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ORIGINAL PAPER

COMPARISON OF THE PROXIMATE COMPOSITION, AMINO ACID COMPOSITION AND GROWTH-RELATED MUSCLE GENE EXPRESSION IN DIPLOID AND TRIPLOID RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) MUSCLES

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Abstract

The aim of the present study was to identify differences in the proximate composition, amino acid composition and expression of the muscle growth-related genes myogenic determining factor (MyoD) and myostatin (MSTN) in muscle between diploid and triploid female rainbow trout (*Oncorhynchus mykiss*) that were reared under the same conditions. The research included two different ages (12- and 16-month) of diploid and triploid rainbow trout. The lipid and protein contents of the triploid fish were significantly higher while their moisture content was significantly lower than those of the diploid fish. The amino acid compositions of the proteins of both diploid and triploid rainbow trout were well balanced. The percentages of total amino acids (W_{TAA}), essential amino acids (W_{EAA}), non-essential amino acids (W_{NEAA}) and delicious amino acids were similar between the triploid and diploid fish. The W_{EAA} to W_{TAA} and W_{EAA} to W_{NEAA} indices were comparable to the reference values of nearly 40% and above 60%, respectively, recommended by the FAO/WHO. According to the amino acid scores, phenylalanine can be described as the first limiting amino acid, and the sum of methionine and cysteine represented the highest protein score in both diploid and triploid rainbow trout. MyoD1 mRNA expression was higher in triploid fish, while MSTN2 mRNA expression was higher in diploid fish. The growth potential was greater in triploid fish than in diploid fish. This study shows that both diploid and triploid rainbow trout are good protein sources; triploid rainbow trout is under further investigation due to presenting higher, balanced nutritional quality and better growth than the diploid fish.

Keywords: diploid rainbow trout, triploid rainbow trout, amino acids, proximate composition, MyoD and MSTN mRNA expression.

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INTRODUCTION

Fish is an important component of the human diet because it is rich in high-quality protein, low in cholesterol, and represents a good source of LC-PUFAs (long-chain polyunsaturated fatty acids), vitamins and essential minerals (Venugopal 1996). For the sake of ecological protection, wild fishing is strictly controlled, and cultured fish are therefore the main source of aquatic products and food. With improvements in aquaculture, the consumption of farmed fish has greatly increased in recent years, which has influenced the world supplies and market prices.

Rainbow trout (*Oncorhynchus mykiss*) is a Pacific trout species belonging to the Salmonidae family. Since its introduction to China in 1959, the aquaculture of rainbow trout has rapidly expanded owing to its excellent taste and high market value, making it one of the most important lead-in and cultured species (Sun 2010). It is also cultured in the USA, Japan, Europe, Russia, Canada and Iran (Rasmussen 2000, Afsana 2004, Dobly 2004, Skinner 2010). In salmonids, mature individuals often exhibit a higher mortality rate after breeding, which is known as the "mature death" phenomenon; however, triploid fish infertility can prevent biological death and decrease production costs due to the development of large individuals, along with improved economic benefits and experimental requirements. Because triploids are sterile, the energy consumption required for sexual maturation can be avoided, and more biological effort can be directed towards improving flesh quality, somatic growth and feed utilisation; thus, the growth performance of triploid fish is expected to be better than that of diploid fish (Tiwary 2004). Hence, triploid rainbow trout is expected to be an aquaculture resource that can satisfy consumer and culturist requirements.

Somatic growth is closely related to muscle tissue, which comprises approximately 65% of the fish body mass (Johnston 1999). Studies on the possible effects of triploidisation on muscle growth could help to explain the variable results in growth performance observed in triploid fish (Bing 2006, Taylor 2013). Although there have been some studies on the proximate composition of flesh as well as its amino acid and fatty acid composition in wild and cultured diploid rainbow trout, none of these studies have focused on triploid rainbow trout. Hence, comparisons of growth and differences in flesh quality between diploid and triploid farmed rainbow trout have not been fully addressed. Additionally, the nutritional value and economic value of triploid rainbow trout for human consumption have yet to be investigated.

