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Short communication

Triploidy does not decrease contents of eicosapentaenoic and docosahexaenoic acids in filets of pink salmon *Oncorhynchus gorbuscha*

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ABSTRACT

Triploid fish has become an important item of commercial aquaculture, but data on its fatty acid (FA) composition are still controversial, especially regarding essential polyunsaturated fatty acids, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). We studied FA composition and content of diploid and triploid pink salmon *Oncorhynchus gorbuscha*, reared in aquaculture in a bay of the White Sea (Russia). FA composition, measured as percentages of total FA of triploids and immature diploid females significantly differed from that of mature diploid fish. Specifically, mature diploids had higher percentage of EPA and DHA in their muscle tissue (filets) compared to that of triploids and immature diploid females. Nevertheless, the contents of EPA and DHA per mass of the filets in diploid and triploid specimens were similar. Thus, no special efforts are needed to improve EPA and DHA contents in filets of triploids.

1. Introduction

Polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are known as essential constituents of human nutrition to prevent cardiovascular deceases and neural disorders (De Caterina, 2011; Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006). A number of international and national health organizations recommend consumption of 0.5–1.0 g of EPA + DHA per day for a healthy diet (Adkins & Kelley, 2010). The main source of EPA and DHA for humans is fish (Gladyshev, Sushchik, & Makhutova, 2013; Robert, 2006).

Aquaculture is an important source of high nutritive fish. In the last decades in commercial aquaculture, production of triploid fish, obtained by a heat shock or pressure, has increased (Flajshans et al., 2010; Ozorio, Escorcio, Bessa, Ramos, & Goncalves, 2012). It is worth to emphasize that triploid organisms are found naturally in wild populations and according to the regulations across the European Union, fish produced by induction of triploidy are not considered as genetically modified organisms (Flajshans et al., 2010). Triploid fish are sterile and at adult stages exhibit better growth than their diploid counterparts, because the sterility prevents energy loss for gamete production and negative effects of sexual maturation for meat quality (Flajshans et al., 2010; Ozorio et al., 2012). Moreover, the use of sterile triploids in aquaculture can reduce the risks of propagation of non-native escapees into the wild (Ozorio et al., 2012; Ribeiro et al., 2012).

Nevertheless, in spite of the better fillet quality of triploids because of the prevention of mobilization of stored lipids for gonad development, they were reported to have a lesser sum of PUFA in the flesh compared to diploid individuals (Flajshans et al., 2010; Manor, Weber, Cleveland, & Kenney, 2014; Manor et al., 2012; Ozorio et al., 2012; Ribeiro et al., 2012). Therefore, it was suggested to improve PUFA content in muscle of triploids by dietary supplementation of PUFA (Ribeiro et al., 2012).

All the above data on less PUFA in triploids were based on measurements of their percentages in total fatty acids (Flajshans et al., 2010; Manor et al., 2012, 2014; Ozorio et al., 2012; Ribeiro et al., 2012). Meanwhile, for estimation of nutritive value for humans, measurements of PUFA per mass of consumed filets, rather than their percentage in total fatty acids should be carried out (Gladyshev, Sushchik, Gubanenko, Demirchieva, & Kalachova, 2007; Gladyshev et al., 2012; Huynh & Kitts, 2009). Unfortunately,

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no data on PUFA content in mass units in triploid vs diploid fish are available in the existing literature.

Among fish species, salmonids are known to have comparatively high content of EPA and DHA (Gladyshev et al., 2013). In commercial salmonid aquaculture, triploid rainbow trout *Oncorhynchus mykiss* are often used and their fatty acid composition is studied (Manor et al., 2012, 2014; Ozorio et al., 2012; Ribeiro et al., 2012). Study of other salmonid species is also desirable to expand and clarify data on nutritive quality of triploid fish.

Hence, the aim of our study was to compare the fatty acid composition and contents in filets of triploid and diploid pink (humpback) salmon *Oncorhynchus gorbuscha* in aquaculture and estimation of their nutritive value in order to obtain the recommended daily intake of EPA and DHA.

2. Materials and methods

2.1. Experimental fish and rearing conditions

Pink salmon *Oncorhynchus gorbuscha* Walbaum was introduced in the White Sea from the Pacific Ocean in the second half of the 20th century (Petryashov, Chernova, Denisenko, & Sundet, 2002). Studied experimental fishes were obtained from spawning of specimens, caught in the Keret River (West coast of the White Sea, Russia). Triploidy was induced by heat shock, performed after fertilization at 28–30 °C during 10–15 min. Simultaneously, three control portions of the eggs were kept at 16 °C (river water temperature). Fries, obtained from the control and experimental eggs, were reared separately, but under the same conditions in an aquaculture farm. Then the young fish were reared in pools with fresh waters during 1.5 years. Then the fish were transferred in the Chupa Bay (West coast of the White Sea, 66°16′ N, 33°03′ E) and reared during 6 months (from May to October 2015) in cages of 12 m^3; installed in inshore of the sea bay with stocking density ~250 individuals per 1 m^3. Water temperature in the bay varied in May–October from 3.1 to 13.8 °C, salinity ranged from 16.8 to 27.5‰. Fish were fed three times per day to satiation with minced three-spined stickleback (*Gasterosteus aculeatus*). The fatty acid profile of the diet is given in Table 1.

