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.

Keywords: essential fatty acids; triploid fish; filets

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 (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006;De Caterina, 2011).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 (Robert, 2006; Gladyshev, Sushchik, & Makhutova, 2013). However, contents of EPA and DHA in diverse fish species differ by more than two orders of magnitude (Gladyshev et al., 2013). Thereby, it is impossible to cover the recommended daily intake by eating certain fish species (e.g., Vasconi et al., 2015).

Aquaculture is known to be an important source of high nutritive fish. In the last decades in aquaculture, commercial 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 represented 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). Due to the sterility, adult triploid fish exhibit better growth than their diploid counterparts 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, Gomes, Vieira, Tabata, Takahashi, & Moreira, 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; Ozorio et al., 2012; Manor, Weber, Salem, Yao, Aussanasuwannakul, & Kenney, 2012; Ribeiro et al., 2012; Manor, Weber, Cleveland, & Kenney, 2014). Therefore, it was suggested to improve PUFA content in muscle of triploids by dietary supplementation of PUFA (Ribeiro et al., 2012).

It is important to note, that All the above data on less PUFA in triploids were based on measurements of their percentages in total fatty acids (Flajshans et al., 2010; Ozorio et al., 2012; Manor et al., 2012, 2014; Ribeiro et al., 2012). Meanwhile, it was demonstrated, that to 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, no data on PUFA content in mass units in triploid vs diploid fish is 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 (Ozorio et al., 2012; Manor et al., 2012, 2014; Ribeiro et al., 2012). Studies of other salmonid species are also desirable to expand and clarify data on nutritive quality of triploid fish.

Hence, the aim of our study was comparison of fatty acid composition and contents in filets of triploid and diploid pink (humpback) salmon *Oncorhynchus gorbuscha* in aquaculture and estimation of their nutritive value for humans 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 XX 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, that performed 10-15 minutes after fertilization at temperature 28-30°C during 10-15 minutes. Simultaneously, three control portions of the eggs were kept at 16°C (river water temperature). The control and experimental fries, obtained from the control and experimental eggs, were reared separately, but under the same conditions in an aquaculture farm. Young fish, obtained during the experiment, were reared in pools with fresh waters during 1.5 years. Then the fish were reared from May to October 2015 in the Chupa Bay (West coast of the White Sea, 66°16′ N and 33°03′ E). ничего не понятно и длинно.

Fish were reared in cages of 12 m^3; installed in inshore of the sea bay with stocking density ~250 individuals per 1 m^3. Это же к предыдущему предложению относится? Нужно совмещать

The control and experimental groups of young fish were separately reared under the same conditions in an aquaculture farm. They were kept in pools with fresh waters during 1.5 years. Then, the fishes were transferred to the Chupa Bay (West coast of the White Sea, 66°16′ N and 33°03′ E), where they were kept in cages of 12 m^3 installing in inshore of the sea bay. The fishes were reared during 5 months (from May to October) 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.

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 fishes, 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, volume/volume) 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, Sushchik, Gubanenko, Kalachova, Rechkina, & Malyshevskaya, 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, Σ16PUFA n4&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 values of percentages of 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 than those of 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). Mean values of percentages of other fatty acids were nearly similar and overlapped in all studied 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 studied groups of fish (Fig. 2). Ratio of n-6/n-3 in immature fish, both triploid and diploid, was significantly higher, than that in mature diploid fish (Fig. 2).

4. Discussion

Fatty acid composition of the pink salmon obtained in this study was close to that of another species of salmonid, *Oncorhynchus mykiss* (rainbow trout), reared in aquaculture (Ribeiro et al., 2012; Manor et al., 2014). Moreover, higher percentage of EPA and DHA in mature diploids compared to that of triploids, found by other authors (Flajshans et al., 2010; Ozorio et al., 2012; Manor et al., 2012, 2014; Ribeiro et al., 2012), was confirmed in our study. It should be emphasized, that immature diploid fish did not differ significantly from triploids concerning EPA and DHA percentage. Indeed, the triploidy prevent gonad development and triploid fish in their biochemical composition appeared to be close to immature diploid fish. In contrast to the percentages, contents of EPA and DHA per mass of the filets (i.e., in edible product) in mature diploid and triploid specimens were similar. Evidently, the similar contents of

EPA and DHA in mature diploids and triploids were due to the significantly higher content of total fatty acids in triploids' biomass.

Similar phenomenon, namely reciprocal ratio of percentages and contents of EPA and DHA in biomass of compared groups of fish, was found for other salmonids. For instance, migrating (anadromous) form of sockeye salmon *Oncorhynchus nerka* had significantly lower percentages, but significantly higher contents of EPA and DHA in filets, than its landlocked form, kokanee (Gladyshev et al., 2012). There are studies for other taxa, where the n-3 PUFA levels were negatively related to the total lipid content, e.g., for Eurasian perch *Perca fluviatilis* (Mairesse, Thomas, Gardeur, & Brun-Bellut, 2006). This phenomenon is believed to have a following explanation.

When the total lipid content or total fatty acid content increases in fish muscles, reserve lipids, i.e., triacylglycerols (TAG) are accumulated that have low content of PUFA, whereas the cellular phospholipids (PL) that are rich in PUFA, remain constant (Kiessling, Pickova, Johansson, Åsgård, Storebakken, & Kiessling,2001; Benedito-Palos, Calduch-Giner, Ballester-Lozano, & Perez-Sanchez, 2013). Therefore, this study confirms that in order to estimate nutritive value of a product for human nutrition, the essential PUFA contents should be expressed on a food mass basis, rather than their percentage in total FA (Gladyshev et al., 2007, 2012; Huynh & Kitts, 2009).

In conclusion, triploid *O. gorbuscha* had nutritive value regarding EPA and DHA contents similar to that of the diploid form. No special efforts to improve PUFA content in muscle of triploids (Ribeiro et al., 2012) are necessary.

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Figure captions

Fig 1. Scatterplots of canonical scores for the two discriminant functions, Root 1 and 2, after multivariate discriminant analysis (MDA) of the fatty acids composition (% of total FA) of *Oncorhynchus gorbuscha*: Fi2n – diploid immature females, J3n – triploid immature fish, M2n – diploid mature males, Fm2n – diploid mature females. Cages in the White Sea coastal water (Chupa village, Russia).

Fig.2. Mean values of indicators of nutritive value, content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, and ratio of sums of n-3/n-6 polyunsaturated acids in muscle tissue of *Oncorhynchus gorbuscha*: J3n – triploid immature fish, Fi2n – diploid immature females, M2n – diploid mature males, Fm2n – diploid mature females. Cages in the White Sea coastal water (Chupa village, Russia). Bars represent standard error. Means labelled with the same letter are not significantly different at $P< 0.05$ after Tukey HSD *post hoc* test for ANOVA. When ANOVA is insignificant, letter labels are absent.