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ORIGINAL ARTICLE

Characterization and dietary regulation of glutamate dehydrogenase in different ploidy fishes

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Abstract Glutamate dehydrogenase (GDH) plays a crucial role in amino acid deamination and has been used as an inductor of nutrients metabolism. In this study, we cloned and analyzed the GDH cDNAs in diploids (red crucian carp), triploids and tetraploids and characterized their expression profiles upon dietary treatments. Results showed a high sequence similarity of GDH among the three kinds of ploidy fishes and other vertebrates. Expression analysis revealed that GDH exhibited a distinct spatial pattern of expression in different types of fishes. The triploids and tetraploids had higher levels of expression than diploids in heart, liver, gill, muscle, foregut and mid-gut. The GDH expression was also developmentally regulated with a stronger expression around blastula stage. The maternal GDH transcripts were first detected from eggs and their expression dropped down from the gastrula stage to heart beat stage. Adult triploids showed the highest levels of GDH expression in liver during breeding season which may contribute to the good appetite and fast growth. In addition, triploids exhibited high growth rates and excess GDH expression compared with other two types of fishes. The liver GDH enzyme activities were also higher in triploids than red crucian carp and tetraploids. Moreover, GDH expression is regulated by dietary protein levels. Fish fed with either high or low protein diets showed higher levels of GDH expression. In summary, our results demonstrated for the first time that the different ploidy fishes had different patterns of GDH mRNA expression during development, breeding and non-breeding seasons, and as well dietary effects from different protein levels in diet. These data indicate that abundant GDH expression may play an important role in growth rates in triploids.

Keywords Glutamate dehydrogenase · Red crucian carp · Triploid · Tetraploid · Embryogenesis · Dietary protein level

Introduction

Compared with other vertebrates, teleosts require higher protein diets to obtain amino acids for protein synthesis, energy metabolism and glucose formation. The demand for high-protein diet to achieve maximum growth in fish is due to the aquatic mode of life, ammonotelism and poilothermy poikilotherm (Kaushik and Seiliez 2010; Li et al. 2009). Thus, the better understanding of amino acids metabolism in fish is beneficial for aquaculture application.

Amino acids metabolism and utilization could be divided into two steps in fish. The first step involves the deamination and/or transamination of amino acids into an intermediate. The second step is the utilization of the intermediate for protein synthesis. Glutamate dehydrogenase (GDH) is an essential enzyme required for glutamate metabolism. GDH reversibly catalyses deamination of glutamate with the production of ammonia as a byproduct participating in nitrogen cycle (Rønnestad et al. 1999).

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Therefore, GDH could be considered as a significant marker/regulator for protein utilization and/or ammonia excretion.

It has been reported that long-term starvation could mobiles muscle protein in several fish species, such as sockeye salmon (*Oncorhynchns nerka*) (Mommsen et al. 1980), rainbow trout (*Oncorhynchus mykiss*) (Smith 1981), red sea bream (*Chrysophrys major*) (Woo and Fung 1981) and American eel (*Anguilla rostrata*) (Moon 1983), and results in high levels of free amino acids in plasma. Furthermore, high-protein diets promote free amino acids in plasma in rainbow trout (Cowey et al. 1977) and Japanese eel (*Anguilla japonica*) (Ogata et al. 1985). High concentration of free amino acids induces deaminated action catalyzed by GDH leading to higher rates of ammonia excretion (Alexis and Papaparaskeva-Papoutsoglou 1986). High levels of GDH deaminating enzyme activities have been observed in rainbow trout and blackspot seabream (*Pagellus bogaraveo*) (Figueiredo-Silva et al. 2010). However, a different response has been reported in gilthead seabream (*Sparus aurata*), that high glutamic acid content dietary reduced GDH activity (Gómez-Requeni et al. 2003).

Recently, researchers have started to intensively focus on connections between growth and metabolism factors (Picha et al. 2009; Stelling et al. 2002; Li et al. 2008). Increase appetite or greater efficiency in nutrients metabolism was reported to enhance growth rate (Silverstein et al. 2000). Nutrients metabolism is linked with muscle growth and protein synthesis (Li et al. 2008, 2011; Chu et al. 2011; Ding et al. 2011; Zhang et al. 2011; Yao et al. 2008). It is obvious that the relation of efficient growth and protein metabolism needs to be elucidated. Up to date, protein utilization indicator, details of GDH expression pattern and its biological function in aquatic animals are still limited.

