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Comparison of taurine biosynthesis ability between juveniles of Japanese flounder and common carp

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Summary. This study was conducted to investigate taurine deficiency and the ability of taurine biosynthesis in both juvenile Japanese flounder (JF) and juvenile common carp (CC) in vivo using low taurine level diets. Three different taurine level diets were prepared by the supplementation of taurine to the basal composition (JF – 0, 0.5 and 1.5% in JF; CC – 0, 1, 3% in CC). The final average body weight and feed efficiency of JF fed the JF – 1.5% was significantly higher than those of fish fed on the JF – 0%. On the other hand, no significant difference was observed in CC fed with CC – 0, 1, and 3% diets. The taurine retention rate was negative in the case of JF-fed with the taurine-free supplement (JF – 0%). On the other hand, the taurine retention rate was about 280% in the case of CC-fed with the taurine-free supplement (CC – 0%). These findings indicate that while taurine is essential for growth of JF, it is not essential for the growth of CC.

Keywords: Taurine-biosynthesis-juvenile – *Paralichthys olivaceus* – *Cyprinus carpio*

Introduction

Taurine (2-amino ethanesulfonic acid) is a free intracellular amino acid that is involved with many important biological functions including membrane protection, detoxification and antioxidation in mammals (Chesney, 1985). Taurine serves as an organic osmolyte in the brain and kidney as the process of cell volume regulation that is especially critical following hypo- or hyper-osmolar stresses (Chesney et al., 1998). Taurine also protects various functions of retinal rod outer segments, due to its role in osmoregulatory, antioxidant and ion regulatory activities (Huxtable, 1992). In lipid metabolism, the roles of taurine include conjugation with bile acids in the liver which increase the use of the bile acids, the degradation of cholesterol metabolites and the participation in micelle formation contributing to fat absorption in the small intestine (Yokogoshi et al., 1999). In mammals, the major pathway for taurine biosynthesis from cysteine involves the oxygenation of cysteine to cysteine sulfinate, followed by decarboxylation to hypotaurine and then to taurine (Griffith, 1987). Cysteine sulfinate decarboxylase (CSD) plays an important role in the pathway. CSD catalyzes of the decarboxylation reaction of cysteine sulfinate to form hypotaurine. Taurine has also been shown to be an essential dietary sulfur amino acid for cats because cats are inherently deficient of CSD, the key enzyme of taurine biosynthesis (Knopf et al., 1978; De La Rosa and Stipanuk, 1985; Sturman et al., 1986). The offspring of taurine-deficient female cats have a large number of neurological defects, including tapetal and retinal degeneration, delayed cerebellar granule cell division and migration and abnormal cortical development (Chesney et al., 1998).

Fish meal is known to be the best source of essential amino acids required by finfish and shrimps (De Silva and Anderson, 1995). Amino acids are also considered to act as a feed attractant, and De Silva and Anderson (1995) suggested that amino acids may act as growth promoters. However, which amino acids have this function has yet to be determined. Taurine is one of the candidate amino acids that have potential as a growth promoter. Taurine is the most abundant amino acid among the free amino acids

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profiled in marine animals and plants (Sakaguchi and Murata, 1988). However, taurine is not contained in alternative protein sources such as soybean meal, cottonseed meal, and casein. Yokoyama and Nakazoe (1991) reported that taurine is accumulated in the whole body, liver and muscle when rainbow trout are fed with dietary sulfur amino acids, and taurine excretion was also detected; and they concluded that rainbow trout has taurine biosynthesis ability. Subsequently, Yokoyama et al. (2001) reported that the activity of cysteine sulfinate decarboxylase differs in vitro among fish species. CSD activity is high in rainbow trout, but is low in Japanese flounder and common carp. However, common carp show no adverse physiological effects in experiments where taurine-free diets were used, such as casein diet in vivo (Fontagne et al., 2000; Carvalho et al., 2004). On the other hand, Kim et al. reported that taurine supplementation improved the growth performance and feeding behavior of juvenile Japanese flounder in vivo (Kim et al., 2003, 2005a, b). This study was conducted to investigate the taurine biosynthesis ability of juvenile Japanese flounder (two different development stages) and common carp by feeding low taurine fish meal diets to these two species whose CSD activities rank low in in vitro studies.

Materials and methods

Diet formulation

The formulation and the percentage of crude protein and lipid in the experimental diets are shown in Table 1. Since brown fish meal contains about 6 mg/g of taurine, the fish meal was washed 3 times with 70% ethanol to remove the taurine. This washing treatment also results in the removal of some kinds of attractants. Free amino acid (FAA), IMP (inosine 5′-monophosphate) and inosine were analyzed in both the washed fish meal and the non-washed fish meal using an amino acid analyzer and the high performance liquid chromatography (HLPC) method of Tsuchimoto et al. (1985). To compensate for the leaching effect of washing, attractants were added to the diets to adjust their contents to the original levels of the non-washed fish meal (Table 2).

