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Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Sexual maturation is an energy demanding, physiological process that alters growth efficiency and compromises muscle quality in many food-fish species. Lipid mobilization supplies energy required for this process. To study the effect of ration level on fatty acid composition, diploid (2N) rainbow trout, approaching ovulation, were fed at 0.25 and 0.50% of tank biomass/day and to apparent satiation (~0.75% of tank biomass/day). In addition, triploid (3N) female trout, which exhibit only minimal ovarian development, were fed at 0.50% of tank biomass/day. The primary objective of this study was to determine effects of ration level on fatty acid composition in different lipid compartments (muscle, visceral adipose tissue, liver, and gonad) during sexual maturation. Lower feeding levels produced smaller fish, but did not affect the onset of sexual maturation. Higher feeding levels resulted in fish muscle with higher relative amounts of saturated fatty acids (SFAs), but monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were not affected by ration level. While ration level affected the fatty acid profile of each of the four tissues analyzed, the number of fatty acids affected was greatest in white muscle. An additional objective was to determine differences in the fatty acid composition of energy stores during maturation in female rainbow trout that were fed at a moderately restricted feeding level (0.50% of tank biomass/day). These differences were determined by comparing mature 2N to sterile 3N females of the same age. Diploid muscle contained higher amounts of PUFAs (44.4 ± 1.0%) than 3N muscle (39.7 ± 0.8%). Saturated fatty acids were in the highest concentrations in muscle and visceral adipose tissue, and 2N liver contained more PUFAs and fewer MUFAs than 3N liver; however these values are relative values. In general, fatty acids affected by ration level were not the same as fatty acids affected by ploidy. Triploid fatty acid profiles did not mimic those of the satiation fed group; which was expected if 3N fish were simply consuming excess energy. Both 2N and 3N muscle fatty acid profiles were similar to that of the diet, except muscle had lower amounts of PUFA precursors (18:3n-3 and 20:5n-3) and higher relative amounts of their product (22:6n-3). Also, 2N muscle had higher 16:1 and 3N muscle had higher 16:0 compared to the diet. It is unclear if these differences are hormonally driven or if there are other physiological dissimilarities between 2N and 3N trout causing these differences. Overall, our data suggest that 2N and 3N fatty acid metabolism is regulated differently.

1. Introduction

Sexual maturation is a dominant physiological process that causes a shift from somatic growth to gonadal growth (Taranger et al., 2010). In many cultured fish species, including salmonids, gonadal development occurs at the expense of stored energy and nutrients, including lipids. During this time period, females cannot assimilate enough nutrients from the diet to support gonadal development (Aussanasuwannakul et al., 2011, 2012; Gorgun and Akpinar, 2007; Jonsson et al., 1997; Kiessling et al., 2001; Manor et al., 2012; Memis and Gun, 2004; Nassour and Leger, 1989; Ribeiro et al., 2011; Salem et al., 2006; Shearer, 1994). This repartitioning alters body composition, in general, and muscle lipid content, specifically. Depletion of intramuscular fat and protein catabolism in cultured rainbow trout results in a reduction in muscle quality; softer fillets with minimal fat are less desirable for food products (Cleveland et al., 2012; Rasmussen, 2001; Salem et al., 2006, 2007), particularly in a species where a fillet with more oil is a standard of identity. During sexual maturation lipid is mobilized initially from visceral adipose tissue; although, in the long term, lipid will be mobilized from secondary storage sites such as muscle (Manor et al., 2012; Tocher, 2003). In disagreement, Kiessling et al. (1991a) suggest that intramuscular fat acts as a short-term fat depot and is mobilized first. However, effects of sexual maturation on composition will likely depend on the size and composition of nutrient reserves, diet composition, and ration levels.

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One method that can be used to avoid deterioration of muscle quality during sexual maturation is induction of triploidy. Triploid (3N) fish have three sets of chromosomes as opposed to two sets of chromosomes in normal diploid (2N) fish. The aquaculture industry induces triploidy in a variety of cultured species to cause sterility and prevent the onset of sexual maturation. In salmonids, such as rainbow trout, 3N females do not undergo sexual maturation and therefore, do not develop large ovaries (Piferrer et al., 2009). Triploid females do not experience the shift from somatic growth to gonadal growth, thus preventing mobilization of lipids and deterioration of muscle quality. Triploid males do, however, undergo sexual maturation but produce non-viable sperm (Piferrer et al., 2009). These characteristics make the production of all female, 3N fish desirable within the aquaculture industry. Nevertheless, little is known about the differences in 2N and 3N fatty acid metabolism.

