Seasonal changes in the fillet fatty acid profile and nutritional characteristics of wild Trasimeno Lake goldfish (Carassius auratus L.)

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abstract

Goldfish (*Carassius auratus* L.) is very diffuse in Trasimeno Lake, and show great adaptability and have become a dominant fish of the lake ecosystem. The aim of this study was to evaluate the seasonal effect on the fatty acid profile and nutritional characteristics of goldfish caught in Trasimeno Lake. Forty fillets per season were used to evaluate the respective proximate composition, fatty acid profile, nutritional indices and protein and lipid oxidative stability. Season significantly affected the chemical traits of the fillets. A lower level of SFA was observed during spring, while a higher proportion of MUFA was observed during the winter. PUFA exceeded 50% of the total fatty acids content during the autumn, spring and summer. The nutritional indices were very good in comparison to those observed in other livestock animals. Goldfish fillets are characterised by a high nutritional value and good oxidative stability; the best results of this study were obtained in autumn and spring fillets.

Introduction

Trasimeno is one of the largest lakes of the Italian peninsula, with a surface area of 128 km^2; the water level significantly fluctu- ates due to rainfall and seasonal demands from the towns, villages and farms that dot the shore. Trasimeno has always been quite shallow, muddy and very rich in fishes (pike, carp and tench).

Goldfish (*Carassius auratus* L.) is an allochthonous species very diffuse at present in the Trasimeno, showing great adaptability. They have become the dominant fish of the lake ecosystem (Lorenz- oni, Corboli, Ghetti, Pedicillo, & Carosi, 2007; Mearelli, Lorenzoni, & Mantilacci, 1990). The high invasive capacity of this species is due to its tolerance to extreme environmental conditions (low temper- ature, high rate of pollution, cloudy water with a low percentage of oxygen), its high fertility and wide alimentary regimen. The tem- perature tolerance is the most important factor for success of gold- fish; in fact it can survive from 0.3 to 43.6 °C (Ford & Beitinger, 2005). This species lives in shallow ponds, lakes rich in vegetation and slow-moving rivers. During the dry season or winter it burrows in mud (Ford & Beitinger, 2005). It feeds on plants, insect larvae and plankton. For these reasons, goldfish, as well as all the allochtho- nous species, alter the equilibrium of the fish community of the Trasimeno Lake, to the detriment mainly of carp and tench.

When caught for food uses, the greatest economic disadvantage of this fish is the large number of fine inter-muscular bones the species possesses, which makes it laborious to eat for the unaccus- tomed. Thus, this meat is used as food only after undergoing

processing that improves the economic value of goldfish. Therefore, it is very unlikely that goldfish production could increase and become important in the international market. On the other hand, there are some advantages of fishing Trasimeno goldfish; in fact, this species is dangerous to the Trasimeno Lake ecosystem, making its capture a solution to reduce its number, especially in springtime before the reproductive phase. Moreover, this solution could increase the income of local fishermen. So, knowledge of this spe- cies, also present in other Italian natural freshwater environments, would be valuable.

A previous study (Dal Bosco, Mourvaki, Mugnai, & Castellini, 2010) found that goldfish meat, processed into croquettes, can be considered a good source of n-3 polyunsaturated fatty acid (PUFA), providing approximately 78.6 mg of Eicosapentaenoic fatty acid (C20:5n-3, EPA) and 137 mg of docosahexaenoic fatty acid (C22:6n-3, DHA) per 100 g of consumed product.

An important issue regarding captured goldfish on a semi-com- mercial scale is the homogeneity of the chemical composition of fish meat. Indeed, the chemical composition of other fish species varies from season to season due to their natural cycling, matura- tion and feeding. Thus, the aim of this study was to evaluate the seasonal effect on the fatty acid profile and nutritional characteris- tics of goldfish caught in Trasimeno Lake.

1. Materials and methods

1.1. *General*

This study was carried out in collaboration with the Fisherman Cooperative ''Albatrasimeno'' of S. Feliciano (Perugia, Italy), utilizing 20 fished goldfish per season (February, April, July and October, 2010), which, after being caught, were transferred to the laboratory of the cooperative.

The average water temperature and the amount of oxygen dis- solved in the above-mentioned periods were, respectively: Febru- ary 9.5 °C, 13.8 mg/L; April 12.6 °C, 10.5 mg/L; July 23.4 °C,
8.9 mg/L; October 18.9 °C, 9.6 mg/L.