Three experimental diets for juvenile Japanese flounder (JF) were prepared based on the washed-fish meal as a protein source supplemented with taurine 0, 0.5 and 1.5% (JF – 0, 0.5 and 1.5%), respectively. Similarly three experimental diets for juvenile common carp (CC) were prepared based on the washed-fish meal as a protein source but supplemented with taurine 0, 1 and 3%, respectively (CC – 0, 1 and 3%), because the requirement for taurine supplementation had not previously been determined for common carp. All ingredients were mixed together with distilled water to make a mash, pelleted with a press machine and then dried for 24 h in a freeze-dryer (Nissei, Tokyo, Japan). The free amino acid contents (mg/g) of the experimental diets are shown in Table 3. Crude protein and lipid contents were adjusted to 55% and 10% in the JF and CC diets. The calibrated taurine levels in JF – 0% diet, JF – 0.5% diet and JF – 1.5% diet were 0.6 mg/g, 5.6 mg/g and 16 mg/g, respectively. The calibrated taurine levels of CC – 0% diet, CC – 1% diet and CC – 3% diet were 0.6 mg/g, 9.5 mg/g and 27.2 mg/g, respectively. Since the taurine content of fish meal is high (about 6 mg/g), the taurine content of washed fish meal remained at a level of about 10% compared with that of the non-washed fish meal.

Table 1. Composition and crude protein and lipid contents of the experimental diets

Composition of the basal diets for Japanese flounder						
Ingredients	JF[1] – 0%	JF – 0.5%	JF – 1.5%	CC[2] – 0%	CC – 1%	CC – 3%
Brown fish meal (washed)	74.0	74.0	74.0	72.0	72.0	72.0
Lipid	2.2	2.2	2.2	2.2	2.2	2.2
Attractant[3]	0.8	0.8	0.8	0.8	0.8	0.8
Taurine	0.0	0.5	1.5	0.0	1.0	3.0
α-Starch	8.0	8.0	8.0	8.0	8.0	8.0
Dextrin	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose	1.9	1.4	0.4	3.9	2.9	0.9
n-3HUFA[4]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[5]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[6]	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E[7]	0.1	0.1	0.1	0.1	0.1	0.1
Chemical analysis						
Crude protein (%)	54.5	55.8	55.4	54.2	55.1	54.9
Crude lipid (%)	9.8	10.5	9.8	9.9	9.8	10.2
Taurine (%)	0.06	0.56	1.60	0.06	0.95	2.72

[1] *JF* Means taurine supplemented experimental diet for Japanese flounder
[2] *CC* Means taurine supplemented experimental diet for common carp
[3] See Table 2
[4] *n-3 HUFA* n-3 highly unsaturated fatty acids (EPA, 7.6%; DHA, 38.5% Nippon Chemical Feed Co. Ltd., Chiba, Japan)
[5,6] From Kim et al. (2003)
[7] *DL*-α-tocopheryl acetate, purity 50% (Nippon Roche, Tokyo, Japan)

Table 2. Attractant composition used in the experimental diets

Attractant	
Supplementation of trace free amino acids	
Histidine	375.2
Alanine	105.8
Glysine	25.2
Glutamic acid	45.1
Proline	25.9
Supplementaion of nucleotide-related compounds	
Inosine	135.4
IMP[1]	87.4
Total	800 mg/100 g in diet

All attractants are produced from Wako Pure Chemical Industries Ltd., Osaka, Japan
[1] *IMP* Means inosine 5′-monophosphate

Experimental fish

Three feeding experiments were conducted at the National Research Institute of Fisheries Science, Yokohama, Japan. Juvenile Japanese flounder used in this experiment were obtained from the Nisshin Marine Tech, Aichi, Japan. Common carp were obtained from the Yoshida Field Station,

Table 3. Free amino acid composition (dry basis mg/g diet, mean ± SD, $n = 5$) of experimental diets

