

PROOF

Comparison of Growth Performances, Nutritional Composition in Muscle of Diploid and Triploid Masu Salmon (*Oncorhynchus masou* B., 1856)

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E-mail: xinjian66627@163.com	Accepted 30 March 2015

Abstract

A study was conducted to compare growth and nutrients in the muscle of diploid and triploid masu salmon (*Oncorhynchus masou* B., 1856). Triploidy was induced by providing a heat shock treatment to the fertilized eggs. When fish grew up to 100 g around, triploids were prepared after triploidy determination prior to the trial. Initial mean weights of diploids and triploids were 103.67 ± 9.66 g and 109.95 ± 8.45 g, respectively. Quadruplicate groups of 30 fish were randomly assigned in each tank fed by water with a flow-through system and were fed to apparent satiation for 140 days. Survival, specific growth rate, feed conversion rate, condition factor, relative growth rate and absolute growth rate were determined in both groups. The contents of moisture, crude protein, lipid, ash, amino acids and fatty acids in muscle were analyzed. It has been determined that the triploid fish showed a higher weight gain than diploid fish despite the difference was not significant (P>0.05). There were no significant differences in crude protein, crude lipid, ash, amino acids and fatty acids between diploids and triploids, and triploids had no negative effects on muscle nutrients under fish farming practices especially for the production of *Oncorhynchus masou* on-growing stages.

Keywords: Diploidy, triploidy, Oncorhynchus masou, growth, nutritional composition.

Introduction

Genome manipulation offers a powerful method to achieve rapid and directional breeding and reproduction in fish. Polyploidy, a way to artificially duplicate the chromosome, is considered to be a classic approach to genome manipulation. In some fish species, growth rates vary among females, males, or infertile individuals. To exploit this, culturists produce monosex or infertile populations to increase productivity (Ocalewicz et al., 2010; Kucharczyk et al., 2014; Nowosad et al., 2015a; Nowosad et al., 2015b). Despite numerous research studies devoted to androgenesis and gynogenesis, the survival rate of offspring produced in this way is still very low (Nowosad et al., 2015b). Thus, a number of researchers have evaluated methods to produce sterile triploid fish. Triploidy fish are of potential benefits for aquaculture and fisheries management because they are sterile and sterilization can overcome the detrimental effects of sexual maturation on normal growth of fish (Gheyas, et al., 2001). Triploidy can be induced by one of shocks such as thermal (heat or or cold), mechanical (pressure) chemical (cytochalasin B) used for the retention of the second polar body during the second meiosis phase, then three chromosome sets can be generated for every embryonic cells (Johnstone, 1992). Inducing triploidy is the practical means in which sterilize large numbers of fish without using potentially harmful chemicals or radiation. The heat shock has extensively been used for inducing triploidy on salmonidae species, such as rainbow trout, *Oncorhynchus mykiss* (Solar, *et al.*, 1984; Guner, *et al.*, 2005) and brown trout, *Salmo trutta* (Kizak *et al.*, 2013).

Masu salmon (*Oncorhynchus masou* B, 1856) is a member of the family of Salmonidae and found in the Western Pacific Ocean along East Asia, ranging from the Kamchatka, Kuril Islands, Sakhalin, Primorsky Kraisouth through Korea, Taiwan and Japan (Masuda, *et al.*, 1984). It was introduced to China in 1996, and also has been an important species in coldwater fish aquaculture (Wang *et al.*, 1998). The price on the market is much higher than that of other salmonids. However, culture of *O. masou* has been less well studied due to some difficulties such as poor weight gain and condition factor, high feed conversion rate and non-aggressive feeding compared

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to rainbow trout. On attaining sexual maturity, 80% of passing fish die after spawning, and those that remain alive (preferentially dwarf males) participate in spawning next year (Bai *et al.*, 2004). Growth performances of triploid fish are expected to be better than those of diploids. Also, triploid females of *O. masou* are sterile, and the testes of triploid males are less developed than diploid males and have no spermatozoa even in the breeding season (Arai and Wilkins, 1987). Wang *et al.* (2005) showed that the mass production of triploid *O. masou* is feasible by heat shocks that induce very high rates of triploidy (close to 95%) without a major reduction of survival. Thus, triploid *O. masou* is also expected to be an aquaculture resource.

