Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*

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Abstract

Two experiments were conducted to investigate the effect of dietary taurine and cystine on growth and body composition of juvenile red sea bream *Pagrus major*. In Experiment I, a casein-based semi-purified diet included a small amount of fish meal were supplemented with taurine at the levels of 0 (control) and 1.0 %. The experimental diets in Experiment II were without fishmeal and supplemented with taurine at 0 (control), 0.5, 1.0 and 2.0 % or cystine at 1.0 and 2.0%. These diets were fed three times a day for 6 weeks to fish (average body weight: 2.3g in Experiment I and 2.5g in Experiment II). In Experiment I, fish fed the taurine supplemented diet showed significantly ($P<0.05$) improved growth, feed efficiency and feed consumption relative to fish fed the unsupplemental diet. The whole body taurine content increased, whereas the non-essential amino acid contents decreased, in fish fed the taurine-supplemental diet compared to fish fed the unsupplemented diet. In Experiment II, the growth, feed efficiency and feed consumption of fish fed the taurine-supplmented diets, irrespective of the dietary taurine levels, were significantly higher than those of fish fed the control diet and the cystine-supplemented diets. Taurine content in the whole body increased with the dietary taurine level, while the taurine contents did not increase by the supplemental cystine. Other free amino acid contents in the taurine supplemented diet groups followed similar trends to those in Experiment I. These results indicate that supplemental taurine to a casein-based semi-purified diet at more than 0.5% improved the growth and feed performance of juvenile red sea bream. It is also suggested that juvenile red sea bream can not metabolize cystine into taurine.

Keywords: casein, cystine, growth, juvenile, metabolism, *Pagrus major*, taurine

1. Introduction

Taurine is one of the most abundant free amino acids in various tissues, and has been implicated to play important roles in numerous physiological functions including bile salt synthesis, osmoregulation, modulation of neurotransmitters, membrance stabilization, and antioxidation in mammals (Huxtable, 1992). Several studies have recently indicated that there are interspecific differences in the pathway and capacity of taurine biosynthesis in fish (Goto et al., 2001; Goto et al., 2003). The enzyme activity of cysteinesulfinate decarboxylase in red sea bream and Japanese flounder is only approximately half the levels of rainbow trout (Yokoyama et al., 2001), and thus this step is considered to be the rate limiting for taurine synthesis in the former species. In addition, taurine content in the muscle of rainbow trout (Yokoyama and Nakazoe, 1992) fed diets supplemented with taurine were remained at similar levels with each other irrespective of the dietary taurine contents. In contrast, taurine content in the whole body of juvenile Japanese flounder (Park et al., 2002; Kim et al., 2005), European sea bass (Martinez et al., 2004) and in the muscle of juvenile yellowtail (Matsunari et al., 2005), each fed diets supplemented with taurine increased with the increase in the dietary taurine level. These results suggest that marine fish depend on dietary taurine to maintain the body taurine pool.

Dietary taurine requirements in juvenile stages of marine fish have been reported in Japanese flounder (1.5-2.0%), yellowtail (>1.0%) and European sea bass (0.2%). In addition, Takagi et al. (2006) reported that the occurrence of green liver induced by highly inclusion of soybean meal in yearling red sea bream was prevented by the

supplementation of taurine. However, dietary taurine requirement of red sea bream has been determined neither in juvenile stage nor young or adult stages.

The present study aimed to investigate the effect of dietary taurine on the growth performance and whole body free amino acid composition including taurine in juvenile red sea bream. Since soybean meal contains various antinutritional factors (Francis et al., 2001), which may affect the physiology of fish, we used casein-based semi-purified diets in two feeding experiments in this study. The 1st experiment (Experiment I) was designed to evaluate the acceptability of a casein-based low-fish meal diet with and without supplemental taurine. As the result, juvenile red sea bream well accepted the diets. Then, the 2nd experiment (Experiment II) was conducted using a fish meal-free casein-based diet with graded levels of taurine. We also investigated the effect of supplemental cystine instead of taurine to determine the ability of taurine biosynthesis from cystine.