In our laboratory, we have successfully obtained different ploidy fishes, including diploids, triploids and tetraploids, by interspecies crossing and reverse crossing technologies of red crucian carp with common carp (*Cyprinus carpio*) (Babiak et al. 2002; Boris 2003; Liu et al. 2001). The triploids population is highly valuable in aquaculture due to its resilience and sterile traits and it displays the faster growth rate compared with diploids and tetraploids. These different ploidy fishes provide an important biological platform for comparative studies of cyprinid fishes in fish biology and aquatic application (Liu et al. 1999, 2000). Though the sterile appearance and genome variation has been investigated, little attention has been paid to understand its mechanism of growth rate and protein metabolism. Especially, the information of GDH expression pattern and regulation in different ploidy fishes is still unveiled. Hence, in the present study, we cloned GDH cDNAs from the livers and studied their expression pattern in different tissues of different kinds of fishes. Embryonic and seasonal GDH expression differentiation in the three kinds of fishes was assayed by realtime PCR. The GDH activity of different ploidy fishes in liver was assayed. We also extended our study to understand the influence of dietary protein. This might also provide molecular evidence to improve our knowledge of the protein metabolism in different ploidy fishes.

Materials and methods

Animals and tissue preparation

The allotetraploid hybrids ($4n = 200$) were produced by the interspecific hybridization of red crucian carp (*Carassius auratus* red var.) (♀) ($2n = 100$) and the common carp (*Cyprinus carpio* L.) (♂) ($2n = 100$) (Liu et al. 2001; Sun et al. 2003; Liu 2010). The triploid hybrids were made by crossing the females of the red crucian carp with the males of the allotetraploid hybrids. The diploid red crucian carp, triploid hybrids and tetraploid hybrids were from the Engineering Centre of Polyploidy Fish Breeding of the National Education Ministry located at Hunan Normal University. After animals were anaesthetized by 2-phenoxyethanol (Sigma), eight different tissues, including heart, liver, kidney, gill, muscle, fore-gut, mid-gut and hind-gut samples were collected and immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

RNA isolation and cDNA synthesis

Total RNAs were extracted from the collected tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The integrality of RNA samples were tested by agarose gel electrophoresis and the quality of RNA samples were determined by calculating through the A260/A280 and A260/A230 ratios spectrophotometer (BioPhotometer Eppendorf). Potential genomic DNA contamination was eliminated by incubation with DNase I for 60 min at 37 °C (Fermentas, Vilnius, Lithuania) eliminated potential genomic DNA contamination. First-strand cDNA synthesis was carried out from 1 μg total RNA using AMV reverse transcriptase (Fermentas, Vilnius, Lithuania) with oligo $(dT)_{12–18}$ primer.

Cloning of full length cDNA of GDH

The degenerated primers were designed based on conserved sequences of GDH in other teleosts (Table 1). Polymerase chain reaction (PCR) amplifications were done for 35 cycles with denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 30 s. The products were separated with 2 % agarose gel and then

Table 1 Primers used for GDH cloning and quantitative real-time PCR

Primer name	Primer sequence (from 5′–3′)	Purpose
GDH-S	GACAACGGVGAATGGGA	CDS
GDH-A	CCTGGCTRATGGGYTTAC	CDS
GDH-3-1	GGGTTTCGGTAATGTGGGTCT	3′ RACE
GDH-3-2	GAGAAACAACTGACCAGGAAGAACG	3′ RACE
GDH-5-1	GCTCCACCAAATGGCACA	5′ RACE
GDH-5-1	GGAGTTCTGTGCTGRCTGT	5′ RACE
GDH-RT-S	CACAACACAGTCAGCACAGAACTC	Real-time PCR
GDH-RT-A	ACCAAATGGCACATCCACAACAG	Real-time PCR
β-Actin-S	GCTCTTCCCCATGCAATCCT	Real-time PCR
β-Actin-A	GGTTCCCATCTCCTGCTCAA	Real-time PCR

cloned and sequenced. Subsequently, the full length cDNA was amplified using the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA). Specific nested PCR primers were designed based on the obtained sequences (Table 1). For 3′ RACE, the amplifications were done under the conditions: 94 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min with 30 cycles. For 5′ RACE, the amplification conditions were: 94 °C for 30 s, 65 °C for 30 s, 72 °C for 1 min with 30 cycles.

Quantitative real-time PCR

GDH mRNA expression was performed using quantitative real-time PCR in a ABI PRISM® 7500 Real Time PCR System (Applied Biosystems). The primers were designed based on the obtained sequences (Table 1) and β-actin was used as an internal control. In order to eliminate endogenous DNA contamination, RNA was digested by DNase I prior to the cDNA synthesis. 5 μL of first-strand cDNAs (in a dilution of 1:20) as templates was added to 20 μL PCR solution containing 20 nmol/L primers and SYBR Green PCR Master Mix (ABI). The procedure was repeated three times for each sample: 50 °C 5 min, 95 °C 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 45 s. The dissociation curve was used to verify single product generation at the end of the assay and the analysis of relative mRNA expression was performed using the $2^{-\Delta\Delta C_t}$ method.

Determination of the tissue distribution of GDH transcripts

RNAs were isolated from 8 different tissues in red crucian carp (body weight 112.57 ± 6.79 g, $n = 5$), triploids (body weight 159.18 ± 2.58, $n = 4$) and tetraploids (body weight 120.58 ± 5.94, $n = 5$). After the cDNAs were synthesized, the GDH differential expressions of eight kinds of tissues in three different ploidy fishes were assayed by quantitative real-time PCR.