Free amino acid composition (mg/g diet) of the Japanese flounder diets						
Amino acid	JF – 0%	JF – 0.5%	JF – 1.5%	CC – 0%	CC – 1%	CC – 3%
Essential amino acid						
Arginine	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.01	0.09 ± 0.00	0.08 ± 0.00
Lysine	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.16 ± 0.01	0.15 ± 0.00	0.15 ± 0.00
Histidine	4.66 ± 0.07	4.22 ± 0.47	4.86 ± 0.63	4.14 ± 0.05	4.13 ± 0.01	4.01 ± 0.01
Phenylalanine	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.08 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
Tyrosine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Leucine	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.05 ± 0.00
Isoleucine	0.11 ± 0.00	0.11 ± 0.01	0.10 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.02
Methionine	0.14 ± 0.00	0.12 ± 0.01	0.13 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Valine	0.25 ± 0.01	0.26 ± 0.00	0.26 ± 0.00	0.06 ± 0.01	0.00 ± 0.00	0.04 ± 0.06
Threonine	0.08 ± 0.00	0.08 ± 0.02	0.08 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01
Non-essential amino acid						
Taurine	0.56 ± 0.00	5.56 ± 0.66	16.00 ± 2.92	0.62 ± 0.01	9.45 ± 0.54	27.20 ± 0.54
Cystathionine	0.23 ± 0.00	0.25 ± 0.01	0.24 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
Alanine	1.22 ± 0.01	1.25 ± 0.05	1.18 ± 0.02	1.09 ± 0.02	1.14 ± 0.04	1.09 ± 0.12
Glycine	0.35 ± 0.00	0.35 ± 0.00	0.35 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01
Glutamic acid	0.65 ± 0.04	0.63 ± 0.02	0.64 ± 0.01	0.58 ± 0.04	0.58 ± 0.01	0.58 ± 0.03
Serine	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Aspartic acid	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00
Proline	0.22 ± 0.01	0.25 ± 0.05	0.26 ± 0.01	0.25 ± 0.04	0.25 ± 0.05	0.28 ± 0.01

Tokyo University of Marine Science and Technology, Shizuoka, Japan. In Experiment I for JF, replicates of 30 fish (average body weight 0.3 g) were randomly placed in each of six 60-L aquariums ($60 \times 35 \times 30\,cm^3$). In Experiment II for JF, replicates of 10 fish (average body weight 3.7 g) were placed randomly into each of six 60-L aquariums. Fish of two tanks in each experiment were fed on one of the three experimental diets, JF – 0, 0.5 and 1.5%, respectively. In the Experiment for CC, replicates of 20 fish (average body weight 4.8 g) were randomly placed in each of six 60-L aquariums. Fish of two tanks in each experiment were fed on one of the three experimental diets, CC – 0, 1, 3%, respectively. In the three experiments, fish were fed three times a day to satiation for 4 weeks at a water temperature of 20 °C. At the end of the feeding trial, fish were weighed and stored at −80 °C for subsequent analysis of free amino acids. Three fish from each aquarium were also fed the respective experimental diet for an additional 2 days. These fish were placed immediately in separate plastic flasks containing 1 L of water at 20 °C and then starved for 24 h. After 24 h, a water sample (50 ml) from each plastic flask was collected to determine the rate of taurine excretion using the method of Yokoyama and Nakazoe (1991).

Free amino acids (FAA) analysis and taurine retention rate

For Experiment I of JF and Experiment of CC, five frozen fish from each tank were dissected and the brain, liver, eyes and muscle were used for free amino acids (FAA) analysis. For Experiment II of JF, three frozen fish were used for this analysis. Another five frozen fish (three frozen fish in Experiment II of JF) were used for FAA analysis of the whole body. The whole body and tissues were homogenized with 2% sulfosalicylic acid and centrifuged at $2300 \times g$ for 15 min at 5 °C. For determination of the excreted taurine and NH_3, water samples from each plastic flask were deproteinized by the addition of 10% sulfosalicylic acid, followed by centrifugation at $2300 \times g$ for 15 min at 5 °C. FAA levels were determined individually with an automatic amino acid analyzer (Model L-8500A, Hitachi, Tokyo, Japan). Taurine retention rates were estimated by subtraction of the initial fish taurine content from the final fish taurine content and divided by the total amount of taurine in diet using the method of Kim et al. (2003). Statistical analysis of growth performances and FAA accumulation in fish fed six diets were performed using one-way ANOVA and Tukey's multiple-range test.

Results

Growth

Growth performances of the juvenile Japanese flounder and juvenile common carp in the feeding experiments are shown in Table 4. The growth of juvenile Japanese flounder was improved by taurine supplementation. In Experiment I for JF, the final average body weight of juvenile flounder reared on the JF – 1.5% (2.4 g) and JF – 0.5% (2.0 g) diets were significantly higher compared to that of fish fed JF – 0% (1.0 g). The percent gain and feed efficiency increased with the increasing level of taurine content in the experimental diets. In Experiment II for JF, the final body weight of juvenile flounder was 14.5 g in the JF – 0.5 and 1.5% groups, respectively. The weight gain of juvenile flounder fed the JF – 0% diet was significantly lower among the weight gains of the experimental groups. Also, the feed efficiency of the JF – 0% group was significantly lower compared with the JF – 0.5 and 1.5% groups. In Experiment II for JF, the growth of juvenile flounder was not significantly different between the JF – 0.5 and 1.5% groups. This indicates that taurine

Table 4. Results of the 4-week feeding trials in the JF and CC experiments[§]

