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FILLET FATTY ACID COMPOSITION, ESTIMATED INDEXES OF LIPID METABOLISM AND OXIDATIVE STATUS OF WILD AND FARMED BROWN TROUT (*SALMO TRUTTA* L.)

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ABSTRACT

The aim of the present study was to compare the fillet fatty acid composition and the oxidative stability of wild and cultivated brown trout (*Salmo trutta* L.). The study was carried out in Valnerina (Perugia, Italy), where 20 two-year-old farmed brown trout (250 ± 15 g) were utilized; another 20 trout were chosen from a sample caught during the same period from the Nera River (Perugia, Italy) based on their weight (± 10% of weight of farmed ones). The fatty acid profile, fatty acid index and oxidative stability of the fish fillets were assessed. The farmed trout had a significantly higher fat (3.16 *vs* 2.80%, $P<0.05$) and energy content than the wild ones, whereas no significant differences were observed with respect to protein and moisture. The linoleic acid content was higher than the linolenic content in farmed trout, while in the wild fish, the inverse situation was observed. The docosahexaenoic and eicosapentaenoic acid contents in farmed trout were higher than those in wild trout. The wild fish showed higher thioesterase and Δ^9-desaturase (18) and lower Δ^5- Δ^6-desaturase activity. Regarding oxidative stability, the wild fish showed a significantly higher development of oxidative processes associated with a lower peroxidability index.

- Keywords: *Salmo trutta*, farmed, wild, fatty acid, oxidative stability -

INTRODUCTION

The total consumption of Brown trout (*Salmo trutta* L.) in Italy amounts to approximately 5,000 tons per year (ISMEA, 2006). In Italian rivers fishing for this species is highly developed and thus there is also a food consumption of wild trout. The demand cannot be satisfied by catching wild fish, essentially because the world fish stocks are limited; thus, the number of aquaculture products is increasing.

Many nutritional and organoleptic characteristics of fish are correlated with the quantity and quality of lipids and are affected by intrinsic (genotype, age, sex, physiological stage) and extrinsic (environment, water temperature, feeding) factors and differences in the lipid composition of various farmed or wild-caught fish species have been described in a number of studies (CAHU *et al.*, 2004). Concerning feeding, the wild brown trout has a typically carnivorous diet and frequently preys on larval and adult insects as well as crustaceans, annelids, and gastropods; as the fish grows, it may also prey on other fish. To our knowledge, only a few studies (BOCCIGNONE *et al.*, 1987; BOCCIGNONE *et al.*, 1988; ZIINO *et al.*, 1991; AKPINAR *et al.*, 2009; BAYIR *et al.*, 2010) have analyzed the fatty acids of different wild *Salmo trutta* sub-species; thus, the fatty acid dynamics of wild fishes is not well known. Accordingly, in this present study, the fatty acid compositions and the oxidative stability of the flesh of wild and farmed brown trout were compared.

MATERIAL AND METHODS

Rearing conditions, diets and fishing conditions

The study was carried out on March 2011 at the Ichthyic Genetic Centre of the Province of Perugia located in Borgo Cerreto in Valnerina (Italy), where brown trout are reared to produce the fish necessary to restock different rivers of Central Italy.

Twenty two-year-old brown trout (approximately 20 cm long and weighing approximately 250 ± 15 g) provided by the Center were utilized, and another 20 were chosen from a sample caught from the Nera River based on their weight (±10% of weight of farmed fish). This river originates in the Sibillini Mountains (Central Italy) and flows for approximately 125 km before joining the Tiber River. The water temperature in the Ichthyic Genetic Centre of the Province of Perugia ranges from 6° to 15°C. The farmed fish fed a commercial diet (Veronesi Verona spa, Italy; 40% of crude protein, 24% lipids, 6.5% Ash, 2% fiber; 7.9% linoleic acid, C18:2n-6 LA, 2.9% linolenic acid, C18:3n-3 ALA and 15.5% total n-3 fatty acid) at approximately 1.5% of their live weight for the last year of life. The main ingredients of the diet were soybean meal, fish meal, fish oil, wheat meal, pea protein concentrate, wheat gluten, minerals, amino acids and antioxidants. After capture, all fish (wild and farmed) were slaughtered in water and ice and immediately transferred (packed into an insulated polystyrene box with ice) to the laboratory of the Department of Applied Biology of Perugia (transport time equal to one hour) for the chemical analyses.