Determination of GDH expression during early development

Embryos of different developmental stages of red crucian carp (for each stage $n = 4$), triploids (for each stage $n = 4$), and tetraploids (for each stage $n = 4$) were collected under the microscope. Total RNAs were isolated with Trizol reagent and subsequently reverse-transcribed to generate cDNAs as described above. GDH differential expression during pattern of embryogenesis was determined by quantitative real-time PCR.

Expression of GDH in adult among different ploidy fishes

In order to analyze the GDH expression in adult fish of the three different ploidy fishes, we analyzed the GDH mRNA levels in the liver during the breeding season and after the reproduction season ($n = 4$ for each different ploidy fishes). For each ploidy fish, three adult individuals were randomly collected in breeding season (April) or non-breeding season (November). After RNA isolation and cDNA synthesis, the transcripts expression levels of GDH were assayed by quantitative real-time PCR.

GDH activity assay among different ploidy fishes in breeding season

The GDH activity assays were detected in breeding season (April) in liver of different ploidy fishes ($n = 4$ for each different ploidy fishes). All the fish were obtained in the same pool. The procedure was performed individually on homogenates of livers of red crucian carp, triploid and tetraploid according to Regnault (1993). The supernatant protein was estimated by Bradford method. Conditions of the assays were 850 μl 80 mM imidazole buffer (pH 8.0) and 10 μl 1 M NaCl. We used 40 μl enzyme extract and 50 μl 20 mM NAD (the final volume is 1 ml). The GDH activity was determined from the slope of NAD reduction to NADH

recorded at 340 nm (=6.22 × 10^{-3}) using a GESYS spectrophotometer at room temperature. Results were expressed as mIU (μmol NADH formed min^{-1} mg^{-1} protein).

Dietary protein regulations

To assay the growth rates and GDH expression in red crucian carp, triploids and tetraploids, the three kinds of fishes (0.5 years old, $n = 8$ for each ploidy fishes) were cultured in one tank. After 1 week adaptation, all the fishes were fed with 32 % protein level diet for 1 month. To calculate the food intake differentiation among red crucian carp, triploid and tetraploid, the fish were fed in excess a weighed amount of pellets and the uneaten feed was siphoned out from the tanks immediately and were dried using an electrical fan at 28 °C. Then the number of uneaten pellets was counted for the calculation of food intake in each tank during each feeding.

To reveal the effects of dietary protein levels on triploids liver GDH expression, five isocaloric diets with five dietary protein levels at 22, 27, 32, 37 and 42 %, respectively were formulated (Table 2). The amino acids analyses of the diets were previously described by Yin et al. (2009) and the amino acids compositions of the diets are summarized in Table 3. Juvenile triploids were cultured in five fiberglass tanks (12.5 m^2) tanks ($n = 10$) and adapted to the experiment diets for 1 week before starting the experiment. Then, fishes were fed with different diets with 22, 27, 32, 37 and 42 % protein levels for 1 month, respectively.

All the diets were prepared by thoroughly mixing dry ingredients with oil and then adding water until a stiff dough resulted. The dough was then passed though a meat-mincer equipped with a 2-mm die, and the resulting strands were dried using an electrical fan at 28 °C. After drying, the material was broken up into regular pieces sieved to a convenient pellet size and stored at −20 °C.

All the fishes used in this study were weighed before and after the experiment. Water quality parameters were monitored throughout the feeding trial. The growth performance was evaluated. The indexes for the assessment of growth performance were calculated as follows: weight gain (%) = 100 × ($W_t - W_0$)/W_0, where, W_0 is the initial weight and W_t is the final weight. Feed conversion ratio (FCR) = food intake (g)/weight gain (g). The GDH mRNA expression levels from livers were estimated using real-time PCR.

Table 2 Experimental diet compositions (g/kg)

Ingredients	Diets (%)				
	22	27	32	37	42
Fish meal	12	20	28	36	44
Soybean meal	32	32	32	32	32
Wheat meal	8	8	8	8	8
CaH_2PO_4	1	1	1	1	1
Wheat starch	37	29	20	12	3
Cod liver oil	2	2	3	3	4
Soybean oil	3	3	3	3	3
Carboxymethyl cellulose	2	2	2	2	2
Vitamin and mineral premix[a]	3	3	3	3	3
Proximate nutritional components					
Crude protein (%)	22	27	32	37	42
Digestible energy (kJ/g)[b]	14.9	14.7	14.5	14.3	14.2

[a] Vitamin and mineral premix provided by DSM, S.A. de C.V.