The aim of breeding programmes has changed from increasing yield to improving meat quality-related traits (Van Wijk 2005). Myogenic regulatory factors (MRFs) are a family of vertebrate proteins that act as potent transcription factors for muscle genes (Pownall 2002). The myogenic determining factor (MyoD) gene, which belongs to the MRF gene family, initiates and maintains the differentiation and development of skeletal muscle during

myogenesis (Weintraub 1994). Previous studies have suggested that MyoD1 is involved in establishing and maintaining slow- and fast-twitch mature muscle fibre phenotypes, which is important because muscle fibre characteristics play a key role in meat quality (Picard 2002). Numerous studies have examined the MyoD1 gene, mainly focusing on its roles in pigs (Knoll 1997, Kim 2009), cattle (Tian 2007) and other animals, and have found it to be correlated with muscle development and growth (Zhang 2007). MSTN is a secreted protein that acts as a negative regulator of skeletal muscle mass. MSTN activity may help to improve the management of muscular growth of animals, as well as to define a new strategy to control both meat quantity and quality. The MSTN gene has direct effects on fat tissue, as it inhibits the fat deposition mechanism possibly by regulating the metabolic process (Mcpherron 2002). Therefore, it is conceivable that increasing the functional activity of MyoD or reducing/suppressing the functional activity of fish MSTN could be significant for improving meat quality (Lee 2003).

Thus, the main objectives of this study were to analyse and compare the differences in the proximate composition, amino acid profiles and MyoD1 and MSTN2 mRNA expression between diploid (2n) and triploid (3n) rainbow trout in Heilongjiang Province, China, to better characterise these fish for culture purposes and to establish a more profitable and sustainable triploid rainbow trout farming industry.

MATERIAL AND METHODS

Sample preparation

All of the fish involved in this study were bred according to the guidelines of the Animal Husbandry Department of Heilongjiang, China, in November 2014. The triploid all-female eggs were obtained via the method reported by Espinosa et al. (Espinosa 2005). Fish were bred in 4 tanks (250 cm × × 250 cm × 100 cm), including 2 tanks of diploids and 2 tanks of triploids. Twenty-five fish were bred in each tank. The water in the tanks had the following characteristics: (1) temperature, 11.8-16.8°C; (2) pH, 7.4-7.8; (3) dissolved oxygen concentration, 8.0-9.0 mg O_2 l^{-1}; and (4) salinity, <0.05%. The fish were fed a commercial diet (4.0-5.5 mm) three times a day; the average proximate composition and amino acid compositions of the feed mixture are shown in Table 1 and Table 2. Fifteen sampled fish from each tank were selected at 12 and 16 months (from November of 2015 to March of 2016), and measurements of their body weight and length were recorded after anaesthetisation with 200 mg l^{-1} MS-222 (Beijing, China). The age and the average weight and length (mean±SD) of the studied fish are shown in Table 3. After obtaining the biological data, the fish were skinned, and the muscle area near the dorsal fin was filleted. A small portion of the muscle was immediately stored in liquid nitrogen and maintained at -80°C until RNA extraction,

Table 1

Comparison of nutrient components in diploid and triploid rainbow trout and commercial feed (%), $n = 15$

Components	Group I		Group II		Commercial feed
	2*n**	3*n*	2*n*	3*n*	
Moisture	73.21±0.53^a	70.06±0.71^b	76.79±0.65^c	70.53±0.49^b	7.03±0.27
Protein	20.47±0.33^a	23.15±0.42^b	17.46±0.39^c	22.32±0.52^b	42.50±0.89
Lipid	3.37±0.01^a	4.14±0.09^b	2.68±0.18^c	4.43±0.16^b	11.49±0.66
Ash	1.49±0.06^a	1.47±0.05^a	1.37±0.01^b	1.33±0.01^b	11.82±0.44

Means with different superscripts in the same row are significantly different ($P < 0.05$);
* n – the number of diploid or triploid samples in each group.

while the remainder of the dorsal muscle was homogenised and separately pooled in a plastic bag. The latter samples were stored at -20°C (within 7 days) and subsequently analysed in duplicate replicates, as described in the next section.

Proximate composition analyses

The proximate composition of the fish was analysed according to procedures indicated by the Association of Official Analytical Chemists (AOAC 1995).

Amino acid analyses

After acid hydrolysis (tryptophan exception), the amino acid composition of the muscle was analysed with an amino acid analyser (L-8900, HITACHI, Japan), as described by Ma et al. (2010). All of the determinations were expressed according to dry matter.

Estimation of fish nutritional values

The amino acid scores (AASs), chemical scores (CSs) and essential amino acid index (EAAI) were calculated using equations (FAO/WHO) described by Wang et al. (2015).