In order for triploids to be accepted by the aquaculture industry, they must be shown to perform as well as, or better than, diploids (O'Keefe and Benfey, 1997). So far, the growth comparison and differences in flesh quality between diploids and triploids of *O. masou* have not been fully investigated. Prior to actual use of triploid fish for *O. masou* farming, these should be fully investigated. The purpose of this study was to investigate the effect of triploidization on growth and nutritional composition in muscle of on-growing *O. masou* and provide information about the possibility of triploid culture for rearing practices.

Materials and Methods

Materials

This study was conducted in Bohai Coldwater Fish Hatchery, Chinese Academy of Fishery Sciences. Masu salmon eggs and sperm were collected from brood fish for triploidy induction. Fish were reared in hatchery tanks (600 L per tank) fed by the flow through spring water. Spring water was filtered through zeolum, corallite and activated carbon, and its quality parameters were monitored daily. Water temperature, dissolved oxygen, pH value, and ammoniacal nitrogen were measured with a water determinator (YSI 6600V2-2). During the experimental period, average water temperature ranged from 10.9 to 11.5 °C, pH value ranged from 7.1 to 7.3, ammoniacal nitrogen was $<0.02 \text{ mg L}^{-1}$ and dissolved oxygen was >6.0 mg L⁻¹. Approximately 2 L s⁻¹ of freshwater was supplied for fish. The photoperiod changed to 12 h light: 12 h dark.

The feed was formulated from commonly available ingredients (Table 1), and the corresponding amino acids and fatty acids profiles were presented in Table 2 and Table 3, respectively. Dry ingredients were sieved through a 60 mesh screen and homogenized by blending thoroughly in a feed mixer. The needed amount of fish oil and lecithin was added to the ingredients and the proper amount of water prepared for dough that allowed pelleting (diameter 3.0 mm). After pelleting, the pellets were air-dried in a workshop until they reached 8%-10% moisture, and then refrigerated at -20 °C until fed to the fish.

Methods

Triploidy was induced by applying a heat shock shortly after fertilization as described by Wang et al. (2005). Fertilized eggs were heat shocked 10 min post-fertilization at approximately 26.0 °C for 20 min duration. The eggs without heat shock treatment were kept as control. When fish grew up to 100 g around, triploids were identified by using the triploidy erythrocyte method as described by Woznicki and Kuzminski (2002) just prior to the experiments. Initial mean weights of diploid (DMS) and triploid O. masou (TMS) were 103.67 \pm 9.66 g and 109.95 \pm 8.45 g, respectively. Comparisons between triploid and diploid fish were conducted, and twelve tanks were randomly allocated into quadruplicate groups of 30 fish. Fish were fed 4 times a day to apparently satiation by hand. The trial was carried out for 140 days.

At the end of this trial, fish were weighed using an electronic balance (precision 0.01 g). Fish were anesthetized with 30 mg L⁻¹ clove oil before sampling. Specific growth rate (SGR), relative growth rate (RGR), absolute growth rate (AGR), survival, feed conversion rate (FCR) and CF were calculated as stated below (Korkut *et al.*, 2007; Kızak *et al.*, 2013).