[b] Digestible energy estimated using the following coefficients: 15 kJ for carbohydrates, 35 kJ for lipids and 23 kJ for protein

Table 3 Analyzed amino acid composition of the experimental diets (%)

Item	Diets (%)				
	22	27	32	37	42
Indispensable amino acids					
Arginine	1.29	1.43	1.83	2.01	2.04
Histidine	0.77	0.97	1.09	1.23	1.34
Isoleucine	1.01	1.24	1.40	1.57	1.72
Leucine	1.63	2.02	2.32	2.59	2.77
Lysine	1.40	1.82	2.17	2.44	2.57
Methionine	0.10	0.17	0.21	0.27	0.28
Phenylalanine	1.02	1.12	1.38	1.52	1.58
Threonine	0.71	0.93	1.05	1.21	1.32
Tyrosine	0.50	0.60	0.72	0.87	0.96
Valine	1.00	1.17	1.38	1.55	1.66
Dispensable amino acids					
Alanine	0.94	1.25	1.45	1.68	1.81
Aspartate + asparagine	2.16	2.55	2.78	3.13	3.51
Glutamate + glutamine	2.52	3.57	3.94	4.20	5.28
Glycine	0.99	1.36	1.56	1.85	2.02
Serine	0.47	0.58	0.73	0.83	0.88

Statistical analysis of data

Data, expressed as means ± SEM, were analyzed by one-way analysis of variance. The normality and constant variance for experimental data were tested by the Levene's test (Wei et al. 2012). If data did not have homogenous variance, they underwent logarithm transformation to meet the necessary assumptions of analysis of variance (Wei et al. 2012). Differences among treatment means were determined by the Tukey's multiple range test. All statistical analyses were performed using SPSS 13.0 software

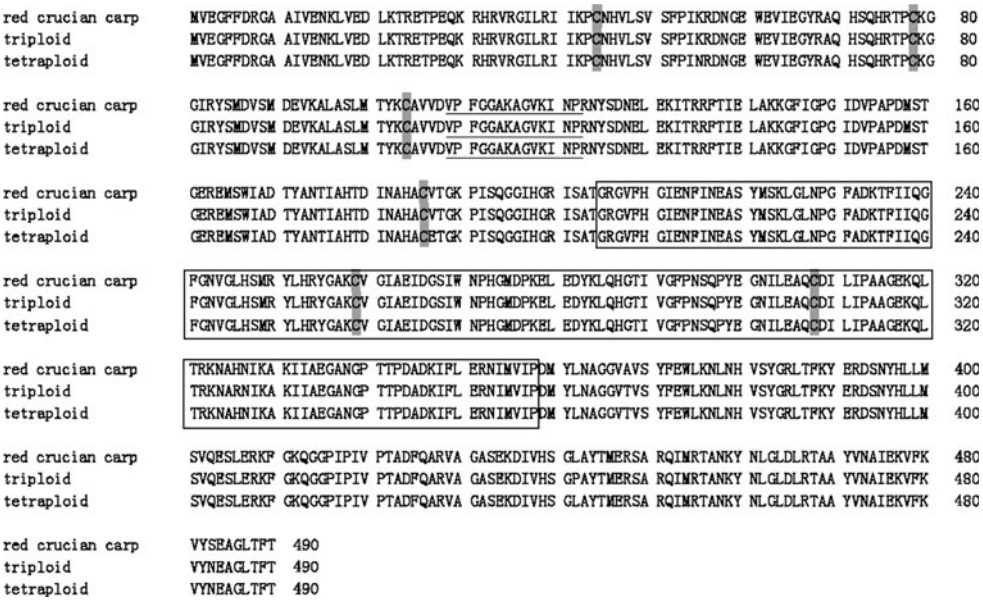

Fig. 1 Amino acid alignment of GDH proteins of red crucian carp, triploid and tetraploid. Six conserved cysteine residues were showed in *gray background*. Glu/Leu/Phe/Val dehydrogenases active site and Glu/Leu/Phe/Val dehydrogenases domain were *underlined* and *boxed*, respectively

(Chicago, IL, USA). P values <0.05 were taken to indicate statistical significance.

Results

Cloning and sequence analysis of GDH cDNAs from the different ploidy fishes

GDH cDNAs were isolated from the liver cDNAs library of diploid red crucian, triploid and tetraploid. The GDH cDNA clones we isolated from diploids, triploids and tetraploids were 2,601, 2,258 and 2,356 bp, respectively (Genbank accession code: JN634757, JN634758, JN634759). Each cDNA contains a 1,470-bp open reading frame which encodes an identical 290 amino acids peptide. A Glu/Leu/Phe/Val dehydrogenases active site and a Glu/Leu/Phe/Val dehydrogenases domain as characters of GDH are present in the deduced protein. Six cysteine residues were found in each GDH proteins (Fig. 1). The 5′ untranslated terminal region (UTR) of red crucian carp, triploids and tetraploids were 310, 212 and 143 bp, respectively. The 3′ UTR were 822, 576 and 733 bp, and each 3′ UTR contains a polyA tail.

Sequence homology and phylogenetic analysis

The deduced amino acid sequences of red crucian carp, triploids and tetraploids were analyzed with ClustalW. The results revealed that a highest sequence homology with other teleosts (91–98.2 % similarity), such as *T. hakonensis*, zebrafish, Atlantic salmon and rainbow trout. Lowest sequence similarity values were found with amphibians (91.6–91.8 %), mammals (87.6–88.6 %) and invertebrates (70.4–74.3 %). A phylogenetic tree was constituted with Mega 4.1 software using Neighbor-joining method (Fig. 2). The tree displayed two main branches, invertebrates and vertebrates.