Fillets and samples preparation

After washing with running water, the fish were manually eviscerated, the tails and heads were removed, and eighty fillets (mean weight 65 ± 2 g) were dissected for analysis.

Chemical composition

Analyses were immediately carried out in duplicate to determine the proximate composition. Moisture, ash and total nitrogen contents were determined using AOAC methods (N. 950.46B, 920.153, and 928.08, respectively). The total protein content was calculated using the Kjeldahl nitrogen method and a conversion factor of 6.25. The total lipids content was extracted (FOLCH *et al.*, 1957) from 5 g of each homogenized sample and determined gravimetrically. Energy content of fillets was determined by an adiabatic bomb calorimeter (model IKA Calorimeter C400, Adiabatic 2800, Bremen, Germany).

Fatty acid profile and indexes

The fatty acids content was determined by gas chromatography after lipid extraction according to the method developed by FOLCH *et al.* (1957). One milliliter of lipid extract (from 0.2 to 0.3 mg lipids) was evaporated under a stream of nitrogen, and the residue was derivatized by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80°C for one hour, methyl esters were extracted with petroleum ether, and 1 μL was injected into a gas chromatograph (Fisions Mega 2 Carlo Erba Gas Chromatograph, model HRGC Milan, Italy) equipped with a flame ionization detector.

The separation of fatty acid methyl esters (FAME) was carried out on an Agilent (J W) capillary column (30 m x 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 and 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 to 130°C and then increased at a rate of 4.0°C/min until reaching a temperature of 180°C, which was held for 5 min;

the temperature was subsequently 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 tridecanoic acid (C13:0) methyl ester added before extraction as an internal standard. The concentration of each fatty acid (mg/100 g of fish) was calculated from the lipid content of the fish and a conversion factor of 0.91 according to JOHANSSON *et al.* (2000). The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and PUFA, and several indexes were used to estimate the desaturase and elongase activity of the muscle tissue (VESSBY *et al.*, 2002). The activities of Δ^9-desaturase and elongase were estimated by relating the percentage of product to the percentage of the precursor (OKADA *et al.*, 2005). Specifically, the Δ^9-desaturase index for the C18:1 was calculated as 100 times the ratio of oleic acid (C18:1) to the sum of C18:1 and stearic acid (C18:0). The total Δ^9-desaturase index (both 16 and 18) was calculated as 100 times the ratio of the sum of C16:1 and C18:1 to the sum of C16:1, C16:0, C18:1 and C18:0.

The elongase index was calculated as the ratio of C18:0 to C16:0, whereas the thioesterase index was calculated as the ratio of C16:0 to myristic acid (C14:0) (ZANG *et al.*, 2006).

To evaluate the activity of both Δ^5-desaturase and Δ^6-desaturase, the enzymes catalyzing the formation of long-chain n-6 and n-3 polyunsaturated fatty acids (PUFAs) starting from the precursors C18:2n-6 and C18:3n-3, the following equation was used (SIRRI *et al.*, 2010):

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

The index of nutritional quality (INQ) was calculated based on the eicosapentaenoic (EPA) + docosahexaenoic (DHA) acid level using the formula suggested by GODBE (1994) on 100 g of fillets used to calculate a ratio of the achieved percentage of the requirement of the sum of these fatty acids to the percentage of a 2000 kcal intake (TESTI *et al.*, 2006).