2. Materials and methods

2.1. Diet

The experimental diets were based on vitamin-free casein and gelatin as the main protein source. In Experiment I, a small amount of fish meal was also included (Table 1). Two experimental diets were supplemented with taurine (Wako Pure Chemical Industries, Osaka, Japan) at the levels of 0 (control) and 1.0 %. The experimental diets in Experiment II were without fishmeal and supplemented with taurine at 0 (control), 0.5, 1.0 and 2.0 % or cystine (Wako) at 1.0 and 2.0% (Table 2). The ingredients were thoroughly mixed, moistened by the addition of warm water (40°C), and made into pellets using a garlic crusher. The pellets were dried for 24h in a freeze-dryer (Nissei

Co., Ltd., Tokyo, Japan). The pellets were sorted with a mesh (1.4mm) to a uniform size and store at -20°C until fed to the fish.

2.2. Fish and feeding

Juvenile red sea bream for Experiments I and II were obtained from the Owase Station, Fisheries Research Division, Mie Prefectural Science and Technology Promotion Center, Mie, Japan and the Fish Nursery Center of Kinki University, Susami, Wakayama, Japan, respectively. Before the feeding experiments, the fish were stocked in 500L polycarbonate (PC) tanks and fed a commercial feed (Marubeni Nisshin Feed, Japan) for 10 days. Then, they were fed the respective control diet for further 11 days. Thirty fish (average body weight, 2.3 ± 0.0g) were allotted into 4 PC tanks with 100L water volume in Experiment I, and 30 fish (average body weight, 2.5 ± 0.5g) were allotted into 12 tanks in Experiment II. Each experimental diet in Experiments I and II were assigned to two replicate tanks of fish. Each rearing trial was conducted in a flow-through system supplied with seawater (3000mL/min), controlled at 20°C. Each experimental diet was given to satiation three times a day throughout the 6-week feeding experiment. At the end of the feeding trial, the fish were weighed individually, 6 fish from each tank were sampled and stored at -20°C until chemical analyses.

2.3. Feeding behavior observation

At the end of the feeding trial, three fish from each experimental tank were placed in a transparent 60L ($60 \times 35 \times 30 cm^3$) square tank and were acclimatized for 18 hours. Then, experimental diet was fed by hand and feeding behavior was video-recorded for 5 min (30 frames/sec) using a charge coupled device camera (Matsushita Electric

Industrial Co., Ltd., Osaka, Japan) from the lateral side of the observation tank. From the sequence of feeding behavior on the experimental diet, feeding behavior of red sea bream were divided into two categories; Ingestion: a fish aimed one particle of diet, captured and swallowed, Reject: once a fish captured a diet but spit out. All frames of the video recordings were used to analyze frequency of ingestion and reject during observation period.

2.4. Chemical analysis

Determinations of moisture, crude protein and crude lipid of the experimental diets, were made by 6 h drying at 110 °C, semi-micro Kjeldahl method (N × 6.25) and chloroform-methanol extraction after Folch et al. (1957). The extraction of free amino acids from the diets and whole body were carried out by homogenization in 2% sulfosalicylic acid and centrifugation at 2,300 ×g for 15 min. Free amino acid concentrations were determined with an automatic amino acid analyzer (JLC-500/v JEOL, Tokyo, Japan).

2.5. Statistical analysis

Date on growth performances and free amino acid concentrations were subjected to one-way ANOVA and Tukey-Kramer multiple comparison test. When two groups were compared, data were analyzed using Student's *t*-test. For all statistical analyses, a SPSS 11.0 microcomputer software package (SPSS, Chicago, IL, USA) was used. In all statistical testing, differences at $P<0.05$ were considered as significant.

3. Results

3.1. Growth and feed performance

Results of the feeding experiments are shown in Table 3. The fish fed the 1.0% taurine supplemented diet showed significantly (P<0.05) higher weight gain (WG), specific growth rate (SGR), feed efficiency and daily food consumption (% BW) compared with fish fed the control diet containing 0.2% taurine. In Experiment II, fish fed any taurine supplemented diet containing 0.4 to 1.6% taurine had significantly higher SGR and WG, feed efficiency and daily food consumption than the control diet group (Table 3). However, no significant differences were found in the growth and feed performance between fish fed the control diet and the two cystine supplemented diets. Frequency of ingestion was significantly higher in fish fed the 1.0 and 2.0% taurine diets than those of control diet (Table 6).