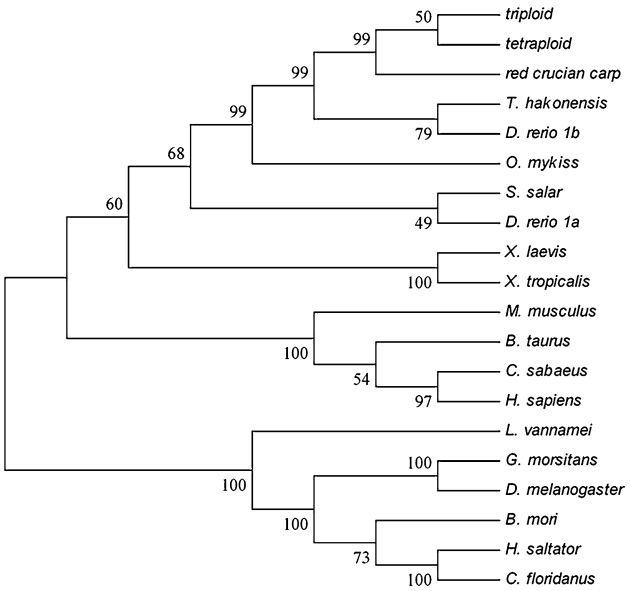

Fig. 2 Neighbor-joining phylogenetic tree of GDH proteins. The GenBank protein accessions were: *Danio rerio* GDH 1a, NP_997741.1; *Danio rerio* GDH 1b, NP_955839.2; *Tribolodon hakonensis*, BAD83654.1; *Salmo salar*, CAD58716.1; *Oncorhynchus mykiss*, CAD11803.1; *Xenopus laevis*, NP_001087023.1; *Xenopus tropicalis*, NP_001011138.1; *Mus musculus*, EDL24875.1; *Chlorocebus sabaeus*, AAU11837.1; *Homo sapiens*, BAD96538.1; *Bos taurus*, AAN15276.1; *Litopenaeus vannamei*, ACC95446.1; *Harpegnathos saltator*, EFN77465.1; *Camponotus floridanus*, EFN70808.1; *Glossina morsitans*, ADD20040.1; *Drosophila melanogaster*, CAA 72173.1; *Bombyx mori*, NP_001040245.1. The number on the branches represents 1,000 bootstrap values

Tissue expression pattern of GDH mRNAs in different ploidy fishes

The tissue expression of GDH was analyzed in different ploidy fishes by quantitative real-time PCR. The GDH mRNAs were expressed in all the assayed tissues. In red crucian carp, GDH mRNAs were highly expressed in liver, kidney and fore-gut, whereas they were less expressed in gill and muscle. In triploid, the high GDH expression were found in liver, fore-gut and mid-gut and lowest expression were found in gill, muscle and hind-gut. In tetraploid, liver and fore-gut showed the highest GDH expression levels, whereas the lowest levels of expression was found in gill, muscle and hind-gut. Overall, GDH expression levels varied among different tissues including heart, liver, gill, muscle, fore-gut and mid-gut and the mRNAs expressions were significantly lower in red crucian carp of these six tissues ($P < 0.05$) (Fig. 3). No significant differential expressions were found between triploid and tetraploid in the tissues assayed ($P > 0.05$).

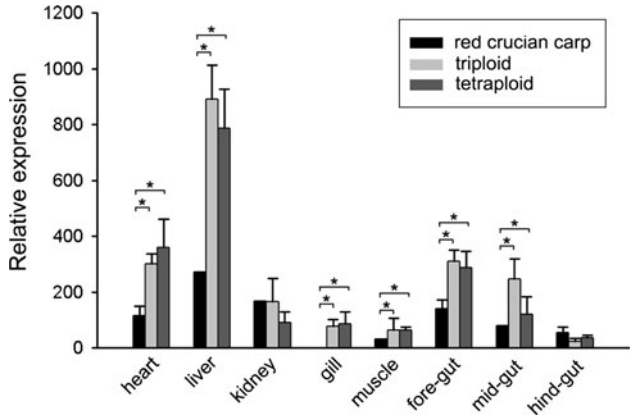

Fig. 3 GDH mRNA expression at different tissues of adult red crucian carp, triploid and tetraploid. β-Actin was used as an endogenous control (mean ± SE of relative expression; red crucian carp, $n = 5$; triploid, $n = 4$; tetraploid, $n = 5$). *Asterisks* indicate statistical differences between different ploidy tissues ($P < 0.05$)

Differential expression of GDH mRNAs during embryonic development

The expression pattern of GDH during embryogenesis was determined in the three kinds of fishes by quantitative real-time PCR. GDH expression persisted throughout the embryogenesis. GDH transcripts were first detected as maternal mRNAs in fertilized (or unfertilized) eggs and the expression levels decreased significantly from the gastrula to heart beat stage in all the three fishes. A small increase was observed after the eye pigment stage. However, the expression levels were much lower than the maternal expression levels.