1.2. *Fillets and samples preparation*

After washing with running water, goldfish with an average live weight of 550 g were gutted; the tails and heads were then removed. The epaxial and ipaxial parts of the fillets were dis- sected and both utilised for the preparation of 40 individual samples (mean weight of 100 ± 13 g), for analysis for each season.

The samples were immediately transferred to the laboratory of the Department of Applied Biology of the University of Perugia for analysis.

1.3. *Chemical composition*

Analyses were immediately carried out in duplicate to deter- mine the proximate composition. Moisture, ash and total nitrogen were obtained using AOAC (1995) methods (N. 950.46B, 920.153 and 928.08, respectively). The total protein content was calculated using Kjeldahl nitrogen and a conversion factor of 6.25. The total lipid content was extracted (Folch, Lees, & Sloane-Stanley, 1957) from 5 g of each homogenised sample and determined gravimetrically.

1.4. *Fatty acid profile and desaturase activity*

Fatty acids contents were determined by gas chromatography after lipid extraction according to the Folch et al. (1957) method. One millilitre of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatised by adding 3 ml of sulphuric acid (3% in methanol). After incubating at 80 °C for 1 h, methyl esters were extracted with petroleum ether, and 1 μl was injected into the gas chromatograph (Fisions Mega 2 Carlo Erba Gas Chromatograph, model HRGC, Milan, Italy) equipped with a flame ionisation detector.

The separation of fatty acid methyl esters (FAME) was carried out on an Agilent (J&W) capillary column (30 m × 0.25 mm I.D, CPS Analitica, Milan, Italy) coated with a DB-Wax stationary phase (film thickness of 0.25 μm). The operating conditions of the column injection were as follows: the temperatures of the injector and detector were 270 and 280 °C, respectively; the detector gas flows were H_2 50 ml/min and air 100 ml/min. The oven temperature was programmed to give good peak separation; the initial temperature was set at 130 °C and it was then in- creased at a rate of 4.0 °C/min until reaching a temperature of 180 °C, which was held for 5 min; the temperature was subse- quently increased at a rate of 5.0 °C/min until it reached 230 °C, which was held for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 ml/min. Individual fatty acid methyl esters were identified with reference to the retention time of tri- decanoic acid (C13:0) methyl ester, added before extraction as an internal standard. The relative proportion of individual fatty acids was expressed as a percentage.

To evaluate the activity of both Δ^5-*desaturase* and Δ^6-*desaturase*, the enzymes catalysing the formation of long-chain n-6 and n-3 PUFA, starting from the precursors C18:2n-6 and C18:3n-3, the following equation was calculated, as suggested by Sirri, Castellini, Roncarati, Franchini, and Meluzzi (2010):

$$\Delta^5 \text{ — desaturase plus } \Delta^6 \text{ — desaturase}$$
$$= [C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3 / C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3] \times 100$$

1.5. *Nutritional characteristics*

The mean value of each fatty acid was used to calculate the sum of the saturated

(SFA), monounsaturated (MUFA) and PUFA, and calculate the peroxidability index (PI) according to the equation proposed by Arakawa and Sagai (1986):

PI ¼ ð%monoenoic × 0:025Þþ ð%dienoic × 1Þþ ð%trienoic × 2Þ þ ð%tetraenoic × 4Þþ ð%pentaenoic × 6Þþ ð%hexaenoic × 8Þ:

The concentration of each fatty acid (mg/100 g of fish) was ob- tained from the lipid content of the fish, using the conversion fac- tor 0.91 according to Johansson, Kiessling, Kiesling, and Berglund (2000).

The amount of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991), and the hypocholesterolaemic/ hypercholesterolaemic ratio (HH), as suggested by Santos-Silva, Bessa, and Santos-Silva (2002):

- $AI = (C12:0 + 4 \times C14:0 + C16:0)/[(\sum MUFA + \sum (n\text{-}6) + \sum (n\text{-}3)];$
- $TI = (C14:0 + C16:0 + C18:0)/[(0.5 \times \sum MUFA + 0.5 \times (n\text{-}6) + 3 \times (n\text{-}3) + (n\text{-}3)/(n\text{-}6)];$

$$HH ¼ ½ðC18 : 1n — 9 þ C18 : 2n — 6 þ C20 : 4n — 6 þ C18 : 3n — 3 þ C20 : 5n — 3 þ C22 : 5n — 3 þ C22 : 6n — 3Þ=ðC14 : 0 \quad þ C16 : 0Þ]$$

1.6. *Assessment of oxidative stability*

The extent of lipid oxidation was evaluated in fillets as thiobar- bituric acid-reactive substances (TBARs), according to the modified method of Ke, Ackman, Linke, and Nash (1977). Oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/ kg muscle).