3.2. Whole body free amino acid composition

The free amino acid compositions in the whole body of juvenile red sea bream are shown in Tables 4 and 5. In Experiment I, taurine content in the whole body of the control diet group was markedly lower compared to the taurine-supplemented diet group (Table 4). The contents of the non-essential amino acid except for proline, and the contents of cystathionine and phenylalanine of the whole body in fish fed the control diet were much higher than those of fish fed the taurine-supplemented diet. In Experiment II, the taurine content of the whole body increased significantly with the increase of dietary taurine levels, however no significant differences were observed in the taurine contents between the control diet group and the two cystine-supplemented groups (Table 5). The contents of the whole body non-essential amino acids followed

similar trends to what was observed in Experiment I. The contents of aspartic acids, serine, glutamic acid, glycine, alanine and proline decreased as the dietary taurine content increased. The contents of these amino acids of the cystine-supplemented diet groups were similar to those of the control diet group. Unlike the case in Experiment I, the cystathionine content of fish fed the control diet was significantly lower than fish fed the 0.5% taurine-supplemented diet. In the taurine-supplemented diet groups, the cystathionine content decreased as the dietary taurine content increased. Although the cystathionine content of fish the 1.0% cystine-supplemented diet was not significantly different from fish fed the control diet, the content of fish fed the 2.0% cystine-supplemented diet increased to a level identical to fish fed the 0.5% taurine diet.

4. Discussion

In the present study, juvenile red sea bream fed the taurine supplemented diets showed significantly superior growth performances to those of fish fed the control unsupplemented diets in both Experiments I and II . This growth promotion effect of taurine can be attributed to the increased feed intake and improved feed efficiency. These observations are similar to the results in juvenile Japanese flounder (Park et al., 2002; Kim et al., 2005) and juvenile yellowtail (Matsunari et al., 2005), where graded levels of taurine were supplemented to the test diets. In addition, the whole body taurine contents of the taurine supplemented-diet groups were higher and increased with the dietary taurine content compared to the control groups in both experiments of the present study. These results indicate that taurine is an important element for the growth of juvenile red sea bream, and the juveniles are able to utilize crystalline taurine. Since the growth and feed efficiency was not significantly different between fish fed 0.5

to 2.0 % taurine supplemented diets (the analytical dietary taurine contents were 0.4 to 1.6 %), the dietary taurine requirement of juvenile red sea bream is assumed to be less than 0.5 %.

Rainbow trout fed a methionine supplemented diet showed a decrease of the serine content and an increase of the cystathionine content in the liver (Yokoyama and Nakazoe, 1992). Serine is one of the important key factors in the trans-sulfuration pathway from homocysteine to cystathionine in mammals (Finkelstein and Martin, 1986). The whole body serine levels of juvenile red sea bream fed the taurine supplemented diets were significantly lower than those of fish fed the taurine-unsupplemented diets in both Experiments I and II. However, the relationships between the dietary taurine level and the whole body cystathionine level did not coincide between Experiments I and II; the cystathionine content was higher in the taurine-unsupplemented group in Experiment I whease the content in Experiment II was lower compared to the taurine-supplemented diet groups. These observations indicate that there are interspecific differences in the capacity of cystathionine biosynthesis from methionine in fish. Since the contents of individual free amino acid, especially those of non-essential amino acids in the whole body of juvenile red sea bream decreased with the increase of whole body taurine content, red sea bream might regulate the size of free amino acid pool. More detailed investigations are needed on the sulfur amino acid metabolism in red sea bream.

The major pathway for taurine synthesis from cysteine in mammals involves the oxygenation of cysteine to cysteinesulfinate, followed by decarboxylation to hypotaurine and then to taurine (Worden and Stipanuk, 1985). The pathway from cysteine or cystine to taurine has been examined in rainbow trout and Japanese flounder.