Among the three types of fishes, the diploid red crucian carp showed the lowest GDH mRNAs levels during embryogenesis compared with the triploid and tetraploid in all developmental stages analyzed. Triploids showed the highest GDH mRNAs expression levels at levels at the blastula stage. In addition, the triploids also showed higher levels of GDH expression at 4-cell, muti-cell and hatching stages ($P < 0.05$) compared with the diploid and tetraploid fish embryos (Fig. 4).

Differential expression of GDH mRNAs in adults among different ploidy fishes

The levels of GDH mRNA expression was determined in non-breeding and breeding seasons in liver of adult red

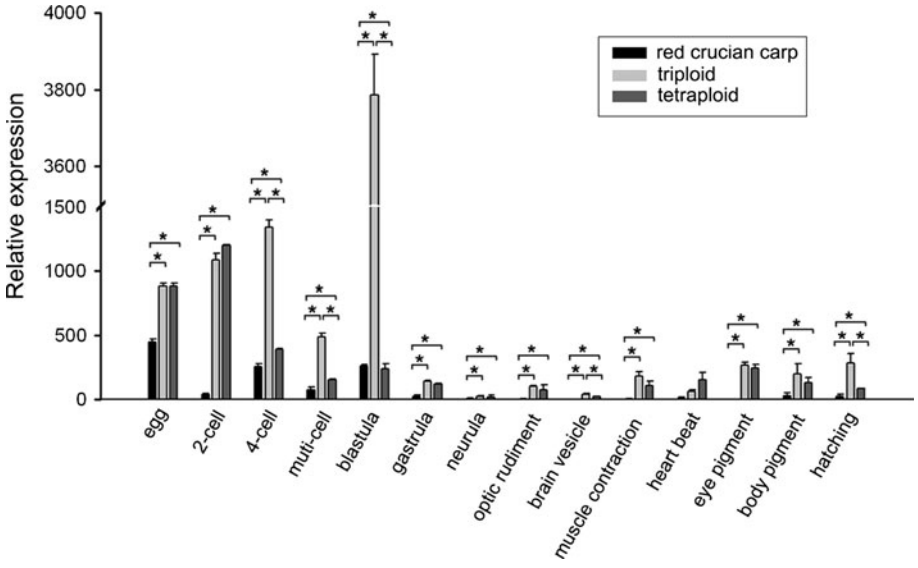

Fig. 4 GDH gene expression in during embryonic development of red crucian carp, triploid and tetraploid (mean ± SE of relative expression; $n = 4$ for each group). *Asterisks* indicate statistical differences between different ploidy tissues ($P < 0.05$)

crucian carp, triploid and tetraploid by real-time PCR analysis. In non-breeding season, red crucian carp showed the lowest levels of GDH expression compared with triploid and tetraploid ($P < 0.05$). However, no significant expression difference was detected between triploid and tetraploid ($P > 0.05$). During breeding season, triploid showed significant high levels of GDH transcripts compared with other two types of fishes ($P < 0.05$) (Fig. 5).

GDH activity assay differentiation among different ploidy fishes

After determined the mRNA GDH expression among the three kinds of fishes, we calculated GDH enzyme activity in these fishes in livers. The triploids showed the highest GDH activity with significant difference compared with red crucian carp and tetraploids. However, no significant difference was found between red crucian carp and tetraploids (Fig. 6).

Growth rates and GDH expression among different ploidy fishes with same protein diets

The growth rates were determined in the three different ploidy fishes fed with the same diets (32 % protein diets) (Table 4). The initial weight of red crucian carp, triploids and tetraploids had no significant difference. However, after one month feeding with the 32 % protein diets, triploids showed a significant faster growth rate compared with other two kinds of fishes. Meanwhile, tetraploids exhibited higher growth rate than red crucian carp. Similarly, triploids showed the highest levels of GDH expression in the liver after one month of feeding with this diet, while lowest

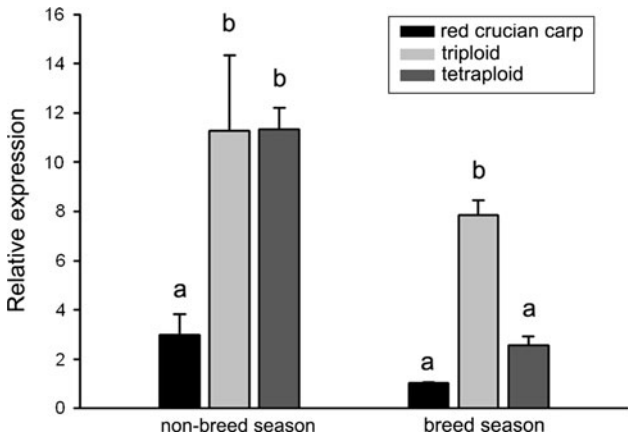

Fig. 5 GDH gene expression pattern in non-breed season and breed season of adult red crucian carp, triploid and tetraploid (mean ± SE of relative expression; $n = 3$ for each group). *Different characters* represent significant difference ($P < 0.05$)