RNA extraction and Reverse transcription

Ten fish from each group were analysed via quantitative real-time PCR. Total RNA was extracted from their muscles with the TRIzol reagent (Invitrogen). Reverse transcription was carried out to generate cDNAs using the PrimeScript RT Reagent Kit (TakaRa).

Table 2

Amino acid composition of muscles from diploid and triploid rainbow trout (dry weight (%), $n = 6$

Amino acid	Group I		Group II		Commercial feed
	2*n*	3*n*	2*n*	3*n*	
Threonine (Thr)	3.64±0.09	3.64±0.01	3.54±0.12	3.81±0.12	1.61±0.00
Valine (Val)	4.38±0.44	4.39±0.29	4.25±0.25	4.25±0.09	1.71±0.03
Methionine (Met)	2.99±0.29	3.11±0.21	3.04±0.14	2.90±0.14	0.83±0.05
Isoleucine (Ile)	3.25±0.15	3.35±0.02	3.16±0.12*b*	3.50±0.10*a*	1.37±0.01
Phenylalanine (Phe)	1.89±0.12	1.86±0.11	1.90±0.11	1.96±0.02	1.83±0.02
Lysine (Lys)	6.47±0.15	6.49±0.10	6.15±0.20*b*	6.75±0.29*a*	2.73±0.08
Leucine (Leu)	6.35±0.14	6.45±0.06	6.15±0.26	6.66±0.26	3.08±0.06
Aspartic acid (Asp)*	7.78±0.15	7.80±0.07	7.46±0.28*b*	8.20±0.32*a*	4.03±0.03
Glutamic acid (Glu)*	11.29±0.17	11.78±0.48	10.87±0.48	11.76±0.55	6.83±0.05
Glycine (Gly)*	3.59±0.33	3.49±0.03	4.24±0.11*b*	3.60±0.20*a*	2.49±0.01
Alanine (Ala)*	4.24±0.06	4.10±0.16	4.07±0.15*b*	4.54±0.12*a*	2.30±0.01
Serine (Ser)	3.18±0.06	3.27±0.11	3.13±0.09	3.34±0.11	1.76±0.00
Cysteine (Cys)	2.85±1.36	4.05±1.14	2.97±0.16*b*	2.02±0.15*a*	0.71±0.00
Tyrosine (Tyr)	2.94±0.07	2.99±0.11	2.78±0.15*b*	3.15±0.13*a*	1.22±0.01
Proline (Pro)	2.57±0.69	2.60±0.48	2.63±0.19*b*	1.96±0.02*a*	1.86±0.02
Histidine (His)	1.68±0.20	1.64±0.19	1.56±0.06	1.63±0.02	1.00±0.01
Arginine (Arg)	4.38±0.14	4.45±0.13	4.26±0.12	4.59±0.17	2.56±0.06
Essential amino acids W_{EAA}	28.97±1.27	29.28±0.42	28.17±0.93	29.82±0.99	13.34±0.11
Half-essential amino acids W_{HEAA}	6.06±0.27	6.09±0.06	5.83±0.17	6.21±0.17	3.55±0.32
Non-essential amino acids W_{NEAA}	38.43±0.89	40.07±0.98	38.15±1.33	38.57±1.56	21.83±0.99
Delicious amino acids W_{DAA}	26.89±0.50	27.17±0.42	26.64±0.92	28.11±1.18	15.64±0.92
Total amino acids W_{TAA}	73.46±1.40	75.44±1.47	72.14±1.42	74.61±1.71	38.17±1.54
W_{EAA}/W_{TAA}	39.44	38.82	39.05	39.97	34.46
W_{EAA}/W_{NEAA}	75.38	73.08	73.84	77.32	61.11
W_{DAA}/ W_{TAA}	36.61	36.01	36.92	37.68	40.97

Values with different superscripts in the same row are significantly different ($P < 0.05$). An * denotes delicious amino acids (DAAs). N is the mean number of diploid or triploid samples in each group.

Table 3

Average weights and lengths of the diploid and triploid rainbow trout

Group	Age	Ploidy	Weight (g)	Length (cm)	Sample number
I	12 months	2n	100.17±10.01	18.88±0.94	15
		3n	98.46±9.88	18.39±0.75	15
II	16 months	2n	302.5±34.79	25.5±2.70	15
		3n	364.7±29.52	29.6±3.11	15

Quantitative real-time PCR

mRNA gene expression levels were determined via quantitative real-time PCR using SYBR Premix Ex Taq (TakaRa) and a 7500 Real-Time PCR System (Applied Biosystems). β-actin was employed as the reference gene.

