not be generalized, since half of the measured PUFA appeared to be higher in wild fish (Table 1). Nakamura *et al*. (2007) argue that there are no specific tendencies of fatty acid compositions and contents among wild and cultured fish groups; the fatty acid composition of Pacific bluefin tuna *Thunnus orientalis* under their investigation did not reflect those of feed, and they consider that it is difficult to control the fatty acid compositions of cultured tuna muscles by feedstuff, raw fish in particular. Also, no

239 significant difference in tissue essential amino acid patterns was found between adult
240 dolphin fish *Coryphaena hippurus* raised in captivity, and adult fish caught in the wild
241 (Ostrowski & Divakaran 1989). The fatty acid composition in marine fish lipids is
242 conditioned by fish nutritional habits, but also by the possibility of transformation in the
243 nutritional chain between sea organisms. As a result, the composition and content of fatty
244 acids may vary not only from species to species but also to an even greater extent from
245 specimen to specimen of the same species (Varljen *et al*. 2003).

246 Fatty acid mixtures in this study were of a complex nature, and of pertinent
247 interest was determination of EPA and DHA in appreciable amounts in bluefin tuna
248 muscle tissue. Due to their medicinal application these two acids have attracted the
249 attention of numerous dieteticians. Human health is directly influenced by the food
250 properties, inferring not only the nutritive quality of meat, but also aquaculture practice
251 consequences such as levels of stress in animals, feeding regimes, antimicrobial
252 administration, and choices of medication (Barisic, Borzic, Kraljevic, Carev, Zoranic &
253 Kaliterna 2005). It is important to note that tuna in this study were not fed antibiotics and
254 the stress levels in its management were reduced to minimum. EPA in this study was,
255 expectedly, higher in farmed tuna. It was established (Saito, Ishihara & Murase 1996)
256 that the mean DHA content in the muscle of yellowfin tuna *Thunnus albacares* accounted
257 for more than 25 % of the total fatty acids, markedly higher than that in other fish
258 species, or for that matter, in farmed and wild bluefin in our study.

259 Total albacore tuna *Thunus alalunga* omega-3 content (Wheeler & Morissey
260 2003), averaged approximately 40 % with mean omega-3 (g/100 g of tissue) ranging
261 from 2.1 to 3.5, which is comparable with our results (21.70 %, or 2.58 g/100 g) for

262 farmed tuna, and (23.61 % or 2.78 g/100 g) for wild tuna. Interestingly, omega-3 fatty
263 acids in wild albacore tuna off New Zealand waters (Vlieg, Murray & Body 1993)
264 reached only 1.1 g/100g of tissue. Wild tuna mean composition of n-3, n-6, MUFA and
265 SAFA of muscle tissue (Soriguer *et al*. 1997) was shown to be up to 10-fold lower than
266 values of bluefin in our investigation.

267 Traditionally, fish with high fat content were considered to be nutritionally
268 important, since they have a relatively high content on n-3 PUFA. However, it has been
269 demonstrated that there is an inverse relationship between the amount of the n-3 fatty
270 acids and total fat content. The n-3/n-6 ratio for our farmed bluefin samples amounted to
271 6.98, for wild 7.56, which emphasizes the importance of this fish as a significant dietary
272 source of n-3 PUFA, as it is suggested that the dietary intake of fish with high ratio of n-
273 3/n-6 would be beneficial (Okland, Stoknes, Remme, Kjerstad & Synnes 2005).