Peroxidability index (PI) was calculated according to the equation proposed by ARAKAWA and SAGAI (1986):

$$PI = (\%\ \text{monoenoic} \times 0.025) + (\%\ \text{dienoic} \times 1) + (\%\ \text{trienoic} \times 2) + (\%\ \text{tetraenoic} \times 4) + (\%\ \text{pentaenoic} \times 6) + (\%\ \text{hexaenoic} \times 8)$$

The amount of each fatty acid was used to calculate the indexes of Atherogenicity (AI) and Thrombogenicity (TI), as proposed by ULBRICHT and SOUTHGATE (1991), and the hypocholesterolaemic/hypercholesterolaemic ratio (HH), as suggested by SANTOS-SILVA *et al.* (2002):

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

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

$$HH = [(C18{:}1n\text{-}9 + C18{:}2n\text{-}6 + C20{:}4n\text{-}6 + C18{:}3n\text{-}3 + C20{:}5n\text{-}3 + C22{:}5n\text{-}3 + C22{:}6n\text{-}3)/(C14{:}0 + C16{:}0)]$$

Assessment of oxidative stability

The extent of lipid oxidation was evaluated in fillets as thiobarbituric acid reactive substances (TBARs) by a spectrophotometer (set at 532 nm, Hitachi U-2000, Theodor - Heuss - Anlage 12, Mannheim, F.R. Germany), which measured the absorbance of thio-barbituric acid-reactive substances, and a tetraethoxypropane calibration curve in sodium acetate buffer (pH=3.5), according to the modified method of KE *et al.* (1977). Oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle).

Statistical analysis

The qualitative traits were analyzed using a one-way ANOVA (STATA, 2005) for the comparison of wild *vs* farmed trout. Least squares means and planned comparisons were used for mean separation when the differences were significant ($P < 0.05$).

RESULTS AND DISCUSSION

In Table 1, the proximate composition of the trout fillets of this study is reported. The wild trout had a significantly lower fat than the farmed ones, most likely due to the high dietary fat level in the feed (24%) and lower activity of the latter. Protein and moisture did not show significant differences.

The observed differences between wild and farmed are in agreement with the data obtained by other authors (RENON *et al.*, 1994a; RENON *et al.*, 1994b; REA *et al.*, 2000), who observed a higher fat content in cultivated fish. Within species, genotype, age, sex and feeding (REA *et al.*, 2000) affect the lipids content, with relevant repercussions on the nutritional characteristics of the meat.

Concerning the fatty acid content, palmitic acid (C16:0) was the major SFA, and myristic (C14:0) and stearic (C18:0) acids followed. Oleic acid (C18:1n-9) was the major MUFA, whereas palmitoleic acid (C16:1n-7) was found only in small quantities. Rainbow trout reared in the same habitat show similar levels of MUFA and oleic acid (DAL BOSCO *et al.*, 2007).

Table 1 - Fillet chemical composition (%) and fatty acid profile (mg/100 g fresh fillet) of cultivated and wild *Salmo trutta*.

		Cultivated	Wild	SED
Moisture	%	73.86	73.93	4.84
Protein	"	21.54	21.92	2.41
Lipids	"	3.16^{b}	2.80^{a}	0.46
Energy	Kcal/100 g fresh fillet	133.3	132.0	4.84
Fatty acids				
C14:0		182.3^{B}	85.17^{A}	25.3
C16:0		547.8	569.5	89.5
C18:0		98.3^{a}	133.3^{b}	10.8
Others		44.6	39.5	8.4
SFA		761.5^{a}	862.7^{b}	73.5
C16:1n-7		2.9^{A}	9.9^{B}	4.1
C18:1n-9		294.2^{A}	394.9^{B}	68.8
Others		136.9	118.7	23.1
MUFA		433.9^{A}	555.2^{B}	92.1
C18:2n-6	LA	123.9	118.7	18.9
C20:4n-6		33.9	40.5	8.2
C18:3n-3	ALA	34.8^{A}	179.9^{B}	26.8
C20:5n-3	EPA	310.8^{B}	179.9^{A}	97.3
C21:5n-3		109.2	100.5	26.5
C22:5n-3		104.9^{a}	59.6^{b}	40.1
C22:6n-3	DHA	675.2^{B}	281.0^{A}	185.3
Others		110.5	100.9	21.6
PUFA		1560.6^{B}	1118.8^{A}	256.3

N=20 per group.
Least square means in the same row with different superscript letters are significantly different A..B: P<0.01; a..b: P<0.05.
SED: Standard Error Deviation.
SFA: Saturated Fatty Acids.
MUFA: Monounsaturated Fatty Acids.
PUFA: Polyunsaturated Fatty Acids.

In the PUFA fraction, EPA and DHA were the major fatty acids detected.