Triploidy was confirmed by measuring the length of the nucleus major axis of erythrocytes (Flajshans et al., 2010; Ribeiro et al., 2012). For each specimen, 50 nuclei were measured.

Table 1
Fatty acid profile of the diet (minced three-spined stickleback): mean ± standard error.

Fatty acid	Percent of total FA (%)
14:0	3.7 ± 0.0
Σ15-17BFA	1.7 ± 0.0
15:0	0.4 ± 0.0
16:0	15.3 ± 0.4
16:1n-9	0.7 ± 0.0
16:1n-7	5.3 ± 0.0
Σ16PUFAn4&n1	0.4 ± 0.0
18:0	3.6 ± 0.1
18:1n-9	14.3 ± 0.2
18:1n-7	4.1 ± 0.1
18:2n-6	1.3 ± 0.0
18:3n-3	0.5 ± 0.0
18:4n-3	1.2 ± 0.0
Σ20:1	7.1 ± 0.2
20:4n-6	0.8 ± 0.0
20:4n-3	0.6 ± 0.0
20:5n-3	7.7 ± 0.0
Σ22:1	4.8 ± 0.3
22:5n-6	0.1 ± 0.0
22:5n-3	3.5 ± 0.1
22:6n-3	19.6 ± 0.1

2.2. Sampling and fatty acid analyses

Fish catch, care and analyses were done according to protocol of Permission No. 78 2013 031972 of the North-West Regional Administration of Federal Fishery Agency of Russian Federation. For following analyses, 10 diploid males (M2n), 6 diploid mature females (Fm2n), 7 juvenile (immature) diploid females (Fi2n) and 9 triploid fish with poorly developed gonads (J3n) were taken. Total length of all fish ranged from 23.4 to 31.8 cm; mass varied from 100 to 345 g.

For fatty acid analyses, samples of the muscle tissues of approximately 2–3 g of wet weight were cut from the right dorsal side of fish, 2–3 cm below the dorsal fin, and weighed. When cutting the muscle samples, we avoided red muscles, skin and bones. Then, the muscle tissue samples were placed into chloroform/methanol mixture (2:1, v/) and kept until further analysis at −20 °C within a month.

Lipid extraction, subsequent preparation of fatty acid methyl esters (FAMEs) and gas chromatography–mass spectrometry were the same as previously described (Gladyshev et al., 2014). The FAMEs were quantified according to the peak area of the internal standard, nonadecanoic acid, which we added to the samples as a chloroform solution prior to the lipid extraction.

2.3. Statistical analysis

One-way ANOVA with Tukey HSD *post hoc* test and multivariate discriminant analysis (MDA) (Legendre & Legendre, 1998) were calculated conventionally, using STATISTICA software, version 9.0 (StatSoft, Inc., Tulsa, OK, USA). Only normally distributed variables (Kolmogorov-Smirnov one-sample test for normality) were included in ANOVA and MDA.

3. Results

Triploid fish and diploid immature females had significantly higher mean percentage of 14:0, Σ15-17BFA, 15:0, 16:1n-7, Σ16PUFAn4&n1, 18:1n-9, 18:4n-3 and Σ22:1 compared to those of mature diploid males and females (Table 2). In turn, the mature diploid specimens (males and females) had significantly higher mean percentages of 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 than the triploid and immature diploid specimens (Table 2). Mean percentages of 16:0 was significantly higher in immature triploid and diploid fish than in mature diploid specimens (Table 2). Mean percentage of 18:2n-6 and 20:4n-3 in diploid males was significantly lower, and percentage of 22:5n-6 was significantly higher, than that in all other groups (Table 2).

Multivariate discriminant analysis (MDA) revealed significant differences in the FA compositions between the fish groups, except that of triploid fish and diploid immature females (Fig. 1). Both MDA discriminant functions (Root 1 and 2) were high and statistically significant (Table 3). The cumulative proportion of variance explained (discriminatory power) by the first two roots was 95.59%. Root 1 had 62.29% of discriminatory power and discriminated best immature diploid females from diploid males (Fig. 1, Table 3). Variables that gave the highest contribution to the first discriminant function (Root 1) were 20:4n-6, on the one hand, and 18:4n-3, on the other (Table 3). Root 2 revealed differences between triploids and diploid mature females primarily due to the contributions of 22:5n-3 and 15:0 in the discriminant function (Fig. 1; Table 3).

Sum of fatty acids per mass of filets in triploid fish was significantly higher, than that in mature diploid specimens, but overlapped with those of immature diploid females (Table 2). Contents of EPA and DHA did not differ significantly between the