SGR (% d⁻¹) = [ln (W₂) - ln (W₁) / t] × 100 RGR (%) = [(W₂ - W₁) / W₁] × 100 AGR = (W₂ - W₁) / t Survival = [number of survived fish/initial number of fish]×100 CF = (W / L³) × 100 FCR = consumed feed (kg) / weight gain (kg)

 Table 1. Feed ingredients and proximate composition of the experimental diet (air-dry basis, %)

Ingredients	Composition
Fish meal	50.0
Soybean meal	20.0
Wheat flour	17.0
Soybean protein concentrate	5.0
Fish oil	4.5
Soy lecithin	2.0
Premix*	1.5
Total	100.0
Analytical composition	
Moisture	11.85
Crude protein	48.05
Crude lipid	8.55
Ash	8.12
Gross energy (MJ kg ⁻¹)	16.43

Note: 1. Premix 1.5%, includes: Choline 0.2%; Antimildew 0.03%; Magnesia 0.2%; Betain 0.10%; Antioxidant 0.02%; Vitamin premix 0.3%; Mineral premix 0.2%; Zeolite 0.45%. 2. Vitamin and mineral mixture provide the following (kg⁻¹ of the diet): V_C 100 mg; V_E 60 mg; V_{K3} 5 mg; V_A 15000 IU; V_{D3} 3000 IU; V_{B1} 15 mg; V_{B2} 30 mg; V_{B6} 15 mg; V_{B12} 0.5 mg; Nicotinic acid 175 mg; Folic acid 5 mg; Inositol 600 mg; Biotin 2.5 mg; Pantothenic acid 50 mg; Fe 25 mg; Cu 3 mg; Mn 15 mg; Zn 30 mg; 10.6 mg.

Table 2. Amino acids profile of the experimental diet (airdry basis, %; EAA: essential amino acids; TAA: total amino acids; DAA: delicious amino acids; E/T: EAA/TAA; D/T: DAA/TAA)

Amino Acids	Composition
	Composition
*Aspartic acid	3.23
Threonine	2.13
Serine	2.53
*Glutamic acid	6.50
*Glycine	3.03
*Alanine	2.45
Cysteine	0.93
Valine	2.08
Methionine	0.97
Isoleucine	1.49
Leucine	3.02
Tyrosine	1.22
Phenylalanine	2.16
Lysine	3.15
Histidine	1.40
Arginine	2.51
Proline	3.14
Tryptophan	0.32
EAA	19.23
TAA	42.26
E/T (%)	45.50
D/T (%)	36.00

* represented delicious amino acids.

Table 3. Fatty acids profile of the experimental diet (g/100 g fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid)

14:06.3214:06.3215:00.2116:018.1317:00.3518:02.1220:00.12 \sum SFA27.2516:15.4318:113.1320:15.0222:16.35 \sum MUFA29.9318:2n-613.4620:4n-60.6818:3n-32.1518:4n-33.0220:5n-3 (EPA)8.1822:5n-30.8222:6n-3 (DHA)12.82 \sum PUFA41.13 \sum n-326.99 \sum n-614.14n-3/n-61.91	Fatty Acids	Composition
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$\begin{array}{cccc} 20:5n-3 & (EPA) & 8.18 \\ 22:5n-3 & 0.82 \\ 22:6n-3 & (DHA) & 12.82 \\ \sum PUFA & 41.13 \\ \sum n-3 & 26.99 \\ \sum n-6 & 14.14 \end{array}$	18:3n-3	2.15
$\begin{array}{cccc} 22:5n-3 & 0.82 \\ 22:6n-3 (DHA) & 12.82 \\ \sum PUFA & 41.13 \\ \sum n-3 & 26.99 \\ \sum n-6 & 14.14 \end{array}$	18:4n-3	3.02
$\begin{array}{ccc} 22:6n-3 (DHA) & 12.82 \\ \sum PUFA & 41.13 \\ \sum n-3 & 26.99 \\ \sum n-6 & 14.14 \end{array}$	20:5n-3 (EPA)	8.18
\sum PUFA 41.13 \sum n-3 26.99 \sum n-6 14.14	22:5n-3	0.82
	22:6n-3 (DHA)	12.82
$\sum_{n=6}^{\infty}$ 14.14	∑PUFA	41.13
	∑n-3	26.99
n-3/n-6 1.91	$\sum n-6$	14.14
	n-3/n-6	1.91

Where W is body weight, W_2 is final weight, W_1 is initial weight, t is the period of trial and L is fork length.