274 When we compared our findings with West Coast albacore tuna (Wheeler &
275 Morissey 2003), where the lipid content ranged from 3.9 ± 0.2 to 36.3 ± 1.1 %, with a
276 distribution of higher lipid towards the head and lower lipid towards the tail, we found
277 our farmed samples of 12.85 % total fat in the tail region as relatively high, however,
278 expected regarding the culture conditions, as opposed to 11.04 % for wild specimens.
279 Compared to the proximate rear part of the ventral ordinary muscle composition of the
280 full cycle cultured Pacific bluefin tuna (Nakamura *et al*. 2007), our data (Table 3) showed
281 lower protein and moisture content, but significantly higher crude fat content. Farmed
282 fish show higher fat and lower moisture than wild specimens, due to high dietary fat level
283 in the feed and reduced activity (Periago, Ayala, Lopez-Albors, Abdel, Martinez, Garcia-
284 Alcazar, Ros & Gil 2005) as confirmed in this study. The Standard Tables of Food

285 Composition in Japan describe proximate composition of the ventral ordinary muscle of
286 bluefin tuna in which moisture, protein and fat contents are 51.4%, 20.1%, and 27.5%,
287 respectively (Kagawa 2001). The protein content is important when considering quality
288 and texture of the fish muscle. Fish muscles that contain small amounts of protein tend to
289 loose much water upon cooking, which ruins the texture of the meat (Okland *et al*. 2005).
290 Our samples demonstrated comparatively high protein content. According to the Food
291 Composition Database in Sugiyama University (Web: http://database.food.sugiyama-
292 u.ac.jp/index_asia.php), raw lean meat of bluefin tuna energy value is 523 kJ, its SAFA,
293 MUFA, PUFA and cholesterol values are listed as 0.25 g, 0.30 g, 0.19 g and 50 mg,
294 respectively, while our results for captive and wild bluefin tuna demonstrate higher both
295 energy and fatty acids values.
296 Normal plasma cholesterol values in most marine fish range from 2.22 to 23.8
297 $mmolL^{-1}$, which is two to six times higher than that of mammalian plasma (Lee, Lee &
298 Kim 2003); mean tuna CHOL level in this assay maintained analogous on a lower range
299 of that scale (4.08 $mmolL^{-1}$), much lower than CHOL values of fattened Atlantic bluefin
300 in Turkey, 6.57 $mmolL^{-1}$ (Percin & Konyalioglu, 2008). GLU values in this study were
301 lower than values of similarly farmed bluefin (11.4 $mmolL^{-1}$) (Percin & Konyalioglu,
302 2008). It is known that excess energy reserves (as GLU, CHOL, TRIG) are required by
303 organism to mediate the effects of stress and to serve as energy buffers. Ji, Takaoka,
304 Kbiswas, Seoka, Ozaki, Kohbara, Ukawa, Shimeno, Hosokawa & Takii (2008) suspect
305 that the imbalance of protein and lipids in diet of Pacific bluefin tuna provides slightly
306 lower growth performance, higher carcass lipid content and higher plasma CHOL levels.
307 Increased concentrations of TP in fish can be caused by structural liver alterations, and

308 can occur from the impaired control of fluid balance that accompanies strenuous exercise,
309 while decreased concentrations may occur due to a failure of protein synthesis, for
310 example starvation (Bernet, Schmidt, Wahli & Burkhardt-Holm 2001). TP in this assay
311 was 54.66 gL^{-1}, while in captured yellowfin and skipjack tuna it maintained at 62 and 58
312 gL^{-1}, respectively (Wells, McIntyre, Morgan & Davie 1986). Interestingly, farmed
313 bluefin TP values (Percin & Konyalioglu, 2008) amounted to 61.6 gL^{-1}. Bonnethead
314 shark *Sphyrna tiburo*, in comparison, had TP values of 29 gL^{-1} (Harms, Ross & Segars
315 2002). In general, animals fed of diets with elevated levels of fat and carbohydrates show
316 a significant increase in TRIG levels in body tissues and plasma. The rise in TRIG could
317 also be an indicator of altered fat metabolism in the liver. TRIG levels in sea bass
318 cultured in the Adriatic Sea were markedly higher than in wild sea bass from the same
319 region (Coz-Rakovac, Strunjak-Perovic, Hacmanjek, Topic Popovic, Lipej & Sostaric
320 2005), while for farmed tuna in this assay they maintained at 4.88 mmol/l. Farmed
321 bluefin in Turkey (Percin & Konyalioglu, 2008) had higher TRIG values than compared
322 to our measurements (6.57 $mmolL^{-1}$). Not surprisingly, the same authors proved that
323 GLU, CHOL, TRIG and TP parameters were all elevated in farmed, when compared with
324 wild bluefin. Interestingly, research on fat cod *Hexagrammos otakii* (Lee & Cho 2009)
325 showed that TP, GLU and CHOL contents were not affected by dietary fatty acids
326 composition. It was demonstrated however (Rehulka & Parova 2000), that chemical
327 composition (esp. lipid and protein content) of the diet impacts metabolic blood response
328 and lipid profiles since fish with higher levels of TP, CHOL, GLU and TRIG were fed
329 with feed with higher dietary lipids and proteins, while well-balanced feed correlates
330 haematological and biochemical parameters with adequate growth potential and feed