The amount of products arising from oxidative damage to pro- teins (protein carbonyl groups (PCGs)) were measured spectropho- tometrically by reacting with 2,4-dinitrophenylhydrazine (DNPH) according to the method proposed by Lushchak et al. (2005). In detail, samples were homogenised (1:10 w/v) using a Potter– Elvehjem glass homogeniser in 50 mM potassium phosphate buf- fer, pH 7.0, containing 0.5 mM EDTA. A few crystals of phenylmeth- ylsulphonyl fluoride were added prior to homogenisation to inhibit proteases. A 250 ll aliquot of this homogenate was then mixed with 0.5 ml of 10% (final concentration) trichloroacetic acid and centrifuged for 5 min at 13,000*g*. The resulting pellets were mixed with 1 ml of 10 mM DNPH in 2 M HCl and incubated for 1 h at room temperature. Control samples were mixed with 1 ml of 2 M HCl. After washing three times with 1 ml of ethanol–butylacetate (1:1 v/v), pellets were dissolved in 1.5 ml of urea (6 M), and PCGs were measured using a spectrophotometer (Hitachi U-2000) set at 370 nm, using a molar extinction coefficient of 22×10^3 M/cm. The values are expressed as nanomoles of PCGs per milligramme of protein in the urea solution. The protein concentration was mea- sured by the Bradford (1976) method with Coomassie Brilliant Blue G-250, using bovine serum albumin as a standard.

The concentration of thiol groups (SH) was spectrophotometri- cally measured at

412 nm (molar extinction coefficient of 13.6 × 10^3 M/cm) in supernatants after reaction with 5,5′-dithio-bis (2- nitrobenzoic acid), as described by Lushchak and Bagnyukova (2006). Sample homogenates were prepared as described for the PCG assay, and supernatants were obtained after centrifugation at 4 °C for 15 min at 15,000*g*. The thiol levels are expressed as micromoles of SH-groups per gramme.

1.7. *Statistical analysis*

The qualitative traits were analysed with a linear model (STATA, 2005) and a fixed effect of season. Least squares means and planned comparisons were used for mean separation when the model was significant ($P < 0.05$).

2. Results and discussion

The chemical characteristics of goldfish captured during differ- ent seasons are listed in Table 1. In general, the fillets of Trasimeno Lake goldfish showed protein contents similar to those observed by Hossain and Jauncey (1989) and Takeuchi (1979) in fillets of carp (*Cyprinus carp*). The season significantly affected the chemical traits of the fillets; in particular, the moisture level was lowest during autumn and did not exhibit significant differences during the other seasons. A higher level of protein was observed during autumn, whereas the lipid contents were lower during winter and spring.

The fatty acid profiles of fillets during the analysed period (Table 2) generally showed some important peculiarities unique to this species. As observed in our previous investigation (Dal Bosco et al., 2010), the major fatty acids in goldfish fillet were palmitic (16:0), oleic (C18:1n-9), linoleic (C18:2n-6), arachidoneic (C20:4n-6), EPA and DHA, according to the results of Vornanen, Tiitu, Kkel, and Aho (1999) for crucian carp (*Carassius carassius*). Independent of the season, these results are very interesting when compared with those observed for other species, such as the much more economically relevant sea bass (*Dicentrarchus labrax*) and gilt- head seabream (*Sparus aurata*), whose levels of EPA and DHA reach 10.9% and 10.9% (EPA) and 10.7% and 14.0% (DHA), respectively (Turchetto et al., 1994). Also, a comparison between the two fish species present in the lakes of central Italy, coregon (*Coregonus lav- aretus*) and perch (*Perca fluviatilis* L.) (PRAL, 1999–2001), confirm the good nutritional properties of goldfish meat. Poli et al. (1994) analysed the fatty acid profiles of this last species caught from Trasimeno Lake and found values of PUFA equal to 33.3%, with 9.78% of EPA and 19.9% DHA.