Statistical analysis

All statistical analyses were conducted using SPSS 19.0 software for Windows. The data are expressed as the mean±SD, and means were analysed with the Duncan's multiple range test and the non-parametric test. $P < 0.05$ was considered to indicate significant differences.

RESULTS

Proximate composition

The results regarding the proximate composition of the muscle determined in the fillets from diploid and triploid rainbow trout are shown in Table 1.

The ash contents of the diploid and triploid rainbow trout did not differ significantly ($P > 0.05$) in any of the groups. The moisture, protein and lipid contents of all of the triploid rainbow trout groups were significantly higher ($P < 0.05$) than those in the diploid groups. Several factors, including the species, age, environment and conditions and, especially, the type and availability of food, are believed to be important factors that contribute to the variations in the nutritional value of the fish. As a reference, lipid content values were determined and were all found to be higher than in wild rainbow trout and lower than in farmed rainbow trout (Fallah 2011) but the commercial feed given to these fish has a higher protein and lipid content than this experiment.

Amino acid composition

The amino acid compositions of the diploid and triploid rainbow trout muscles are listed in Table 2.

In group II, the contents of three non-essential amino acids (Gly, Cys and Pro) were significantly higher ($P < 0.05$) in the diploid rainbow trout than in the triploid fish. In contrast, two essential amino acids (Lys and Ile) and three non-essential amino acids (Asp, Ala and Tyr) displayed significantly higher contents ($P < 0.05$) in the triploid rainbow trout than in the diploid fish. The contents of the other amino acids did not differ between the fish.

The amino acid composition is one of the most important nutritional qualities of protein, and AASs are used to evaluate protein quality worldwide (IQBAL 2006). The AASs and CSs obtained are shown in Table 4. Seven AASs

Table 4

Comparative analysis of diploid and triploid rainbow trout AASs and CSs (mg g^{-1})

Amino acids	Whole egg protein	FAO/WHO	Group I				Group II			
			2n		3n		2n		3n	
			AAS	CS	AAS	CS	AAS	CS	AAS	CS
Threonine (Thr)	2.92	2.50	0.91	0.78	0.91	0.78	0.89	0.76	0.95	0.82
Valine (Val)	4.11	3.10	0.88	0.67	0.89	0.67	0.86	0.65	0.86	0.65
Methionine (Met) + Cysteine (Cys)	3.86	2.20	1.66	0.95	2.03	1.16	1.71	0.97	1.40	0.80
Isoleucine (Ile)	3.31	2.50	0.81	0.61	0.84	0.63	0.79	0.60	0.87	0.66
Leucine (Leu)	5.34	4.40	0.90	0.74	0.92	0.75	0.87	0.72	0.95	0.78
Phenylalanine (Phe) + Tyrosine (Tyr)	5.65	3.80	0.79	0.53	0.80	0.54	0.77	0.52	0.84	0.57
Lysine (Lys)	4.41	3.40	1.19	0.92	1.19	0.92	1.13	0.87	1.24	0.96
Total	29.6	21.9	21.72		22.7		19.95		21.87	
EAAI	—	—	72.68		75.56		71.11		73.56	

were similar or slightly higher in the triploid fish than in the diploid fish. The W_{EAA} contents of the diploid and triploid rainbow trout were similar to the FAO/WHO patterns, and the W_{EAA} contents of the triploid fish were slightly higher than those of the diploid fish.

Gene expression

The specific growth rate (SGR) from 12 to 16 months of diploid and triploid rainbow trout is 0.39% and 0.47%. And both diploid and triploid rainbow trout muscle displayed decreased MyoD1 and MATN2 expression from 12 to 16 months. MyoD1 mRNA expression was higher in triploid rainbow trout than in diploid rainbow trout in both groups ($P < 0.05$). There were no

significant differences in MyoD expression between groups I and II for the triploid fish ($P > 0.05$), whereas the diploid fish in group II showed significantly lower MyoD expression than those in group I ($P < 0.05$) – Figure 1*a*. Similarly, MSTN2 mRNA expression was higher in the diploid fish than the triploid fish in both groups ($P < 0.05$); however, no significant differences were observed between groups I and II in either the diploid or triploid rainbow trout ($P > 0.05$) – Figure 1*b*.