The taurine levels in the liver and plasma increased as the dietary level of cystine increased in rainbow trout, suggesting an active synthesis of taurine from cystine (Walton et al., 1982; Yokoyama and Nakazoe, 1998). A considerable amount of taurine and hypotaurine was synthesized from radiochemically labeled cysteine injected into the peritoneal cavity of rainbow trout (Yokoyama et al., 1997). In addition, a large dose of cystine increased the hypotaurine contents of liver and kidney in rainbow trout (Yokoyama and Nakazoe, 1998). These results suggested that there is a metabolic pathway from cystine to taurine in rainbow trout. On the other hand, detailed studies similar to the ones in trout have not been done in red sea bream. Juvenile flounder are unable to use dietary cystine for taurine biosynthesis, and the taurine levels of the whole body, liver and muscle increased only by the supplementation of taurine (Park et al., 2002). In the present study, supplementation of cystine to the casine-based semi-purified diet did not promoted the growth of juvenile red sea bream, and the whole body taurine content did not increase at all. The activity of cysteinesulfinate decarboxylase, which is located in the trans-sulfuration pathway from cysteinesulfinate to hypotaurine, in red sea bream is only approximately half the levels of rainbow trout (Yokoyama et al., 2001). Therefore the red sea bream might have low capacity of taurine biosynthesis from cystine.

Some studies on the use of alternative protein sources have revealed that no or low fish meal diets are poorly palatable (Day and Plascenica González, 2000; Kissil et al., 2000). The reduction in diet palatability usually results in a decrease in feed intake. In this study, the feed consumption rate (% BW/ day) of the taurine supplemented groups increased compared to the control diet and the cystine-supplemented diet groups. In addition, similar results were observed in frequency of ingestion. However, fish

size was different between treatments, because the observation for feeding frequency was conducted after 6 weeks feeding trial. Therefore, it is somewhat unclear from the results of the present study whether diet palatability or fish size is responsible for the increase in frequency of ingestion. It is known that feeding of fish is accelerated or appetite is stimulated by the presence of certain free amino acids. Ina and Matsui (1980) reported that the appetite of red sea bream was stimulated by the mixture of glycine, alanine and valine, which were rich in a polychaete worm. Although either of glycine, alanine, lysine, valine, glutamic acid and arginine acted as strong chemical stimulants, taurine was proved to be a weak stimulant for red sea bream (Fuke et al., 1981). The feeding of red sea bream was stimulated mainly by the mixture of proline, glycine and glucosamine (Shimizu et al., 1990). In addition, taurine has been reported to have an adverse effect on palatability in marbled rockfish (Takaoka et al., 1990). According to these results, it cannot reasonably be assumed that taurine had acted as a chemical stimulant in juvenile red sea bream in the present study. Depression of feed intake in rainbow trout is induced by a deficiency or imbalance of essential amino acids (Yamamoto et al., 2000, 2001). As discussed before, since taurine is an essential element for the growth of juvenile red sea bream, this result indicates that the reduced feed intake in red sea bream offered the taurine-unsupplemented diets in the present study could be attributed to a deficiency of dietary taurine per se.

The results of the present study suggest that supplemental taurine to a casein-based semi-purified diet promote the fed intake, feed efficiency and growth of juvenile red sea bream. Whole body taurine contents increased with dietary taurine levels. However, supplemental cystine did not metabolized into taurine and had no growth promotion effect. Further research is needed on the sulfur amino acid metabolism and the

physiological role of taurine in red sea bream.

Acknowledgements

We are grateful to the Owase Station, Fisheries Research Division, Mie Prefectural Science and Technology Promotion Center, for providing the juvenile red sea bream. This study was finically supported by the Ministry of Education, Culture, Sports, Science and Technology and Fisheries Agency, Government of Japan.

References

Day, O.J., Plascencia González, H.G., 2000. Soybean protein concentrate as a protein source for turbot *Scophthalmus maximus L.* Aquacult. Nutri. 6, 221-228.

Finkelstein, J.D., Martin, J.J., 1986. Methionine metabolism in mammals: adaptation to methionine excess. J. Biol. Chem. 261, 1582-1587.