At the end of this trial, six fish from each tank were dissected to remove the muscle for nutritional composition analysis. The samples of formulated feed and muscle were analyzed for the contents of moisture, crude protein, crude lipid, and ash according to AOAC (1995) methods. After acid hydrolysis (tryptophan exception) or alkaline hydrolysis (tryptophan), amino acids of muscle were analyzed by amino acid analyzer (L-8900, HITACHI, Japan), as described by Ma *et al.* (2010). The fatty acids of muscle analysis were performed using a gas chromatograph (Shimadzu GC-2010, Japan), as described by Lee *et al.* (2010).

Nutrition Evaluation Method

According to the assessment standards mode suggested by the FAO/WHO and the amino acid mode of whole egg protein, the mode of the amino acid score (AAS), chemical score (CS) and essential amino acid index (EAAI) were calculated as follows (Pellett and Yong, 1980; Qiaoben, 1980).

$$AAS = \frac{aa}{AA_{(FAO/WHO)}} \times 100$$
$$CS = \frac{aa}{AA_{(Egg)}} \times 100$$
$$EAAI = \sqrt[n]{\frac{100A}{A_{E}}} \times \frac{100B}{B_{E}} \times \frac{100C}{C_{E}} \times \cdots \frac{100I}{I_{E}}$$

In the above formula, aa means content of amino acids (%) of the samples. AA $_{(FAO/WHO)}$ means the same amino acid content of FAO/WHO score standard mode (%). AA $_{(Egg)}$ means the content of the whole eggs in the same protein amino acid content (%). n denotes the number of the comparative essential amino acids. A, B, C, ... I represent the content of essential amino acids for samples of muscle protein, and A_E, B_E, C_E, ... I_E are the measurement of the essential amino acids for the whole eggs protein content.

Statistical Analysis

Data were expressed as mean \pm SE and means were analyzed by independent-samples T test. A significance level of 95% was considered to indicate statistical differences (P<0.05). All statistics were conducted using the specific software SPSS 19.0 for Windows.

Results

Growth Performances

At the end of this trial, the survival of CMS and TMS was recorded as 91.67% \pm 3.06 and 90.33% \pm

3.79, respectively. No significant difference was observed in survival of both groups (P>0.05), although it was higher in CMS group. The final mean live weight of CMS and TMS was measured as 204.35 ± 55.76 g and 229.20 ± 73.13 g, respectively. Overall, there were no differences in growth between CMS and TMS (P>0.05). The growth parameters, SGR, CF, RGR, AGR and FCR were presented in Table 4. Similarly, growth parameters of CMS and TMS were not statistically different (P>0.05).

Proximate Composition

The moisture, crude protein, crude lipid and ash in muscle of both groups were given in Table 5. Crude protein was 17.72% (CMS) and 17.98% (TMS), respectively. Crude lipid was 2.05% (CMS) and 2.08% (TMS), respectively. No differences observed in proximate composition (moisture, crude protein, crude lipid and ash) of muscle between CMS and TMS (P>0.05).

Amino Acids Profile

The amino acids profile in muscle of both groups was presented in Table 6. Glutamine (CMS $2.57\% \pm 0.04$; TMS $2.70\% \pm 0.11$) was highest and cysteine (CMS $0.16\% \pm 0.03$; TMS $0.15\% \pm 0.02$) was lowest in both groups. Essential amino acids (EAA) contents in the muscle of CMS and TMS were high, as $50.05\% \pm 0.24$ and $49.54\% \pm 0.21$ of total amino acids (TAA), respectively. Similarly, delicious amino acids (DAA) contents in the muscle of CMS and TMS were also high, as $37.31\% \pm 0.20$ and $37.46\% \pm 0.18$ of the TAA, respectively. No significant difference was observed in EAA/TAA and DAA/TAA in both groups (P>0.05). Overall, there were no differences in amino acids profile between CMS and TMS (P>0.05).