Since total lipid and specific fatty acid contents are important attributes of fillet quality, regulation of fatty acid profiles has received much attention. Considering variables that impact fatty acid composition, studies have addressed 1) cultured versus wild fish, 2) seasonal variations, 3) altered diet composition, 4) fasting, and 5) basic physiology (Gorgun and Akpinar, 2007; Haugen et al., 2006; Kandemir and Polat, 2007; Kiessling et al., 1989, 1991b, 2001; Memis and Gun, 2004; Menoyo et al., 2004; Regost et al., 2001; Turchini and Francis, 2008). Of these variables, diet is the major contributor to muscle fatty acid composition. In general, white muscle saturated fat (SFA) and omega 6 (ω6) fatty acids are relatively stable while muscle monounsaturated (MUFA) and omega 3 (ω3) fatty acids exhibit greater sensitivity to changes in ration level. However, information on the responses of various lipid stores in fish to various ration levels is limited (Kiessling et al., 2001). In addition, little is known about differences in lipid metabolism between 2N and 3N rainbow trout. In our previous study, Manor et al. (2012) investigated fatty acid and proximate compositions of lipid stores in 2N and 3N rainbow trout on a high nutritional plane throughout sexual maturation and ovulation. We found that female rainbow trout on a high nutritional plane, with large visceral adipose tissue energy stores, did not mobilize lipid from muscle energy stores during sexual maturation. These findings are in contrast to studies using fish on lower nutritional planes (Gorgun and Akpinar, 2007; Kiessling et al., 1989, 1991a, 2001; Memis and Gun, 2004; Salem et al., 2007). Albeit, most research has focused on muscle fatty acid composition, with less emphasis on other lipid stores (i.e. visceral adipose tissue). This follow-up study investigates effects of ration level on carcass characteristics and fatty acid composition of energy stores in female rainbow trout. Additionally, effects of sexual maturation on the fatty acid profiles were determined by comparing maturing 2N to sterile 3N, female rainbow trout on a moderately restricted feeding level (0.50% of tank biomass/day). Additional data from this study on growth, fillet quality, and indices of protein degradation are reported in Cleveland et al. (2012). The objective of this paper is to determine the effects of sexual maturation and ration level on fatty acid composition of four distinct tissues (white muscle, visceral fat, liver, and gonad) representing primary fat depots that are central to lipid metabolism, redistribution, and storage.

2. Materials and methods

2.1. Experimental design

Fish care and experimentation followed guidelines outlined by the U.S. Department of Agriculture (USDA) and the National Center for Cool and Cold Water Aquaculture (NCCCWA; U.S. Department of Agriculture, Agricultural Research Service) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals*. Diploid and triploid, female rainbow trout from 4 families (families A, B, C, and D) were generated and maintained at the NCCCWA. At the fingerling stage (~50 g) and for individual identification, fish were implanted with passive integrated transponders (PIT-tags; Avid Identification Systems Inc., Norco, CA) in the dorsal musculature. Fish were confirmed 2N or 3N by flow cytometry (Allen, 1983; Hershberger and Hostuttler, 2007). Multiple families were used to ensure genetic diversity. Fish were maintained indoors, under simulated ambient photoperiod, and supplied with partially-recirculated and treated spring water throughout the study. Water temperatures ranged from 12.4 °C to 14.0 °C.

One month prior to onset of this study, fish were fed at 0.75% of tank biomass/day. Initial ration levels for 2N females were 1) 0.50% of tank biomass/day, 2) 0.75% of tank biomass/day, and 3) apparent satiation, and 3N females were fed at 0.75% of tank biomass/day. Two, 1000 L tanks were assigned to each of the four treatments, with a total of 7 fish per family per treatment. Families were split between two tanks, with the first tank containing 4 fish from families A and B, and 3 fish from families C and D. The second tank contained 3 fish from families A and B, and 4 fish each from families C and D. Therefore, each tank contained an equal number of fish ($n = 14$). Two weeks into the 12 week study, it was calculated that fish fed to satiation were consuming feed equivalent to 0.80–0.90% of tank biomass/day. At this time, 2N feeding levels were adjusted to 1) 0.25% tank biomass/day, 2) 0.50% of tank biomass/day, and 3) apparent satiation (~0.75% of tank biomass/day) for the remaining 10 weeks to increase potential differences between the satiation and the next-lowest feeding level. The 3N fish feeding level was also decreased from 0.75% tank biomass/day to 0.50% tank biomass/day. Triploid fish were only fed at 0.50% of tank biomass/day, a moderately restricted feeding level, because our previous study (Manor et al., 2012) examined 3N females fed to satiation. Moreover, the moderately restricted feeding level employed in the 2N portion of this study was applied to 3N fish in order to test for the effect of ploidy. Although all fish were expected to be female, males were found in two families. In family C, 8 of the 28 fish were males, and in family D, 13 of the 28 fish were males. Since there were not enough females in family D to allow for sampling, this family was excluded from the study. Only data from female fish were included in the analysis of this study. This criterion resulted in 2 fish per family per tank per ration (48 total fish) sampled in January at 22 M of age.

Fish were fed Zeigler G, floating, 5.0 mm (3/16″) pelleted feed (42% protein, 16% fat, 2% fiber; Zeigler Brothers, Inc.; Gardners, PA) dispensed by automatic feeders (Arvotec, Huutokoski, Finland) that adjust feed released daily based on the predicted mass of the fish in the tank. The fatty acid profile of the feed is provided in Table 1. Feeders dispensed feed in multiple feeding events between 7 am and 2 pm. Fish from each tank were weighed monthly to maintain the accuracy of the feeding regimen. Feeders for those tanks fed to satiation dispensed feed at 0.50% of tank biomass/day, followed by hand-feeding at the end of day to apparent satiation. Feeding procedures were modified one month after the start of the experiment to reduce the number of feeding events; these modifications reduced competition for available feed by increasing the amount of feed provided per feeding. This approach promotes a more

Table 1
Fatty acid profile of the diet.

Fatty acid	Percent fatty acid (%)
14:0	7.1 ± 0.8
16:0	19.8 ± 0.5
16:1	7.1 ± 0.1
18:0	3.7 ± 0.2
18:1n-9	16.8 ± 0.2
18:2n-6	21.5 ± 0.1
18:3n-6	0.1 ± 0.1
20:1	9.1 ± 0.1
18:3n-3	3.0 ± 0.1
20:4n-6	0.8 ± 0.1
20:5n-3	6.5 ± 1.0
22:6n-3	5.3 ± 0.2

Percent fatty acid of all measured fatty acids. All measurements were conducted in duplicate.