Folch, J., Lee, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipid from animal tissues. J. Biol. Chem. 226, 497– 507.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227.

Fuke, S., Konosu, S., Ina, K., 1981. Identification of feeding stimulants for red sea bream in the extract of marine worm *Perinereis brevicirrus*. Nippon Suisan Gakkaishi 47, 1631-1635.

Goto, T., Matsumoto, T., Takagi, S., 2001. Distribution of the hepatic cysteamine dioxygenase activities in fish. Fish. Sci. 67, 1187-1189.

Goto, T., Matsumoto, T., Murakami, S., Takagi, S., Hasumi, F., 2003. Conversion of cysteate into taurine in liver of fish. Fish. Sci. 69, 216-218.

Huxtable, R.J., 1992. Physiological actions of taurine. Physiol. Rev. 72, 101-163.

Ina, I., Matsui, H., 1980. Survey of feeding stimulants for the sea bream (*Chrysophrys major)* in the marine worm (*Perinereis vancaurica tetradentata*) Nippon Nogeikagaku Kaishi 54, 7-12.

Kim, S.K., Takeuchi, T., Yokoyama, M., Murata Y., Kaneniwa, M., Sakakura, Y., 2005. Effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder *Paralichthys olivaceus*. Aquaculture 250, 765-774.

Kissil G.W., Lupatsch, I., Higgs, D.A., Hardy, R.W., 2000. Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata L*. Aquacult. Res. 31, 595-601.

Martinez, J.B., Chatzifotis, S., Divanach, P., Takeuchi, T., 2004. Effect on dietary taurine supplementation on growth performance and feed selection of sea bass *Dicentrarchus labrax* fry fed with demand-feeders. Fish. Sci. 70, 74-79.

Matsunari, H., Takeuchi, T., Takahashi, M., Mushiake, K., 2005. Effect of dietary taurine supplementation on growth performance of yellowtail juveniles *Seriola quinqueradiata*. Fish. Sci. 71, 1131-1135.

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.

Shimizu, C., Ibrahim, A., Tokoro, T., and Shirakawa, Y., 1990. Feeding stimulation in sea bream, *Pagrus major,* fed diets supplemented with Antarctic krill meals. Aquaculture 89, 43-53.

Takaoka, O., Takii, K., Nakamura, M., Kumai, H., Takeda, M., 1990. Identification of feeding stimulants for marbled rockfish. Nippon Suisan Gakkaishi 56, 345-351.

Takagi, S., Murata, H., Goto, T., Ichiki, T., Endo, M., Hatate, H., Yoshida, T., Sakai, T., Yamashita, H., Ukawa, M., 2006. Efficacy of taurine supplementation for preventing green liver syndrome and improvimg growth performance in yearling red sea bream *Pagrus major* fed low-fishmeal diet. Fish. Sci. 72, 1191-1199.

Walton, M.J., Cowey, C.B., Adron, J. W., 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr. 112, 1525-1535.

Worden, J.A., Stipanuk, M.H., 1985. A comparison by species, age and sex of cysteinesulfinate decarboxylase activity and taurine concentration in liver and brain of animals. Comp. Biochem. Physiol. 82B, 233-239.

Yamamoto, T., Shima, T., Furuita, H., Shiraishi, M., Sánchez-Vázquez, F.J., Tabata, M., 2000. Self-selection of diets with different amino acid profiles by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 187, 375-386.

Yamamoto, T., Shima, T., Furuita, H., Suzuki, N., Sánchez-Vázquez, F.J. and Tabata, M., 2001. Self-selection and feed consumption of diets with a complete amino acid composition and a composition deficient in either methionine or lysine by rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 32 Suppl. 1, pp. 83–91.

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., Kaneniwa, M., Sakaguchi, M., 1997. Metabolites of L- [35S] cysteine injected into the peritoneal cavity of rainbow trout. Fish. Sci. 63, 799-801.

Yokoyama, M., Nakazoe, J., 1998. Effect of oral administration of L-cystine on hyotaurine level in rainbow trout. Fish. Sci. 64, 144-147.