Concerning the comparison between wild and reared fish, the former had a lower LA content than ALA content, while in cultivated fish, the inverse situation was observed.

Wild trout showed lower levels of myristic acid (C14:0) and higher levels of stearic acid (C18:0), SFA and MUFA. These results are in accordance with those observed by REA *et al.* (2000) in fillets of farmed and wild sea bream.

The results regarding ALA are in agreement with those of BOCCIGNONE *et al.* (1988), who reported higher values in wild than in farmed brown trout (2.09 *vs* 1.36%; 7.70 *vs* 1.26% in our trial; data not shown).

The absolute contents of DHA and EPA in wild trout were lower than those in farmed trout. DE FRANCESCO *et al.* (2004) found lower values (479 and 182 mg/100 g, respectively) in rainbow trout than in farmed brown trout of our study.

The literature regarding the EPA and DHA content in wild and farmed fishes shows some conflicting results. Many authors, in agreement with our findings, reported lower levels of EPA and DHA in wild fish with respect to farmed ones (BOCCIGNONE *et al.*, 1988; REA *et al.*, 2000; SAGLIK *et al.*, 2003). In contrast, RENON *et al.* (1994a) and RENON *et al.* (1994b) found higher EPA and DHA contents in wild sea bream, as did KRAJNOVIC-OZRETIC *et al.* (1994) and REA *et al.* (2000) in sea bass fillets.

The assessment of standard fatty acid profiles is more difficult for wild fish for larger variations among samples due to size/age, reproductive status, geographic location and season (NETTLETON and EXLER, 1985; ACKMAN, 1989; SAITO *et al.*, 1999; DAL BOSCO *et al.*, 2012).

Concerning the indexes of fatty acid metabolism, wild subjects showed higher thioesterase and Δ^9-desaturase (18) and lower Δ^5-Δ^6-desaturase activities (Table 2), which are mainly due to the feeding regimen.

Also BAYIR *et al.* (2010) affirmed that the fatty compositions of neutral and phospholipids of wild *Salmo trutta caspius*, *Salmo trutta labrax* and *Salmo trutta macrostigma* were strongly affected by many factors, especially reproduction and diet.

FOCHETTI *et al.* (2003), studying the eating habits of brown trout of the Nera River, observed selective feeding behavior, with a negative selection for some species of Ephemeroptera and Diptera and a positive selection for species of Trichoptera. In another study on the same river, FOCHETTI *et al.* (2008) observed that Plecopteran nymphs were more preyed upon by older subjects, while ephemeropteran nymphs were also preyed upon by young fish. Trichopteran larvae were the most abundant prey in trout younger than two years, while their percentage decreased considerably in older fish. The remaining aquatic prey (except Dipteran larvae) was scarce, and terrestrial prey was consumed more by older individuals.

Table 2 - Indexes of lipid metabolism, oxidative status and nutritional traits of in fillets of cultivated and wild *Salmo trutta*.

		Cultivated	Wild	SED
Elongase		0.18	0.23	0.08
Thioesterase		3.00^{A}	6.69^{B}	1.01
Δ^9-desaturase (16+18)		74.95	74.77	3.24
Δ^9-desaturase (18)		0.52^{A}	1.70^{B}	0.42
Δ^5/Δ^6-desaturase		88.71^{b}	70.65^{a}	3.81
INQ		22.82^{b}	10.07^{a}	4.15
H/H		2.16^{b}	1.88^{a}	0.21
n-6/n-3		0.18^{A}	0.27^{B}	0.04
Peroxidability index		301.05^{B}	189.79^{A}	54.19
Atherogenic index		0.72^{b}	0.64^{a}	0.11
Thrombogenic index		0.21	0.30	0.10
TBARs	mg MDA/kg	0.65^{A}	2.06^{B}	0.99

N=20 per group.
Least square means in the same row with different superscript letters are significantly different A..B: P<0.01; a..b: P<0.05.
IQN: Index of Nutritional Quality.
H/H: Hypocholesterolaemic/Hypercholesterolaemic acids ratio.
TBARs: Thio-Barbituric Acid Reactive substances.