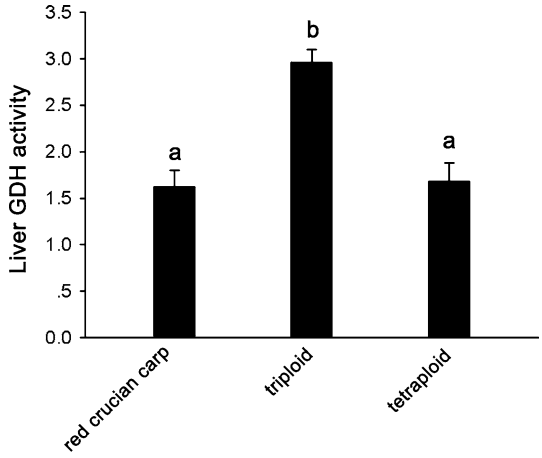

Fig. 6 Liver GDH activity differentiation among different ploidy fishes (mean ± SE of relative expression; $n = 4$ for each group). *Different characters* represent significant difference ($P < 0.05$)

levels of GDH mRNA expression were found in red crucian carp (Table 4).

Effects of diets with different protein levels on triploids

To determine the effect of diets with different protein levels on GDH expression, we fed triploids with five different types of diet with various concentrations of dietary protein. After feed with different protein level diets for 1 month, the GDH mRNAs expression levels in triploids were assayed by real-time PCR. The data showed that fish fed with the low dietary protein (22 %) showed highest levels of GDH transcripts, 32 % protein diet had the lowest levels of GDH expression (Fig. 7). Diets with 27, 37 and 42 % protein concentration had a intermediate levels of GDH transcripts expression.

Discussion

The present study reported the full GDH cDNA sequences from diploid, triploid and tetraploid fish. All the putative proteins contain 290 amino acids which include the Glu/Leu/Phe/Val dehydrogenases active site and the Glu/Leu/Phe/Val dehydrogenases domain. The GDH cDNAs of the three different ploidies exhibited high similarity with other vertebrates sharing over 70.4 % identity with invertebrates and more than 90.8 % with other teleosts. The results strongly suggest that the proteins are evolutionarily conserved. As earlier reports, zebrafish contains two isoforms (GDH 1a and GDH 1b) (Cambier et al. 2012). The topology of the phylogenetic tree indicated that the GDH cDNAs we cloned were more similar to zebrafish GDH 1a. Accordingly, the phylogenetic tree constructed for different

Table 4 Growth rates, daily feed intake, feed conversion ratio, and GDH mRNA expression with 32 % protein diets among different ploidy fishes

Indexes	Groups		
	Red crucian carp	Triploids	Tetraploids
Initial weight (g)	58.12 ± 0.71[a]	59.62 ± 0.41[a]	59.35 ± 0.51[a]
Final weight (g)	108.37 ± 3.34[a]	166.21 ± 1.02[b]	119.13 ± 2.20[c]
Growth rates (100 %)	83.6 ± 7.40[a]	178.5 ± 9.64[b]	101.1 ± 5.03[b]
Daily feed intake (g)	3.82 ± 0.81[a]	5.89 ± 0.94[b]	3.95 ± 0.42[a]
Feed conversion ratio (FCR) (g/g)	2.16 ± 0.17[a]	1.66 ± 0.22[b]	1.98 ± 0.12[a]
The relative abundance of GDH mRNA	2.48 ± 0.09[a]	2.86 ± 0.24[b]	1.04 ± 0.12[c]

Different characters represent significant difference ($P < 0.05$)

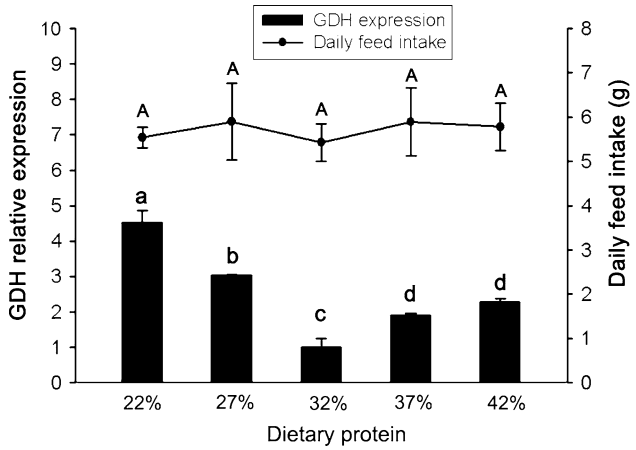

Fig. 7 Triploid GDH gene expression and daily feed intake with different protein levels (mean ± SE of relative expression; $n = 10$). *Different characters* represent significant difference ($P < 0.05$)

animals grouped the diploid, triploid and tetraploid fish among other teleosts, indicating high evolutionary conservation of GDH in fish species.