A lower level of SFA was observed during spring, while a higher proportion of MUFA was observed during winter. PUFA exceeded 50% of the total fatty acid content in autumn, spring and summer, with very good levels of EPA (6.8%, 6.5% and 8.1%) and DHA (14.6%, 13.3% and 14.5%, respectively). Accordingly, the activities of Δ^5-desaturase and Δ^6-desaturase were significantly lower during winter, intermediate during spring and higher during autumn and summer.

The seasonal differences in the chemical composition and fatty acid profiles can be ascribed to different factors, such as the avail- ability of aquatic plants, water

temperature and the physiological phases of fish (Haard, 1992). With respect to the first factor, it is useful to remember that the Trasimeno Lake ecosystem is charac- terised by a winter scarcity of vegetal species, followed by a quick spring resurgence; subsequently, a slow decline takes place, which culminates in the summer. Afterwards, an autumnal recycling occurs but generally does not reach the spring peak. Although Trasimeno Lake has a laminar structure, there are important changes in water temperature and dissolved oxygen that occur throughout the year: the temperature varies from 8 to 9 °C in December and January to 26–27 °C in August (Mearelli et al., 1990). Finally, from a biological point of view, Lorenzoni et al. (2007) reported that, in winter, goldfish reduce their basal metabolism and growth by re-using body resources in order to survive such that their condition worsens until February. At the beginning of spring, their overall body condition starts to improve, due to the weight gain attributed to the development of the gonads but, as the season progresses, there is a new decline that occurs due to spawning and then the emptying of the gonads. The somatic condition, as opposed to the overall body condition, continues to decline during spring, when the fish faces the maximum reproduc- tive effort. The spring weight loss is not only due to the emptying of the gonads but also to the loss of muscle mass and tissue reserves, resulting in significant waste energy attributed to repro- duction. During the summer, the increased availability of food and Mendez and Gonzales (1997) also observed a decrease in the lipid content of fillets from southwest Atlantic hake during favourable climatic conditions allow for the rapid growth and accumulation of fat reserves. In this way, goldfish reach an optimal body condition during the autumn season. Then, with respect to physiological status, it is possible to observe two opposite condi tions, ranging from a semi-hibernation state during winter to intense swimming activity in spring/summer.
spawning months. During these periods, the protein and lipid con- tents decreased due to the use of muscle lipids and proteins as en- ergy reserves. In autumn, the protein and lipid contents increased and, during winter, when the hake were not feeding, the lipid con- tent decreased, indicating the use of lipids as fuel during food shortage.

Tocher et al. (2004) and Ninno, De Torrengo, Costuma, and Brenner (1974) pointed out, in rainbow trout (*Oncorynchus miykiss*) and *Pimelodus maculatus*, respectively, the important role of water temperature in the regulation of the desaturation/elongation pro- cesses during b-oxidation in epatocytes and enterocytes: the activ- ities of D^6 and D^5 *desaturase* increased many times when the temperature ranged from 30 to 16 °C. The results regarding gold- fish seem to contrast with this affirmation, probably because, as has already been mentioned, this is a fish species that has a very slow metabolism during winter, as it exhibits a lower value of D^5 plus D^6 *desaturase* activity.

In our study, the good fatty acid profile exhibited during spring, summer and autumn is justified by the great availability of vegeta- tion and thus by a higher probable antioxidant intake.

Table 3 shows the seasonal changes of the meat nutritional indices. As expected, due to the highest lipid content, the SFA, sum of n-3, EPA and DHA were highest during the autumn and summer; content of PUFA was highest in the autumn.

The AI and TI showed very low values in comparison to those observed in other livestock animals. In particular, AI and TI were both lower than those reported by Dal Bosco, Castellini, Bianchi, and Mugnai (2004) for rabbit meat (0.70 and 0.99), Dal Bosco, Castellini, and Cardinali (2005) for pigeon meat (0.41 and 0.94) and Castellini, Dal Bosco, Mugnai, and Pedrazzoli (2005) for poultry meat (0.49 and 0.88). The best AI was found during autumn and spring.

The HH ratio observed in autumn and winter was lower than that found by Testi, Bonaldo, Gatta, and Badiani (2006) in the dor- sal (2.93 *vs*. 2.40) and ventral rainbow trout fillets (2.93 *vs*. 2.46). These data confirm the optimal nutritional quality of goldfish fillets (Dal Bosco et al., 2010) as well as the seasonal affect on the studied traits.