Yokoyama, M., Takeuchi, T., Park, GS., Nakazoe J., 2001. Hepatic cysteinesulphinate decarboxylase activity in fish. Aquacult. Res. 32, 216-220.

Table 1 Composition of the experimental diet for juvenile red sea bream in Experiment I

Ingredients (% wet weight)	Control	Taurine
Fish meal	10.0	10.0
Casein	42.0	42.0
Gelatin	10.0	10.0
α-Starch	7.0	7.0
Feed oil	12.0	12.0
Soybean lecithin	3.0	3.0
Vitamin mix[*1]	2.0	2.0
Mineral mix[*2]	5.0	5.0
Choline chloride	0.9	0.9
Vitamin E (purity: 50%)	0.1	0.1
Ascorbic acid calcium	0.2	0.2
Cellulose	7.3	6.3
Feeding stimulant[*3]	0.5	0.5
Taurine	0.0	1.0

Analytical contents (dry matter basis)		
Taurine (mg/100g)	24.0	924.0
Crude protein (%)	57.4	58.2
Crude lipid (%)	14.8	15.5

*1 Vitamin mixture ingredient (mg/100g): Vitamin B_1 900 mg, Vitamin B_2 1500 mg, Vitamin B_6 600mg, Vitamin B_{12} 1.5 mg, Niacin 6×10^3 mg, Ca-pantotenate 1500 mg, Inositol 30×10^3 mg, Biotine 90 mg, Folic acid 225 mg, *p* -Aminobenzoic acid 750 mg, Vitamin K_3 750 mg, Vitamin A 600,000 IU, Vitamin D_3 600,000 IU.

*2 Mineral mixture ingredients (g/100g): NaCl 1.0 g, $MgSO_4\cdot7H_2O$ 15.0 g, $NaH_2PO_4\cdot2H_2O$ 25.0 g, KH_2PO_4 32.0 g, $Ca(H_2PO_4)_2\cdot H_2O$ 32.0g, Fe-citrate 2.5 g, Ca-lactate 3.5 g, Trace element mixture 1.0 g, Cellulose 13.0 g.

Trace element mixture ingredients (mg/100mg): $ZnSO_4\cdot7H_2O$ 35.3mg, $MnSO_4\cdot4H_2O$ 17.5mg, $CuSO_4\cdot5H_2O$ 3.1mg, $AlCl_3\cdot6H_2O$ 1.5mg, KIO_3 0.3mg, $CoCl_2\cdot6H_2O$ 0.1mg, Cellulose 42.2 mg.

*3 Proline, 354; Alanine, 232; Inosine 5'-monophosphate, 414 (mg/g).

Table 2 Composition of the experimental diet for juvenile red sea bream in Experiment II

Ingredients (% wet weight)	Control	Tau-0.5	Tau-1.0	Tau-2.0	Cys-1.0	Cys-2.0
Casein	51.0	51.0	51.0	51.0	51.0	51.0
Gelatin	11.0	11.0	11.0	11.0	11.0	11.0
α-Starch	7.0	7.0	7.0	7.0	7.0	7.0
Feed oil	5.0	5.0	5.0	5.0	5.0	5.0
Cuttlefish lecithin	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix[*1]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[*2]	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.9	0.9	0.9	0.9	0.9	0.9
Vitamin E (purity: 50%)	0.1	0.1	0.1	0.1	0.1	0.1
Ascorbic acid calcium	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose	7.3	6.8	6.3	5.3	6.3	6.3
Feeding stimulant[*3]	0.5	0.5	0.5	0.5	0.5	0.5
Taurine	0.0	0.5	1.0	2.0		
Cystine					1.0	2.0
Analytical contents (dry matter basis)						
Taurine (mg/100g)	5.3	422.0	993.4	1598.4	6.5	5.0
Cystine (mg/100g)	0.0	0.0	0.0	0.0	994.5	1895.2
Crude protein (%)	61.2	61.3	61.7	62.6	62.0	62.3
Crude lipid (%)	15.4	15.4	15.3	15.4	15.5	15.4

*1 Vitamin mixture ingredient (mg/100g): Vitamin B_1 900 mg, Vitamin B_2 1500 mg, Vitamin B_6 600mg, Vitamin B_{12} 1.5 mg, Niacin 6×10^3 mg, Ca-pantotenate 1500 mg, Inositol 30×10^3 mg, Biotine 90 mg, Folic acid 225 mg, *p* -Aminobenzoic acid 750 mg, Vitamin K_3 750 mg, Vitamin A 600,000 IU, Vitamin D_3 600,000 IU.