Kind of diets	Taurine content in diet (%)	Mean body weight* (g)		Percent gain (%)	Feed efficiency	Mortality (%)
		Initial	Final			
Experiment I of Japanese flounder (JF)						
0%	0.06	0.3 ± 0.1	1.0 ± 0.4[c]	294[c]	0.74[c]	1.6
0.5%	0.56	0.3 ± 0.1	2.0 ± 0.8[b]	656[b]	0.99[b]	3.3
1.5%	1.60	0.3 ± 0.1	2.4 ± 0.9[a]	818[a]	1.24[a]	3.3
Experiment II of Japanese flounder (JF)						
0%	0.06	3.7 ± 0.2	11.3 ± 2.8[B]	306[B]	1.69[C]	0
0.5%	0.56	3.7 ± 0.2	14.5 ± 2.4[A]	393[A]	1.86[B]	5.0
1.5%	1.60	3.7 ± 0.2	14.5 ± 2.3[A]	393[A]	1.91[A]	0
Common carp (CC)						
0%	0.06	4.8 ± 0.8	28.3 ± 8.0	595	1.20	0
1%	0.95	4.9 ± 0.7	28.2 ± 5.8	574	1.20	0
3%	2.72	4.8 ± 0.7	27.7 ± 6.7	573	1.20	0

[§] Water temperature 20.0 ± 0.2 °C

[A, B, C, a, b, c] Means with different superscripts within the same column are significantly different ($P < 0.05$)