Kminkova, Winterova, and Kucera (1993) found similar values of EPA and DHA contents in pond-reared carp muscle only during the summer (149 and 214 mg/100 g tissue, respectively); during the other seasons, the values were very low, probably due to the diets based on wheat, rye, barley and pea. For the wild carp of Ivriz Dam Lake (Turkey), Kalyoncu, Yaman, and Aktumsek (2010) obtained results similar to ours; in particular, these authors found higher values of EPA and DHA and lower of LA in fillets during au- tumn. In fillets of the wild-caught carp of another Turkish lake, Guler, Kiztanir, Aktumsek, Citil, and Ozparlak (2008) found very high values of EPA and DHA during summer.

Vujkovic, Karlovic, Vujkovic, Vörösbaranyic, and Jovanovic (1999) found EPA and DHA contents equal to 150 and 89 mg/ 100 g fillet for silver carp (*Hypophthalmichtys molitrix* Val.) and big- head carp (*Aristichthys nobilis* Val.) harvested in the Despotovo Fishfarm (Vojvodina, Yugoslavia).

From a nutritional point of view, the EPA + DHA content is of great interest because of the role of these fatty acids in the therapy and prevention of cardiovascular diseases (Uauy & Valenzuela, 1992). The mean values for the EPA + DHA contents were 430, 251, 338 and 426 mg per 100 g fillet tissue, respectively, in autumn, winter, spring and summer (data not shown). This means that, for the recommended daily ingestion of 1 g of EPA + DHA (Ackman, 1989), about 250–300 g of goldfish fillets are needed. Moreover, intakes of EPA and DHA of about 250 mg per day are required to maintain normal cardiac function (EFSA Journal, 2010). The best values for the n-6/n-3 ratio were observed during autumn, spring and summer. A decrease in the n-6/n-3 ratio in the human diet is essential to prevent coronary heart disease by reducing plasma lipids and to reduce the risk of cancer (Kinsella, Lokesh, & Stone, 1990). Nutrition advisers recommend an in- creased intake of n-3 in human diets with a reduction of n-6/n-3 ratio to values below 4 (Kark, Kaufmann, Binka, Goldberger, & Berry, 2003). In the present trial, the n-6/n-3 ratio values were always much below 4.

Regarding the oxidative status, TBARs, carbonyl and thiol con- tents were higher during the summer. These results reflect the highest amounts of long-chain n-3 fatty acids. Considering the high degree of unsaturation found during all seasons and the low values of TBARs and PI, the oxidative status was good. This is evident if compared with those found, in our previous study, on rainbow trout (*Oncorhynchus*

mykiss) reared in Valnerina (Italy), where val- ues of TBARs of 0.79 mg of MDA per kg of fresh fillet and a PI of 239, were found. The situation related in the present study could be due to the good capture technique, to the high ingestion of antioxidants during spring and autumn and semi-hibernation during winter.

Some authors (Lushchak & Bagnyukova, 2006; Lushchak et al., 2005), have demonstrated that Goldfish tissues possess high con- stitutive activities of antioxidant enzymes which might be enough to cope with short-term disturbance of free radical processes.

Finally, the trend found in TBARs, carbonyls and thiols suggests possible relationships among these oxidative stress markers Hence, it is likely that the production of MDA during lipid oxidation might trigger the protein carbonylation. In nature, a gradual increase in some antioxidant enzyme activ ities during the transition from cold winter conditions to warmer summer conditions would reflect the needs of an organism to cope with elevated oxidative stress that accompanies enhanced oxygen consumption and metabolic rate at higher environmental temper atures. This hypothesis might be even more probable if based on the fact that goldfish are a wild species with a more accentuated muscular oxidative metabolism (Lushchak & Bagnyukova, 2006). In conclusion, this study reveals that, during different the environment and physiological conditions change, and cause seasons, caught during these periods should increase. This should be advan- tageous for both healthy characteristics of fillets obtained from fish and for re-equilibration of the fish ecosystem of Trasimeno Lake.

The characterisation and valorisation of this product could represent a good economic opportunity for local fishermen.

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Table 1
Chemical composition of goldfish fillets in different seasons.

		Season				SEM
		Autumn	Winter	Spring	Summer	
Moisture	%	77.54[a]	78.96[b]	78.92[b]	78.17[a,b]	1.25
Protein	%	18.7[b]	17.5[a]	17.4[a]	17.9[a]	0.69
Lipids	%	2.01[b]	1.59[a]	1.70[a]	1.89[b]	0.10
Ash	%	1.80	1.95	2.01	2.03	0.48

$N = 20$ per season.
[a,b] $P < 0.05$.