Evaluation of Nutritional Quality

The data were converted into AA/protein (mg/g), and were compared with standard mode suggested by the FAO/WHO and amino acid mode of

the egg protein. Then AAS and CS of both groups were calculated, respectively (Table 7). AAS and CS results showed that lysine was the highest and tryptophan was the lowest in both groups. Based on AAS and CS, the first and second restrictive amino acids of both groups were tryptophan and methionine + cysteine, respectively.

Fatty Acids Profile

The fatty acids profile in muscle of both groups was shown as percent of total fatty acids in Table 8. Total saturated fatty acids (\sum SFA) contents in the muscle of CMS and TMS were 38.04% ± 0.28 and 37.78% ± 1.13, respectively. Total monounsaturated fatty acids (\sum MUFA) contents in the muscle of CMS and TMS were 30.18% ± 1.51 and 30.63% ± 1.71, respectively. Total polyunsaturated fatty acids (\sum PUFA) contents in the muscle of CMS and TMS were 29.14% ± 2.04 and 30.14% ± 0.49, respectively. No significant differences were found in \sum n-3PUFA, \sum n-6PUFA and \sum n-6PUFA/ \sum n-3PUFA between two groups (P>0.05). Overall, there were no differences in fatty acids profile between CMS and TMS (P>0.05).

Discussion

This study showed that despite survival was lower in triploids than that in diploids, no differences were observed between groups (P>0.05). Survival was in accordance with some reports of salmonids such as *O. mykiss* (Bonnet *et al.*, 1999; Muller-Belecke *et al.*, 2006), Atlantic salmon, *Salmo salar* (Oppedal *et al.*, 2003), but contradicted with other reports (Solar *et al.*, 1984; Sutterlin *et al.*, 1987). This might be related to different triploidy inducing methods.

There was also no difference in growth performances between diploids and triploids in this study (P>0.05), so triploids showed no superiority in growth over diploids. At the end of the experiment, diploids and triploids reached to 204.35 g and 229.20 g, respectively. Although there was no statistical difference in weight gain between triploids and diploids of *O. masou*, it might be concluded that

Table 4. Growth performances of diploid and triploid O. masou at the end of the 140-day growing trial

Parameters	DMS	TMS
IBW (g)	103.67±9.66	109.95±8.45
FBW (g)	204.35±55.76	229.20±73.13
SGR $(\% d^{-1})$	0.48±0.15	0.51±0.14
AGR	0.72 ± 0.30	0.85 ± 0.40
RGR (%)	99.47±44.81	107.39±39.78
CF	1.18 ± 0.06	1.19±0.03
FCR	1.52±0.15	1.38 ± 0.13
Survival (%)	91.67±3.06	90.33±3.79

(IBW, initial body weight; FBW, final body weight; SGR: specific growth rate, CF: condition factor; RGR: relative growth rate, AGR: absolute growth rate, FCR: feed conversion rate)

Values are represented as mean \pm SE of pooled data from triplicates per treatment (n=3). Means in the same row with different subscripts are significantly different (P<0.05).

triploids showed better growth than diploids. Similarly, triploid salmonids such as S.salar (Carter et al., 1999; Oppedal et al., 2003) and O. mykiss (Sheehan et al., 1999) often show growth performance as good as or better than that of diploids when reared separately. Generally, growth advantage of triploid fish is expected after sexual maturity, when somatic growth of diploid fish is usually suppressed by the reproductive process (Koedprang and Nakorn, 2000). Thus, more growth trials containing the whole life stage should be carried out to have more sound data in further research. Moreover, FCR of triploids was slightly lower than that of diploids (P>0.05), which suggests that triploids may have more efficiency at converting feed into live weight for O. masou. This was contradicted with O.mykiss, which might be related to different biochemical, enzymatic and metabolic processes of specific species (Muller-Belecke et al., 2006).