* Mean ± SD

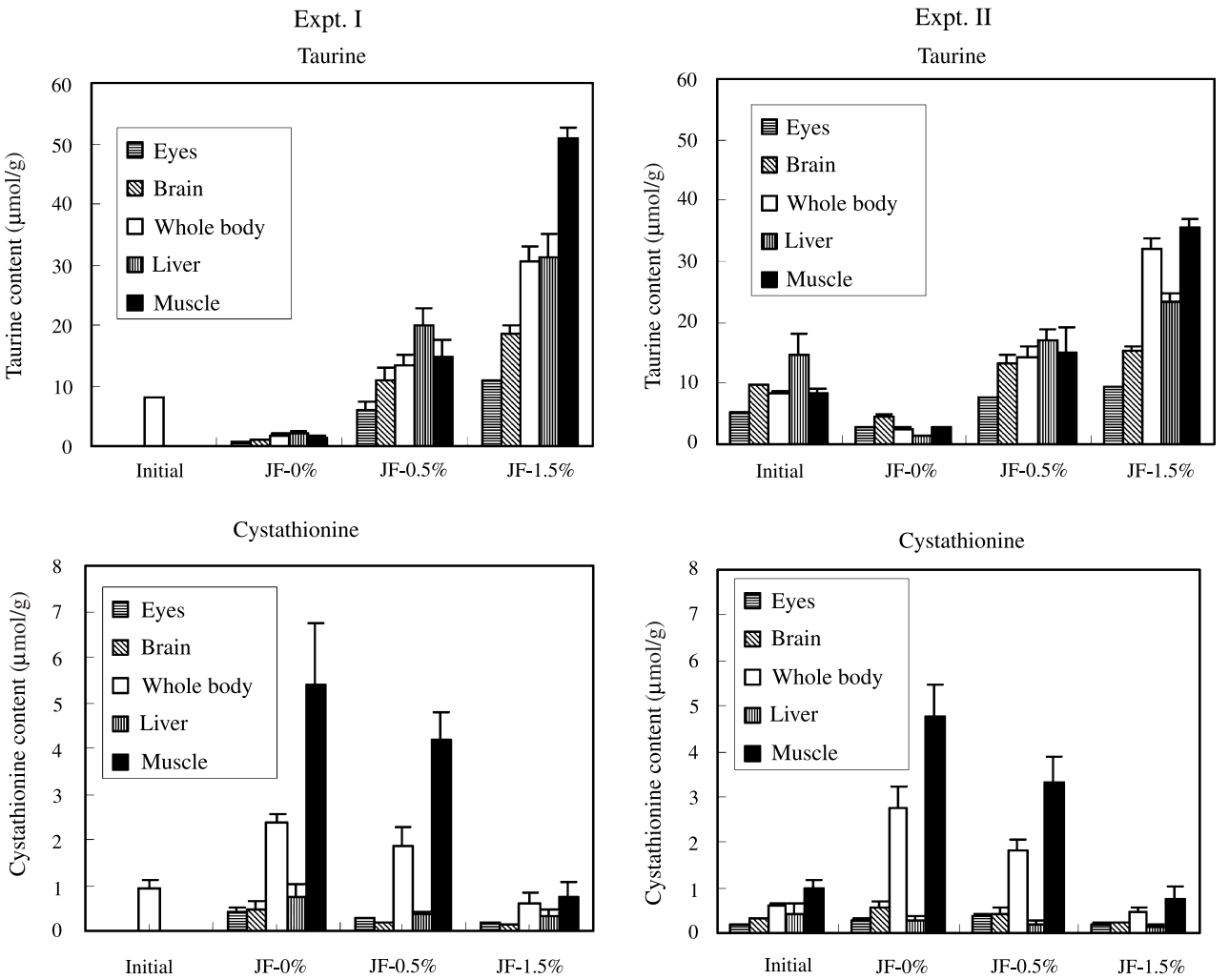

Fig. 1. Taurine and cystathionine content in the brain, liver, muscle, eyes, whole body of fish fed the experimental diets and initial whole body of juvenile Japanese flounder. Data are the means ± SD ($n = 5$ in Experiment I; $n = 3$ in Experiment II)

is more effective in the early growth stages than in the later, advanced growth stages of juvenile flounder. However, in the Experiment for CC, the growth and feed efficiency of juvenile common carp were not significantly different among CC – 0, 1 and 3% groups.

Accumulation and retention rate of taurine in juvenile flounder and carp

The taurine and cystathionine contents in the brain, liver, muscle, eyes and whole body of JF studied in Experiments I and II are shown in Fig. 1. Almost similar patterns can be observed in Experiments I and II for JF. Compared to the juvenile JF, fed JF – 0 and JF – 0.5% taurine supplemented diets, the taurine contents in the whole body and tissues of the JF – 1.5% group show marked increase. The taurine contents in the whole body and tissues of juvenile Japanese flounder increased with the increase in dietary taurine level. However the cystathionine contents in the whole body and tissues of juvenile Japanese flounder decreased with the increase in dietary taurine level. In Experiments I and II for JF, tau-

Table 5. Taurine and NH_3 excretion of Japanese flounder (JF) and common carp (CC)[§]

	Kind of diets		
	JF – 0%	JF – 0.5%	JF – 1.0%
Experiment I of JF			
Taurine	ND	ND	ND
NH_3	9.3 ± 2.7	8.7 ± 2.4	8.4 ± 2.7
Experiment II of JF			
Taurine	ND	ND	ND
NH_3	7.9 ± 2.9	8.2 ± 2.5	8.5 ± 2.4
	CC – 0%	CC – 1%	CC – 3%
Experiment of CC			
Taurine	ND	ND	ND
NH_3	14.2 ± 6.7	15.7 ± 4.4	14.9 ± 5.7

[§] µmol/g body weight per 24 h
Data are the mean ± SD ($n = 3$)
ND Not detected

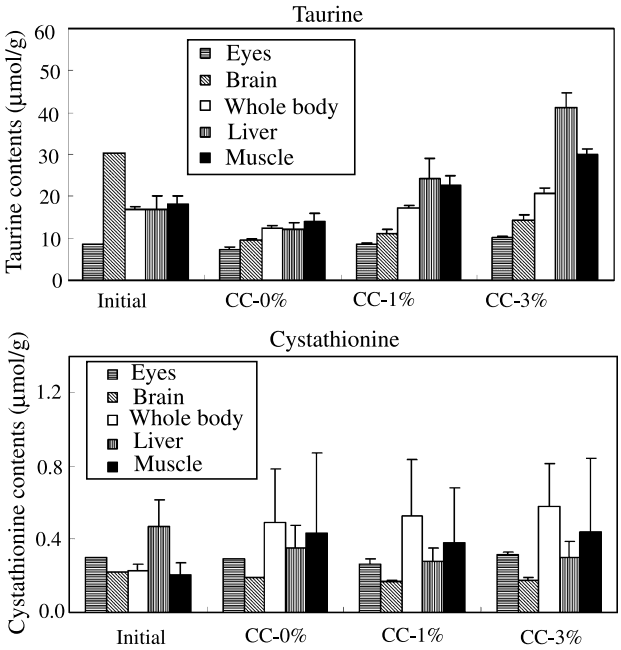

Fig. 2. Taurine and cystathionine content in the brain, liver, muscle, eyes and whole body of the initial and at the end of the feeding experiment for juvenile common carp. Data are the means ± SD ($n = 5$)

Table 6. Retention rates of taurine in the whole body in JF and CC experiments

Diet Taurine content (mg/g)	Total amount of taurine in diet (g)	Taurine content		Retention rate of taurine (%)
		Initial fish (g)	Final fish (g)	
Experiment I of JF				
0% (0.56)	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	−3.0
0.5% (5.56)	0.56 ± 0.07	0.01 ± 0.00	0.19 ± 0.03	31.4
1.5% (16.00)	1.62 ± 0.30	0.01 ± 0.00	0.53 ± 0.04	31.8
Experiment II of JF				
0% (0.56)	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.01	−7.9
0.5% (5.56)	0.87 ± 0.10	0.08 ± 0.00	0.49 ± 0.06	47.3
1.5%(16.00)	2.53 ± 0.46	0.08 ± 0.00	1.17 ± 0.07	43.0
Experiment of CC				
0% (0.62)	0.48 ± 0.01	0.40 ± 0.01	1.75 ± 0.08	279.0
1% (9.45)	7.23 ± 0.41	0.42 ± 0.02	2.45 ± 0.09	28.1
3% (27.20)	20.63 ± 0.41	0.41 ± 0.01	2.88 ± 0.15	12.0