Additionally, SALAVATIAN *et al.* (2011) observed in brown trout a diet that consisted mainly of benthic animals, chiefly represented by Oligocheta, Gastropoda, Diptera, Coelifera, Trichoptera, Lepidopetra, Hemiptera, Coleoptera, Odonata, Homoptera and Archnida, and the feeding behavior of these fish was directly related to the abundance of prey in the river.

GHIONI *et al.* (1996), in analyzing the fatty acid composition of neutral lipids and phospholipids of some freshwater insects, observed that in the above-mentioned classes the major fatty acid present was EPA.

Considering that the feeding behavior of young wild trout (< 2 years of age) is based on insects with a high content of n-3 long-chain fatty acids, it is probable that these fish developed a low elongase and desaturase capacity, which could explain the lower DHA content compared to that of farmed trout. Paradoxically, the diet of wild fish, rich in EPA, renders DHA biosynthesis less efficient when compared with the feed administered to the farmed trout, which is partially composed of vegetal components and contain 7.9% of LA and 2.9% of ALA. According to BELL and DICK (2004), to optimize the synthesis of DHA from ALA, in farmed trout, it would be appropriate to avoid supplementation with fish oil, especially at an early age, and the presence of the long-chain n-3 PUFA in fish oil and meal reduces the synthesis of DHA from ALA by approximately 10 fold. The ability to convert LA and ALA, abundant in vegetable oils, into C20 and C22 PUFAs is well established in some species, such as rainbow trout (KANAZAWA *et al.*, 1978; BUZZI *et al.*, 1996; MOURENTE and TOCHER, 1998). These statements are reinforced by the study of TURCHINI and FRANCIS (2009), who observed that when a diet is characterized by high EPA levels, a large proportion of this fatty acid is used for energy production and only a small quantity is further bioconverted in DHA.

The nutritional properties of brown trout fillets are reported in Table 2.

The INQ index of wild trout was significantly lower respect that of the farmed ones (22.82 *vs* 10.07), that considering the equal energy value, indicates a better achievement of dietary recommendations for EPA + DHA for fillets of farmed trout.

Also the HH ratio was significantly lower in wild brown trout, and in general lower than that found by TESTI *et al.* (2006) in rainbow trout fillets and by us (DAL BOSCO *et al.*, 2007). The atherogenic indexes were both higher than those reported by TURCHINI *et al.* (2003) for brown trout (0.45), whereas the thrombogenic index was similar.

The n-6/n-3 ratio of the fillets was 0.27 and 0.18, respectively for wild and farmed trout, similar to the results of another study in brow trout (TURCHINI *et al.*, 2003).

Regarding the differences in n-6/n-3 ratio between wild and farmed fish, the results of this experiment are in disagreement with most of those found in the literature because in this trial, the wild subjects showed higher values than the farmed, contrary to what was observed in other fish specimen by RENON *et al.* (1994a), KRAJNOVIC-OZRETIC *et al.* (1994) and REA *et al.* (2000).

Concerning oxidative stability, wild subjects showed a lower oxidative stability accompanied by a lower PI and this situation could be related to different factors:

- the different stresses that fish have suffered at the time of capture, which were certainly lower in farmed fishes with respect to wild ones because the wild ones had to fight at the moment of being fished (DIGGLES and ERNST, 1997);
- farmed trout fed a diet fortified in antioxidants, specifically, 11,500 IU vit. A, 1.800 IU vitamin D3, 150 mg Vitamin E, 100 mg vit. C per kg;
- some studies (BASTROP *et al.*, 2002; PASCUAL *et al.*, 2003) have reported that dietary restriction can increase oxidative stress in fish; ELLIOTT and HURLEY (1998; 2000) observed that in severe environments, such as streams at high altitude, where temperatures frequently reach below 10°C and feed resources are scarce, brown trout eat with lower frequencies and are satiated with lower quantities of food compared with farmed fish.

CONCLUSIONS

Wild and farmed brown trout analysed in this study differ mainly in their total lipid content and fatty acid profile. These results, to be confirmed by further investigations on a larger number of fish during different seasons, can be explained mainly by considering the availability and composition of feed, particularly regarding the fatty acid profile and antioxidants, which are the main factors affecting the lipid metabolism and consequently the fatty acid profile of fillets and their nutritional properties.

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