No differences observed in proximate composition (moisture, crude protein, crude lipid and ash) of muscle between diploids and triploids (P>0.05). Thus, proximate composition of muscle generally appears to be stable, and triploid inducing

might not change the proximate composition of O. masou on-growing stages. This result is consistent with the previous reports (Muller-Belecke et al., 2006; Kizak et al., 2013). The combination of high protein and high lipid would lead to good flesh quality with favorable sensory characteristics such as flavor and texture (Xiang et al., 2006). Crude protein was 17.72% (diploids) and 17.98% (triploids) in muscle of O. masou, and higher than that of some other commercial freshwater fish such as grass carp 16.60%, silver carp 17.80% and catfish 14.03-15.87% (Yue et al., 2002). However, Crude lipid in the muscle of diploids (2.05%) and triploids (2.08%) was lower, which was closely related to diets contained relatively lower crude lipid in this study (8.55%). Thus, crude lipid of farmed O. masou diploids or trilploids should be appropriately manipulated by dietary lipid content in order to meet the flesh quality requirement.

The evaluation of protein nutritional value must be based on the type of content and composition of amino acids (Bing *et al.* 2005). Quantitative changes in the DNA content in cells also occur in genetically modified polyploid fast-growing fish, which, in

Table 5. Proximate composition in muscle of diploid and triploid O. masou (wet weight, %)

Parameters	DMS	TMS
Moisture	78.22±1.54	77.89±3.07
Crude protein	17.72±0.69	17.98±2.14
Crude lipid	2.05±0.14	$2.08{\pm}0.30$
Ash	1.35±0.77	1.39±0.70

Values are represented as mean \pm SE of pooled data from triplicates per treatment (n=3). Means in the same row with different subscripts are significantly different (P<0.05).

Amino Acids	DMS	TMS
*Aspartic acid	1.63±0.03	1.69±0.06
Threonine	0.77±0.01	$0.80{\pm}0.04$
Serine	0.67 ± 0.04	0.71±0.02
*Glutamic acid	2.57±0.04	2.70±0.11
*Glycine	0.79 ± 0.06	$0.80{\pm}0.04$
*Alanine	$0.94{\pm}0.05$	$0.98{\pm}0.04$
Cysteine	0.16±0.01	0.19±0.02
Valine	$0.84{\pm}0.02$	$0.87{\pm}0.04$
Methionine	0.57±0.06	0.59±0.02
Isoleucine	0.72±0.03	0.75±0.03
Leucine	1.35±0.05	$1.40{\pm}0.06$
Tyrosine	0.55±0.01	$0.58{\pm}0.02$
Phenylalanine	0.75±0.03	0.71 ± 0.04
Lysine	1.46 ± 0.04	1.52 ± 0.08
Histidine	0.38±0.06	0.38±0.02
Arginine	0.98±0.02	1.01 ± 0.04
Proline	0.63±0.06	0.67 ± 0.06
Tryptophan	0.16±0.03	0.15±0.02
EAA	7.96±0.09	8.14±0.32
TAA	15.90±0.11	16.43 ± 0.62
E/T (%)	50.05±0.24	49.54±0.21
D/T (%)	37.31±0.20	37.46±0.18

Table 6. Amino acids profile in the muscle of diploid and triploid O. masou

(wet weight, %; EAA: essential amino acids; TAA: total amino acids; DAA: delicious amino acids; E/T: EAA/TAA; D/T: DAA/TAA) Values are represented as mean \pm SE of pooled data from triplicates per treatment (n=3). Means in the same row with different subscripts are significantly different (P<0.05). * represented delicious amino acids.