Retention rate = (total taurine content of final fish – total taurine content of initial fish)/total amount of taurine in diet × 100

rine excretion was not detected in all groups (Table 5). The initial taurine contents of the whole body and tissues were different in Experiments I and II (JF) and Experiment CC. This can be attributed to differing taurine accumulation levels due to varying taurine composition levels in the feed diets and the period before the commencement of experiments.

In the Experiment for CC, the taurine and cystathionine contents in the brain, liver, muscle, eyes and whole body are shown in Fig. 2. The taurine contents in the whole body and tissues of juvenile common carp increased with the increase in dietary taurine level. The cystathionine contents in the whole body and tissues of juvenile common carp were not significantly different among the CC – 0, 1 and 3% groups. Furthermore, in the Experiment for CC, taurine excretion was not detected in all groups (Table 5).

The retention rates of taurine are shown in Table 6. The retention rates of taurine in juvenile Japanese flounder were calculated to be in the range of 31 to 47% for the JF – 0.5% and 1.5% groups and in the range of −8 to −3% for the JF – 0% group in Experiments I and II. However, the retention rates of taurine in juvenile common carp were calculated to be up to 12.0% for the CC – 3% group, 28.1% for the CC – 1% group and 279.0% for the CC – 0% group, respectively.

Discussion

In Experiment I for JF, the growth, percent body weight gain and feed efficiency were improved with the increase of taurine contents in the experimental diets. In Experiment II for JF, the growth and percent body weight gain were not significantly different between the JF – 1.5% and the JF – 0.5% group, although these feeding performances were higher than those of the JF – 0% group. On the other hand, the growth and feed efficiency of juvenile common carp were not significantly different among CC – 0, 1 and 3% groups. In this study, we found that taurine supplementation improved the growth and feed efficiency of juvenile Japanese flounder, but not juvenile common carp. Previously Park et al. (2000) reported that juvenile Japanese flounder fed a mysid meal diet contained higher levels of taurine than juvenile Japanese flounder fed on a fish meal diet. Park et al. (2001) also reported that fish fed a taurine-supplemented diet showed significantly better growth than those fed a non-supplemented fish meal diet. Kim et al. (2003) reported that taurine supplementation improved the growth of juvenile Japanese flounder but γ-amino butyric acid (GABA) or β-alanine supplementation did not lead to an improvement, and only taurine, not GABA or β-alanine, was accumulated in the whole body and tissues of juvenile Japanese flounder. Kim et al. (2005b) also suggested that the feeding behavior of juvenile flounder was affected by the dietary taurine supplementation and multiple feeding behaviors (Non-normal behavior) were observed in the taurine 0% group. Omura and Yoshimura (1999) suggested that the abundant taurine localization in the retinal photoreceptor and neural layers of juvenile Japanese flounder may be involved in the protection of the photoreceptor outer segment, the regulation of neural transmission, and photoreceptor differentiation during the developmental stages. This means that taurine is an essential amino acid for normal feeding behavior and predator avoidance during the ontogenetic development of juvenile Japanese flounder. Common carp however have a normal growth performance in experiments using casein diets (casein diets which do not include taurine) (Fontagne et al., 2000; Carvalho et al., 2004). In this study, common carp also showed normal growth and feeding behavior using the taurine non-supplemented diet. This indicates that taurine is an essential amino acid for juvenile Japanese flounder, but not for juvenile common carp.

In the present study, the taurine contents in the whole body and tissues of the JF and CC groups increased with the increase in the dietary taurine level. The taurine retention rates were −8 to −3% in the JF – 0% groups of Experiments I and II. That the taurine retention rate was 278% in the CC – 0% group indicates that the taurine accumulation of juvenile common carp at the end of experiment increased 3-fold compared to the taurine content supplied in the fed experimental diet. The major pathway of taurine synthesis from methionine and cysteine in