Table 2
Fatty acid profile of goldfish fillets in different seasons.

		Season				SEM
		Autumn	Winter	Spring	Summer	
C14:0	%	1.14[a]	2.58[b]	2.17[b]	3.17[c]	0.25
C16:0	%	22.1[b]	21.8[b]	15.2[a]	17.1[a]	2.47
C18:0	%	7.19[b]	4.80[a]	6.74[a,b]	7.70[b]	0.98
Others	%	2.81[a]	3.49[a,b]	4.60[c]	3.61[b]	1.01
SFA	%	33.2[b]	32.6[b]	29.7[a]	33.6[b]	2.08
C14:1n-6	%	0.11	0.32	0.13	0.11	0.22
C16:1n-7	%	2.54[a]	4.86[b]	3.56[a,b]	4.47[b]	1.47
C18:1n-9	%	9.59	11.0	11.3	10.4	2.56
Others	%	4.14	4.76	3.38	3.82	1.45
MUFA	%	16.4[a]	20.9[b]	18.3[a,b]	18.8[a,b]	3.01
C18:2n-6 *LA*	%	13.8[a,b]	17.1[c]	15.6[b]	12.5[a]	1.43
C20:2n-6	%	0.20	0.19	0.18	0.26	0.35
C20:3n-6	%	0.12	0.28	0.20	0.46	0.35
C20:4n-6	%	8.05[b]	6.78[b]	5.91[a]	5.60[a]	2.41
C18:3n-3 *LNA*	%	3.13a	2.61[a]	4.67[b]	5.31[b]	0.99
C20:3n-3	%	0.27	0.42	0.21	0.23	0.26
C20:5n-3 *EPA*	%	6.81[a,b]	5.15[a]	6.54[a,b]	8.09[b]	1.11
C21:5n-3	%	0.48[a]	1.11[b]	0.10[a]	0.11[a]	0.08
C22:5n-3	%	2.41[a,b]	1.84[a]	3.44[b]	2.41[a,b]	0.47
C22:6n-3 *DHA*	%	14.6[b]	10.6[a]	13.3[b]	14.5[b]	2.08
Others	%	0.61	0.34	0.80	0.14	0.64
PUFA	%	50.4[b]	46.5[a]	51.9[c]	50.0[b]	4.52
Δ^5 plus Δ^{6*}		65.5[c]	55.5[a]	58.1[b]	63.4[c]	2.15

$N = 20$ per season.
[a–c] $P < 0.05$.
[*] Desaturase index.

Table 3
Quantitative fatty acid contents, nutritional indices and oxidative status of goldfish fillets in different seasons.

		Season				SEM
		Autumn	Winter	Spring	Summer	
SFA	mg/100 g	668[b]	519[a]	506[a]	635[b]	50.4
MUFA	mg/100 g	329	333	312	357	51.1
∑ n-3	mg/100 g	543[b,c]	345[a]	498[b]	578[c]	42.4
∑ n-6	mg/100 g	443	391	371	353	82.4
EPA	mg/100 g	137[b]	81.9[a]	111[a]	153[b]	14.5
DHA	mg/100 g	293[c]	169[a]	226[b]	273[c]	29.4
PUFA	mg/100 g	1014[c]	739[a]	883[b]	937[b]	65.3
n-6/n-3		0.81[a]	1.13[b]	0.74[a]	0.68[a]	0.22
TBARs	mgMDA/kg	0.20[b]	0.11[a]	0.18[b]	0.27[c]	0.11
Carbonyls	nmol/mg proteins	3.13[b]	1.43[a]	4.22[b]	6.23[c]	1.21
Thiols	µg/g	15.9[a]	16.7[a]	15.8[a]	18.4[b]	2.45
Peroxidability index		228[b]	185[a]	220[b]	227[b]	15.9
Atherogenicity index		0.41[a,b]	0.48[b]	0.35[a]	0.44[b]	0.10
Thrombogenicity index		0.30[b]	0.32[b]	0.22[a]	0.24[a]	0.08
HH*		1.78[a]	2.19[a]	3.35[b]	2.78[a,b]	0.49

$N = 20$ per season.
[a–c] $P < 0.05$
* Hypocholesterolaemic/hypercholesterolaemic ratio.