theory, might influence the composition of amino acids in body tissues of the fish. Several amino acids (Thr, Ile, Pro, Gly, Arg) in muscle of tech (Tinca tinca) were of a significant change by triploidy inducing (Buchtová et al., 2005). However, the muscle amino acids content and proportion of diploids and triploids were basically the same, which was similar to the report of Kizak et al. (2013). Thus, the amino acids profile of O. masou muscle had a conservative pattern. No differences were found in muscle EAAI of diploids (93.58%) and triploids (93.82%) (P>0.05), and amino acids were higher than WHO / FAO standard (35.38%) and egg protein standard (48.08%). Therefore, the nutritional value of amino acids indicated that the muscle proteins of diploid and triploid O. masou had better balance in amino acid composition and higher protein quality.

The knowledge of fatty acids changes and their dynamics will help to elucidate the process of vitellogenesis and final oocyte maturation (Nowosad et al., 2015b). For instance, the EPA/DHA and PUFA n-3/n-6 (P<0.05) in muscles decreased and the content of saturated fatty acids remained unchanged both in the gonads and in the muscles (Mazzeo et al., 2010; Nowosad et al., 2015b). Regretly, the gonad didn't mature during this study and further research needs to be done. There were no significant differences in muscle fatty acids between diploid and triploid O. masou, indicating that no considerable movement of fatty acids from muscle to gonads before sexual maturity. Maybe the significant differences of fatty acids would appear during the process of sexual maturity and the direct reason is that the triploids are infertile and little or no fatty acids move from muscle

Table 7. Evaluation of essential amino acids in muscle of diploid and triploid O. masou

Amino Acids	Evalua	Evaluation Mode		AAS		CS	
Allillo Acids	FAO/WHO	Egg Protein	DMS	TMS	DMS	TMS	
Threonine	250	292	127	130	109	111	
Valine	310	411	112	114	85	86	
Isoleucine	250	331	119	123	90	93	
Leucine	440	534	127	130	105	107	
Lysine	340	441	178	182	137	140	
Tryptophan	60	38	111	99	67	60	
Methionine+Cysteine	220	386	137	143	78	82	
Phenylalanine+Tyrosine	380	565	142	138	95	93	
EAAI (%)					93.58±1.35	93.82±5.40	

(mg/g N; AAS: amino acid score; CS: chemical score; EAAI: essential amino acid index)

Table 8. Fa	tty acids	profile in	the muscle	e of diploid	and triploid	O. masou
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Fatty Acids	DMS	TMS
14:0	6.17±0.16	6.42±0.44
15:0	0.58 ± 0.07	0.59±0.18
16:0	21.85±0.75	21.33±2.38
17:0	0.63 ± 0.13	0.72 ± 0.14
18:0	8.23±0.28	8.10±0.34
20:0	0.59±0.15	0.62 ± 0.14
∑SFA	38.04±0.28	37.78±1.13
16:1	6.37±0.64	6.68±0.63
18:1	22.11±1.10	22.30±2.07
20:1	1.19 ± 0.23	1.15±0.24
22:1	0.52±0.01	0.51±0.03
∑MUFA	30.18±1.51	30.63±1.71
18:2n-6	13.88±2.33	14.69±1.23
20:4n-6	0.58 ± 0.09	0.53±0.23
18:3n-3	0.74±0.13	0.75±0.24
18:4n-3	0.38 ± 0.04	$0.48{\pm}0.06$
20:5n-3 (EPA)	4.43±0.16	4.56±0.13
22:5n-3	1.25 ± 0.09	1.26 ± 0.06
22:6n-3 (DHA)	7.90±0.01	7.88±0.28
∑PUFA	29.14±2.04	30.14±0.49
$\overline{\Sigma}$ n-3	14.69±0.36	14.92±0.51
$\overline{\Sigma}$ n-6	14.45±2.42	15.22±1.00
<u>n-6/n-3</u>	0.98	1.02
Unidentified	2.66±0.83	1.46±0.09

(g/100 g fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid)

Values are represented as mean ± SE of pooled data from triplicates per treatment (n=3). Means in the same row with different subscripts are

to gonads. No such studies have been conducted with *O. masou.* However, it cannot be ruled out that muscle fatty acids would change during the process of gonad development of triploid *O. masou.* In addition, Buchtová *et al.* (2004) in *T.tinca* both demonstrated significant differences in the distribution of SFA and PUFA in muscle lipids.