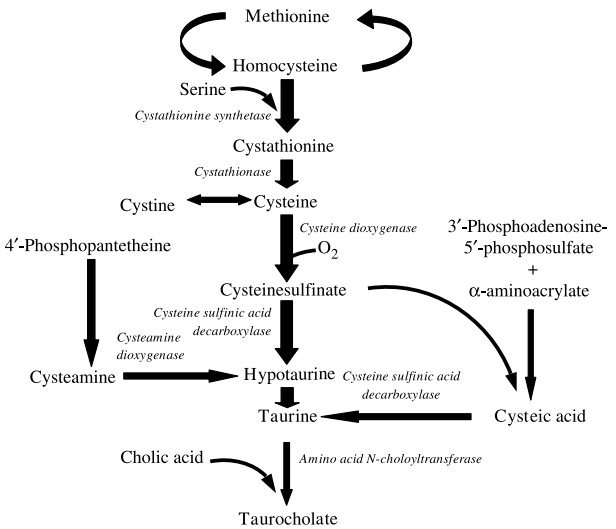

Fig. 3. Metabolic pathway of sulfur amino acids

mammals involves the oxidation of cysteine to cysteinesulfinic acid, the decarboxylation of cysteinesulfinic acid to hypotaurine and further oxidation of hypotaurine to taurine (Fig. 3; Griffith, 1987). From an experiment using methionine, cystine and taurine supplemented diets in vivo, Yokoyama and Nakazoe (1992) had demonstrated that rainbow trout synthesizes taurine from cysteine as a precursor. This result suggests that the cysteine metabolism in rainbow trout is similar to that in mammals. However, it has been found that the growth of juvenile Japanese flounder is improved by dietary taurine (Park et al., 2002; Kim et al., 2003, 2005a, b). Park et al. (2002) suggested that the taurine content of juvenile flounder is changed only by feeding of a taurine supplemented diet not that of a cystine supplemented diet. Yokoyama et al. (2001) reported that the activity of cysteine sulfinate decarboxylase (CSD), a key enzyme in the taurine biosynthesis, is different among fish species in vitro (yellowtail, bluefin tuna, skipjack, Japanese flounder, opaleye, rainbow trout and common carp). In general, CSD activity of fish is lower than that of a mammal, such as mouse or rat; the CSD activities are high in rainbow trout and opaleye; but very low in Japanese flounder and common carp; CSD seems lacking in yellowtail, bluefin tuna and skipjack.

In Experiments I and II for JF, the concentration of cystathionine in the whole body and muscle decreased with the increase of taurine in the experimental diets. But in the Experiment for CC, cystathionine contents were not significantly different among the CC – 0, 1 and 3% groups. This indicates that cystathionine may accumulate in the whole body and muscle of juvenile flounder when the fish is fed a diet without taurine supplementation. However taurine supplementation did not affect the cystathionine contents of the whole body and tissues of the CC groups. Cystathionine is the one of the intermediate products in the trans-sulfuration pathway from methionine to taurine (Finkelstein and Martin, 1986). In Japanese flounder, cystathionine might be accumulated due to taurine deficiency in the metabolic pathway of sulfur amino acids. This result also indicates that juvenile Japanese flounder do not have the ability to synthesize sufficient taurine for growth, although juvenile common carp have the ability to synthesize taurine. Juvenile common carp may use another pathway for taurine biosynthesis such as the cysteamine pathway or cysteic acid pathway (Fig. 3). Further studies are necessary to understand the pathway of taurine biosynthesis and excretion in juvenile common carp.

In the present study, the taurine retention rates were $-8\sim-3\%$ in JF – 0% group and 279% in CC – 0% group, respectively. Taurine excretion was not detected in all groups (Table 5). It is possible that the taurine was not synthesize or eliminated by conjugated formation. It is well known that taurine is the sole amino acid for conjugation with bile acid in the production of taurocholic acid or taurochenodeoxycholic acid which constitutes the major bile acids in the gall-bladder of Japanese flounder (Goto et al., 1996). Yeh and Hwang (2001) reported that the main bile acid in common carp was cyprinol sulfate with a value of more that 94% of the total bile component. Taurocholic acid and taurochenodeoxycholic acid are the main bile acids in flounder. This indicates that taurine may be eliminated by the taurine-conjugated bile acid system and dietary taurine improves the bile acid composition of juvenile Japanese flounder. Further studies are necessary to understand the physiological roles of taurine in the bile acid composition related with growth, feed efficiency and taurine accumulation in juvenile Japanese flounder.

In conclusion, we present evidence that the taurine supplementation of the experimental diet is related to the growth performance of only juvenile Japanese flounder, but not juvenile common carp. Taurine contents of the whole body and tissues increased with the increase in the dietary taurine level. Juvenile Japanese flounder have a low ability for taurine biosynthesis compared with that of juvenile common carp. This result indicates that taurine is an essential amino acid for juvenile Japanese flounder, but not essential for juvenile common carp.