As glutamine is the most concentrated free amino acid in plasma. The metabolic pathways and physiological process factors involved in glutamine metabolism were found in many tissues, including brain, skeletal muscle, liver, kidney, intestine, adipose tissue, lung and lymphocytes in mammals (Curthoys and Watford 1995; Stumvoll et al. 1999; Wu et al. 2007, 2009; Li et al. 2007; Boutry et al. 2012). The widespread patterns of GDH expression in various tissues of red crucian carp, triploids and tetraploids were very similar to that in mammalians. GDH mRNAs were ubiquitously expressed in all the tissues analyzed in this study with the highest levels of expression in livers, hearts and fore-guts. Unlike mammals, ploidy fish could be produced by hybridization in teleosts (Otto and Whitton 2000; Dowling and Secor 1997) which may be due to their effective compensation or a lack necessity for dosage-compensation mechanism. Pala and colleagues reported that in a triploids hybrid fish one of three alleles were silenced (Pala et al. 2008), which provided an evidence of gene-copy silencing in triploid teleost. However, our data suggested that both triploids and tetraploids had higher levels of GDH expression compared with diploid red crucian carp. We presume that gene-copy silencing mechanism was depended on tissues or genes in triploids and tetraploids teleosts. However, more data in teleosts should be presented to prove the conclusion. Furthermore, we detected GDH activity in liver among the fishes. The result showed that the highest GDH activity in triploids. The high activity of GDH may contribute to food appetite and result in faster growth rates.

It is known that liver plays a central role in nitrogen metabolism and it is the main expression site of GDH. High levels of GDH mRNAs expression were found in livers from three different ploidy fishes in different seasons. Both in non-breeding season and breeding season, triploids showed significant expression compared with red crucian carp. The triploid population displayed the fastest growth rate comparing with diploid and tetraploid (Liu et al. 2000). GDH has been confirmed as marker of nutrients metabolism, especially in protein metabolism. Hernandez et al. (2009) reported that amino acids and their metabolites play a key role in growth and feed intake. Recent researches suggested that high activities of GDH could conduce to greater efficiency of protein metabolism, which may enhance growth (Silverstein et al. 2000). Leggatt et al. (2009) confirmed that that in growth hormone transgenic coho salmon (*Oncorhynchus kisutch*) had higher activities of GDH than that in control. Another study reported that dietary supplementation of glutamate could significantly increase growth rates (Allen 1994). However, no affect of GDH transcripts differentiation were found in the study. Our data revealed that triploids with fast growth rate had a significant high GDH expression in liver. The results suggested that the fast growth rate of triploids was related with excess GDH expression. Interestingly, tetraploids showed no significant difference of GDH expression during non-breeding season, but in the triploids a significant difference was found during breeding season. It had been proved that triploids are sterile, while tetraploids are fertile. During breeding season,

tetraploids has normal developed gonad in both males and females. Thus, triploids consume less energy in reproduction compared with tetraploids and the protein metabolism activities of triploids remains on a higher level.

GDH could be a useful indicator of the metabolic utilization of dietary components by fish. We analyzed the growth rates and levels of GDH mRNA expression among different ploidy fishes fed with 32 % dietary protein diets. Triploids displayed the highest growth rates and GDH mRNA levels, which in consistent with the previous results that high GDH mRNAs expression may contribute to the fast growth in triploids.

The effect of dietary on the activity of GDH is contradictory to some other fish species (Cowey and Walton 1989). In gilthead seabream, a reduction on the specific activity of GDH was observed after feeding with high dietary glutamic acid content (Gómez-Requeni et al. 2003). In contrast, rainbow trout showed an increasing GDH activity after feeding with high dietary glutamic acid (Moyano et al. 1991). However, there is still lack information of sterile triploids with different protein level diets. Our study indicated that the mRNA expressions of GDH were related with protein dosage in triploids. Hepatic GDH expressions were higher in fish fed with high-protein diet, which suggested that higher levels of dietary protein accounts for considerable amount of amino acids synthesis or energy production (Treberg et al. 2010). Meanwhile, after long time malnourished, with low protein diet, the GDH expressions were raised to accelerate the protein metabolism and meet the compensatory growth (Cowey and Walton 1989). These results showed that different dietary protein levels could regulate GDH mRNA expression in triploids.

In conclusion, this study first reported the cDNA sequences and ontogenetic expression of GDH in three ploidy fishes. Triploids and tetraploids had higher expression than diploids in all the studied tissues and embryonic stages. Furthermore, triploids showed the highest expression during different seasons, which may contribute to the good appetite and fast growth. The diets containing 32 % protein after one month feeding exhibited a fast growth rates and higher GDH expression in triploids. Therefore, high expressions of GDH mRNAs were confirmed in triploids which may be important for the fast growth rates and protein utilization.

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