The muscle of dilploid and triploid O. masou contained higher content of C16:0 and C18:0 in this study, which is similar to rainbow trout, Oncorhynchus mykiss (Haliloglu et al., 2004), S.salar (Gómez-Guillén et al., 2000), and etc. C16:1 and C18:1 were the major MUFA detected in both groups. High level of C16:1 is one of the main features of freshwater fish muscle (Oliveira et al. 2003) and C18:1 is an important indicator of flesh quality assessment (Rey et al., 2004). For the general public, the desirable dietary lipid had the composition as follows: ratio of SFA, MUFA and PUFA (S: M: P) being 1:1:1, which basically met the demand of fatty acids in the human body (Xue et al., 2012). According to this, the S: M: P ratio was close to 1:1:1 in both diploid and triploid O. masou, which met the requirement of fatty acids for humans.

The n-6PUFA is important in lipid metabolism, being the basis for the formation of arachidonic acid (C20:4n-6, ARA). ARA and eicosaheptaenoic acid (C20:5n-3, EPA) are the precursors for eicosanoids and the relative levels of these two fatty acids will have a profound effect on the formation of these metabolically very active substances (Sargent *et al.*, 1995). In our fish the n-6PUFA remained relatively stable. High levels of linoleic acid (C18:2n-6) were observed in muscle of both diploid and triploid *O. masou.* Kiessling *et al.* (2001) considered that this is the effect of lipid metabolism change and a higher mobilization from phospholipids in muscle.

The diploid and triploid O. masou, similar to other salmonids, have a higher content of n-3PUFA in muscle. The n-3PUFA could eliminate the adverse effects of it to some extent such as lowering serum triglycerides and cholesterol (Guler et al., 2011). Clinical studies have found EPA and docosahexaenoic acid (C22:6n3, DHA) were essential fatty acids of human and animal growth and could effectively prevent human cardiovascular disease (Zimmer et al., 2000), which indicated the diploid and triploid O. masou had a higher food value and health effects. However, the EPA+DHA content of diploid (12.33%) and triploid (12.44%) O. masou is lower than that lived in seawater (Li and Yamanda, 1992), similar to O. mykiss (İbrahim Haliloğlu et al., 2004). The salmon lives in a freshwater environment low in n-3HUFA and migrates to a sea water environment rich in these fatty acids (Bell et al., 1997; Kiessling et al., 2001). It is therefore tempting to speculate this decrease of n-3HUFA, reflects an adaptation to a lower need of these long chained and unsaturated fatty acids in the freshwater environment (Pickova, 1997).

Generally, it is believed that the content and composition of fatty acid of the feed directly affect the fatty acid content and composition of fish muscle (Martins *et al.*, 2007; Hansen *et al.* 2008). Maintaining high levels of n-3 PUFA, as well as low levels of n-6 fatty acids, in farmed fish, is a major concern for producers in order to provide a high nutritional value of the product for human consumption (Simopoulos, 2004). Therefore, in order to improve the flesh quality, selecting the appropriate oil raw materials to increase the PUFA levels of the feed could improve the muscle PUFA ratio of farming diploid and triploid *O. masou*.

In conclusion, triploid *O. masou* could not show a significant increase in the growth performances compared to diploids. Nutritional composition in muscle of triploids was similar to diploids. Triploids were also rich in balanced essential amino acids and polyunsaturated fatty acids.

Acknowledgments

This study was supported by the National Key Technology Research and Development Program in the 12th Five Year Plan of China (2012BAD25B10) and the Special Fund for Agro-scientific Research in the Public Interest (201003055).

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