References

Carvalho AP, Sa R, Oliva-Teles A, Bergot P (2004) Solubility and peptide profile affect the utilization of dietary protein by common carp (*Cyprinus carpio*) during early larval stages. Aquaculture 234: 319–333

Chesney RW (1985) Taurine: its biological role and clinical implications. Adv Pediatr 32: 1–42

Chesney RW, Helms RA, Christensen M, Budreau AM, Han X, Sturman JA (1998) The role of taurine in infant nutrition. Adv Exp Med Biol 442: 463–476

De La Rosa J, Stipanuk MH (1985) Evidence for a rate-limiting role of cysteine sulfinate decarboxylase activity in taurine biosynthesis in vivo. Comp Biochem Physiol 81B: 565–571

De Silva SS, Anderson TA (1995) The feed industry. In: De Silva SS, Anderson TA (eds) Fish nutrition in aquaculture. Chapman and Hall, London, pp 248–268

Finkelstein JD, Martin J (1986) Methionine metabolism in mammals: adaptation to methionine excess. J Biol Chem 261: 1582–1587

Fontagne S, Burtaire L, Corraze G, Bergot P (2000) Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth and lipid metabolism in common carp (*Cyprinus carpio* L.) larvae. Aquaculture 190: 289–303

Goto T, Ui T, Une M, Kuramoto T, Kihira K, Hoshita T (1996) Bile salt composition and distribution of the D-cysteinolic acid conjugated bile salts in fish. Fish Sci 62: 606–609

Griffith OW (1987) Mammalian sulfur amino acid metabolism: an overview. In: Jakoby WB, Griffith OW (eds) Methods in enzymology, vol. 143. Academic Press, New York, pp 366–376

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Kim SK, Takeuchi T, Yokoyama M, Murata Y (2003) Effect of dietary supplementation with taurine, β-alanine and GABA on the growth of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. Fish Sci 69: 242–248

Kim SK, Takeuchi T, Akimoto A, Furuita H, Yamamoto T, Yokoyama M, Murata Y (2005a) Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder *Paralichthys olivaceus*. Fish Sci 71: 627–632

Kim SK, Takeuchi T, Yokoyama M, Murata Y, Kaneniwa M, Sakakura Y (2005b) Effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder *Paralichthys olivaceus*. Aquaculture 250: 765–774

Knopf K, Sturman JA, Armstrong M, Hayes KC (1978) Taurine: an essential nutrient for the cat. J Nutr 108: 773–778

Omura Y, Yoshimura R (1999) Immunocytochemical localization of taurine in the developing retina of the lefteye flounder *Paralichthys olivaceus*. Arch Histol Cytol 62: 441–446

Park GS, Takeuchi T, Seikai T, Yoshinaga T (2000) The effect of residual salts and free amino acids in mysid meal on growth of juvenile Japanese flounder *Paralichthys olivaceus*. Nippon Suisan Gakkaishi 66: 697–704 (in Japanese)

Park GS, Takeuchi T, Seikai T, Yokoyama M (2001) The effects of dietary taurine on growth and taurine levels in whole body of juvenile Japanese flounder *Paralichthys olivaceus*. Nippon Suisan Gakkaishi 67: 238–243 (in Japanese)

Park GS, Takeuchi T, Yokoyama M, Seikai T (2002) Optimal dietary taurine level for growth of juvenile Japanese flounder *Paralichthys olivaceus*. Fish Sci 68: 824–829

Sakaguchi M, Murata M (1988) Taurine. In: Sakaguchi M (ed) Extractive components of fish and shellfish. Kouseisha-Kouseikaku, Tokyo, pp 56–65 (in Japanese)

Sturman JA, Gargano AD, Messing JM, Imaki H (1986) Feline maternal taurine deficiency: effect on mother and off-spring. J Nutr 116: 655–667

Tsuchimoto M, Misima T, Utsugi T, Kitajima S, Yada S, Yasuda M (1985) Method of quantitative analysis ATP related compounds on the rough sea-method of high-performance liquid chromatography using reversed-phase column. Nippon Suisan Gakkaishi 51: 1363–1369 (in Japanese)

Yeh YH, Hwang DF (2001) High-performance liquid chromatographic determination for bile components in fish, chicken and duck. J Chromatogr B Biomed Sci Appl 751: 1–8

Yokogoshi H, Mochizuki H, Nanami K, Hida Y, Miyachi F, Oda H (1999) Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. J Nutr 129: 1705–1712

Yokoyama M, Nakazoe J (1991) Effects of dietary protein levels on free amino acid and glutathione contents in the tissues of rainbow trout. Comp Biochem Physiol 99A: 203–206

Yokoyama M, Nakazoe J (1992) Accumulation and excretion of taurine in rainbow trout *Oncorhynchus mykiss* fed diets supplemented with methionine, cystine and taurine. Comp Biochem Physiol 102A: 565–568

Yokoyama M, Takeuchi T, Park GS, Nakazoe J (2001) Hepatic cysteinesulphinate decarboxylase activity in fish. Aquacult Res 32 (Suppl 1): 216–220

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