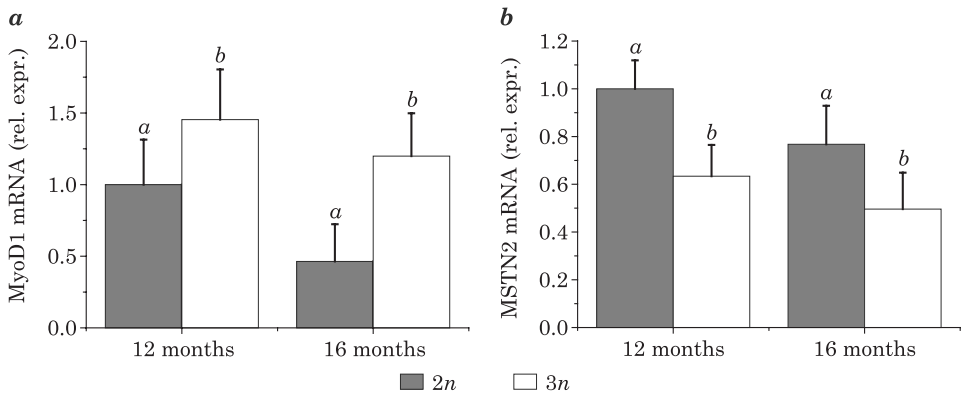

Fig. 1. MyoD1 (a) and MSTN2 mRNA (*b*) expression in diploid and triploid fish. Different superscripts indicate significant differences between the groups ($P < 0.05$)

DISCUSSION

Proximate composition

A combination of high protein and lipid contents leads to good flesh quality with favourable sensory characteristics, such as flavour and texture (Liu 2008). Generally, triploid fish are expected to exhibit a growth advantage after sexual maturity, as the somatic growth of diploid fish is usually suppressed by the reproductive process (Koedprang 2000). Thus, the high lipid contents of triploid fish may be due to this difference. Fish present good palatability when the fresh muscle lipid content ranges from 3.5% - 4.5% (Liu 2002). The analyses conducted in the present study indicated that the lipid contents of all groups of triploid rainbow trout conformed to these levels, while those of the diploid groups did not. Thus, triploid rainbow trout exhibit better protein and lipid contents than diploid rainbow trout.

Amino acid composition

The protein compositions of the examined rainbow trout contained the highest levels of Glu (10.87% -11.78%), followed by Asp, Lys and Leu, in de-

creasing amounts, with His (1.49%-1.68%) showing the lowest level in both diploid and triploid rainbow trout. As a nitrogen donor during purine and pyrimidine synthesis, glutamine is essential for cell proliferation. The rainbow trout proteins were also rich in L-aspartic acid and lysine, which is the limiting amino acid in cereal-based diets. However, the contents and proportions of muscle amino acids were basically the same in the diploid and triploid fish, which was in accord with a report on tench (Kizak 2013). Thus, the amino acid profile of rainbow trout muscle displayed a conservative pattern. The differences in the amino acid profiles could be related to different aspects, such as feed utilisation, the retention of amino acids and the AA profile in the fish body (Rodehutscord 1997) as well as temperature, salinity and storage time.

The nutritional value of a protein source is primarily based on its essential amino acid contents and bioavailability. In this study, the total amino acid (W_{TAA}), total essential amino acid (W_{EAA}), total non-essential amino acid (W_{NEAA}) and total delicious amino acid (W_{DAA}) contents did not differ significantly ($P > 0.05$) between the diploid and triploid rainbow trout. The W_{EAA} to W_{NEAA} and W_{EAA} to W_{TAA} indices were higher in the triploid fish than in the diploid fish. These results showed that the W_{EAA} to W_{TAA} and W_{EAA} to W_{NEAA} indices for all of the groups were comparable to the reference values of nearly 40% and above 60%, respectively, recommended by the FAO/WHO, which indicates that both diploid and triploid rainbow trout may be considered high-quality protein food sources. The level of total delicious amino acid (W_{DAA}) components, including Glu, Asp, Ala and Gly, was higher in triploid fish in groups I and II than in diploid fish (Table 2). It has been suggested that these free amino acids are related to the characteristic fish flavour (Ruiz-Capillas 2004) and that different contents of these AAs may cause variations in fish flavour. The ratio of W_{DAA} to W_{TAA} ranged from 36.61% to 36.92% in the diploid fish and 36.01% to 37.68% in the triploid fish (Table 4). These values were similar to those reported for Masu salmon (37.31%-37.46%) (Wang et al. 2015) but higher than those reported for *Silurus asotus* L. (31.83-32.33%) (Jiang 2012). The living environment, including salinity and rearing temperatures, might affect the nutritional composition of farmed fish, including their flavour (Gomes 2001). These results demonstrate that the protein contents of the rainbow trout muscle (especially the triploid rainbow trout muscle) were of high quality and displayed a well-balanced essential amino acid composition.