*2 Mineral mixture ingredients (g/100g): NaCl 1.0 g, $MgSO_4\cdot7H_2O$ 15.0 g, $NaH_2PO_4\cdot2H_2O$ 25.0 g, KH_2PO_4 32.0 g, $Ca(H_2PO_4)_2\cdot H_2O$ 32.0g, Fe-citrate 2.5 g, Ca-lactate 3.5 g, Trace element mixture 1.0 g, Cellulose 13.0 g.

Trace element mixture ingredients (mg/100mg): $ZnSO_4\cdot7H_2O$ 35.3mg, $MnSO_4\cdot4H_2O$ 17.5mg, $CuSO_4\cdot5H_2O$ 3.1mg, $AlCl_3\cdot6H_2O$ 1.5mg, KIO_3 0.3mg, $CoCl_2\cdot6H_2O$ 0.1mg, Cellulose 42.2 mg.

*3 Proline, 354; Alanine, 232; Inosine 5'-monophosphate, 414 (mg/g).

Table 3 Growth and feed performance of juvenile red sea bream[*1]

	Intitial BW (g)	Final BW (g)	Specific growth rate[*2]	Weight gain[*3]	Feed efficiency[*4]	Daily food consumption[*5] (%BW/day)	Mortality (%)
Experiment I							
Control	2.3 ± 0.0	6.9 ± 0.3 *6	2.5 *	194.2 *	74.9 *	3.1 *	0
Taurine	2.3 ± 0.0	11.9 ± 0.3	3.8	408.3	92.4	3.5	0
Experiment II							
Control	2.5 ± 0.2	6.6 ± 2.2[a*7]	2.3[a]	165.0[a]	91.5[a]	2.4[a]	1.7
Tau-0.5	2.5 ± 0.3	15.7 ± 3.3[b]	4.4[b]	529.2[b]	137.0[b]	3.1[b]	1.7
Tau-1.0	2.5 ± 0.2	16.5 ± 2.8[b]	4.5[b]	559.8[b]	123.9[b]	3.5[b]	0
Tau-2.0	2.5 ± 0.3	17.1 ± 3.0[b]	4.6[b]	581.0[b]	126.2[b]	3.5[b]	0
Cys-1.0	2.5 ± 0.3	7.1 ± 2.3[a]	2.5[a]	181.5[a]	94.8[a]	2.5[a]	1.7
Cys-2.0	2.5 ± 0.3	7.2 ± 2.3[a]	2.5[a]	187.9[a]	98.4[a]	2.4[a]	6.7

*1 Values are means±SD of duplicate groups.

*2 Specific growth rate = 100×(ln(final BW)-ln(initial BW))/days.

*3 Weight gain = 100×（final BW-initial BW）/initial BW.

*4 Feed efficiency = 100 ×(final BW-initial BW)/DM intake.

*5 Daily food consumption = 100 ×food intake/[{(initial BW+final BW)/2}×rearng period (days)].

*6 The asterisk means a significant difference between the treatment groups (Student's t-test, $P < 0.05$).

*7 Values with the same superscript letter within the same column in Experiment II are not significantly different (Tukey-kramer test, $P < 0.05$).