conversion rates. It is therefore reasonable to conclude that farmed tuna in this assay fed with sardine and herring (8.5 and 12.5 % total lipids; 13.0 and 18.1 % total proteins, respectively (Marti da Castro *et al*. 1997; Geirsdottir *et al*. 2007; Huynh *et al*. 2007; Zlatanos & Laskaridis 2007)) had favourable blood analytes levels, when compared to similarly farmed bluefin in the Eastern Mediterranean (Percin & Konyalioglu, 2008), indicating to a balance of plasma biochemical parameters and fatty acid composition of its rear ventral ordinary muscle. Measuring blood biochemical parameters showed an excellent indicator to the, along with the general condition of the organisms, overall metabolic equilibrium in bluefin tuna farmed for human consumption, which in proximate composition differs from wild specimens only in energy value, while significant differences are observable in their fatty acid profiles.

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507

508 Figure 1.

509

510 Comparison of fatty acid composition between wild and farmed bluefin tuna ventral

511 ordinary tail muscle. Values are means ± standard deviation.

512

513 Table 1.

514

515 Fatty acid composition of the rear ventral ordinary muscle of bluefin tuna (*Thunnus*
516 *thynnus*). Values are weight percentage of total fatty acids. Values are means of three
517 determinations.

518

	Farmed tuna	Wild tuna
14:0	8.08	2.92
16:0	18.17	16.21
16:1	12.79	3.98
16:2	5.44	0.58
16:4	1.36	0.68
18:0	2.97	4.95
18:1	15.86	25.85
18:2n-6	2.14	1.25
18:3n-3	0.44	0.86
18:4n-3	2.03	1.54
20:0	0.29	trace
20:1n-7	4.00	4.29
20:2	0.71	trace
20:4n-6	0.18	1.05
20:4n-3	0.22	0.87

20:5n-3	8.66	6.51
22:1n-7	5.15	3.51
22:5n-6	0.80	1.74
22:6n-3	10.44	16.24

trace = < 0.1 %

519

520

521 Table 2.

522

523 Proximate composition of the bluefin tuna ventral ordinary tail muscle.

524

	Farmed tuna	Wild tuna
Total lipid	12.85 ± 0.90	11.04 ± 0.78
Moisture	61.03 ± 0.63	63.28 ± 0.92
Protein	21.09 ± 0.37	20.96 ± 0.45
Energy value	862.02 ± 37.30	789.00 ± 21.42

525

526

527 Table 3.

528

529 Descriptive statistics of glucose (GLU), cholesterol (CHOL), triglyceride (TRIG) and

530 total proteins (TP) plasma parameters of 65 farmed bluefin tuna.

531

Blood parameter	Mean	Median	S.D.	S.M.	Rng. Diff.	25%	75%
GLU ($mmolL^{-1}$)	6.90	6.10	5.27	0.65	38.90	4.90	7.83
CHOL ($mmolL^{-1}$)	4.08	4.32	1.37	0.17	5.82	3.06	5.16
TRIG ($mmolL^{-1}$)	4.88	5.11	1.77	0.22	7.91	3.71	6.23
TP (gL^{-1})	54.66	60.00	16.20	2.03	66.00	40.50	66.50

532