According to the AASs obtained, the sum of the sulphur-containing amino acids (Met and Cys) presented the highest score among both the diploid and triploid rainbow trout proteins (Table 4), indicating that rainbow trout is rich in Met and Cys. Met is important for the synthesis of cysteine and S-adenosyl methionine, which is a methyl group donor for methylation reactions. Met is essential in the diet for producing taurine, which exhibits clear antihypertensive effects (Wiley 1986). Lys is an important amino acid in

salmonids and can supplement the corresponding deficiency in plant proteins. The sum of the aromatic amino acids (Phe and Tyr) presented the lowest score among both the diploid and triploid rainbow trout proteins. Phe can be described as the first limiting amino acid in both diploid and triploid rainbow trout. The essential amino acid index (EAAI) can reflect the proximity of the essential amino acid content to standard proteins. The EAAIs of both the diploid and triploid rainbow trout decreased gradually with age, although the EAAIs of the triploid fish remained higher than those of the diploid fish in each group. Thus, the muscle protein quality of triploid rainbow trout is better than that of diploid rainbow trout, suggesting that triploid rainbow trout is an ideal nutritional food source.

Gene expression

Muscle growth continues for the duration of a fish's life due to both myoblast proliferation and myotube hypertrophy (Rowlerson 2001). Decreased MyoD1 mRNA expression in muscle with increasing age has also been observed in Pacu (Almeida 2008). The opposite pattern was observed for MSTN2 expression levels. In zebrafish, MSTN1 is expressed at extremely low levels in the early embryonic development, whereas MSTN2 is expressed throughout the growth development. MSTN gene expression differs within a fish organism, with higher expression being detected in muscle, suggesting that its basic function is associated with muscle growth (Terova 2013). The main function of MSTN in fish is similar to that in mammals: inhibiting the production and growth of muscle. In the present study, triploidisation may have affected MyoD1 and MSTN2 mRNA transcription in muscle tissues. Based on results reported in the literature, MSTN mRNA transcription is influenced by MyoD (Wagner 2005). The findings of the present study are relevant to the above hypothesis, especially considering the observation that MyoD1 mRNA expression was higher, while MSTN2 mRNA expression was lower in triploid fish than in diploid fish of the same age. The MyoD1 and MATN2 expression may also associated with SGR. These results also demonstrate that triploid fish exhibit better growth than diploid fish.

We demonstrated the differences in MyoD and MSTN gene expression between diploid and triploid rainbow trout for the first time. These findings may be useful for studies aimed at evaluating nutrient regulation related to fillet growth in other fish species. Although the underlying mechanisms remain to be thoroughly elucidated through future research, the elimination/reduction of the inhibitory action of MSTN could be utilised to greatly augment muscle growth, including growth hormone – GH, insulin-like growth factor I – IGF1 (Biga 2004, Garikipati 2012). It is also possible that MSTN gene deletion may be the most favourable mechanism for enhancing flesh composition. The pursuit of these goals will require further studies and investigation of the MSTN gene.

CONCLUSIONS

This study showed that triploid rainbow trout grew better than diploid rainbow trout. Triploid rainbow trout specimens (12 and 16 months of age) were found to exhibit significantly higher muscle lipid and protein contents than their diploid counterparts. Rainbow trout protein presents a well-balanced composition of essential amino acids. The methionine and cysteine score was the highest among the proteins of both diploid and triploid fish. MyoD1 expression is higher while MSTN2 expression is lower in triploid than in diploid rainbow trout. The differences in MyoD1 and MSTN2 expression are due to the sterility of triploid fish. In conclusion, triploid rainbow trout exhibit higher nutritional quality, better protein resources and a greater growth potential than diploid rainbow trout.

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