Table 4 Free amino acid contents in the whole body of juvenile red sea bream in Experiment I (mg/100g, d.b.)[*1]

	Initial	Final	
		Control	Taurine
Taurine	214.8	38.1[a2]	964.5[b]
Aspartic acid	190.3	156.4[b]	60.8[a]
Threonine	38.7	45.6	46.1
Serine	72.2	96.2[b]	47.9[a]
Glutamic acid	193.0	126.8	122.3
Glycine	260.7	319.7[b]	179.2[a]
Alanine	239.0	276.2[b]	232.0[a]
Valine	19.5	19.1	17.3
Methionine	16.4	11.2	11.7
Cystathionine	15.4	16.9[b]	12.8[a]
Isoleucine	11.5	10.5	9.6
Leucine	21.2	18.3	17.0
Tyrosine	16.8	13.6[b]	11.3[a]
Phenylalanine	17.0	14.7[b]	12.4[a]
Histidine	123.0	103.9	118.1
Lysine	37.4	39.6	49.6
Arginine	27.5	25.2	24.6
Proline	431.9	170.0[a]	323.1[b]

*1 Average value of six samples (3 fish / tank) from duplicate groups.

*2 Values with the same superscript letter within the same row are not significantly different (Student's t-test, $P < 0.05$).

Table 5 Free amino acid contents in the whole body of juvenile red sea bream in Experiment II (mg/100g, d.b.)[*1]

	Initial	Final					
		Control	Tau-0.5	Tau-1.0	Tau-2.0	Cys-1.0	Cys-2.0
Taurine	250.5	74.3[a2]	413.2[b]	894.2[c]	1584.4[d]	79.1[a]	76.4[a]
Aspartic acid	145.0	152.7[c]	92.8[b]	76.1[ab]	52.5[a]	170.3[c]	165.1[c]
Threonine	29.0	36.7	36.7	41.2	34.4	36.7	32.2
Serine	46.8	77.4[b]	44.8[a]	31.0[a]	20.6[a]	93.3[b]	76.1[b]
Glutamic acid	259.1	179.3[c]	114.7[ab]	110.0[ab]	106.7[a]	188.9[c]	163.0[bc]
Glycine	91.6	212.4[e]	134.4[bc]	116.5[ab]	76.2[a]	182.8[cd]	166.1[bcd]
Alanine	228.5	248.7[bc]	204.4[ab]	189.1[a]	155.5[a]	279.2[c]	258.6[c]
Valine	15.1	13.8[b]	10.9[a]	11.3[ab]	11.3[ab]	13.7[b]	12.9[ab]
Methionine	11.8	7.1	5.8	7.5	6.2	7.9	6.9
Cystathionine	9.3	7.8[a]	14.9[b]	11.8[ab]	9.6[ab]	9.3[a]	15.1[b]
Isoleucine	10.0	8.2	8.7	7.4	7.5	9.2	9.4
Leucine	18.5	15.4	14.1	13.9	13.7	15.6	14.9
Tyrosine	14.1	9.9	8.0	8.9	9.1	10.3	10.4
Phenylalanine	12.9	8.2	8.0	9.0	9.5	10.3	9.4
Histidine	94.9	88.2	101.3	111.4	106.8	104.6	90.5
Lysine	58.7	58.8	76.9	54.5	41.6	48.0	49.5
Arginine	26.7	16.6	15.6	18.0	16.3	17.0	16.1
Proline	552.4	344.4[b]	274.2[ab]	218.2[ab]	67.7[a]	378.1[b]	321.6[b]

*1 Average value of six samples (3 fish / tank) from duplicate groups.

*2 Values with the same superscript letter within the same row are not significantly different (Tukey-kramer test, $P < 0.05$).

Table 6 Effects of dietary amino acid components on the feeding behavior of red sea bream

	Behavioral traits (frequency $fish^{-1}$ 5 $minutes^{-1}$)	
	Ingestion	Reject
Control	$13.7 \pm 3.2^{bc*1}$	2.3 ± 1.2
Tau-0.5	28.3 ± 5.1^{ab}	2.3 ± 0.6
Tau-1.0	31.3 ± 8.7^{a}	1.7 ± 2.1
Tau-2.0	33.3 ± 4.5^{a}	2.0 ± 2.0
Cys-1.0	13.7 ± 7.2^{c}	6.7 ± 4.7
Cys-2.0	10.3 ± 0.6^{c}	5.7 ± 1.5

*1 Values with the same superscript letter within the same row are not significantly different (Tukey-kramer test, $P < 0.05$).