Investigación e Innovación en Nutrición Acuícola

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Effect of Taurine Precursor on Growth and Taurine Content of Marine Fish

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Abstract

Taurine is thought to be synthesized from methionine and cysteine by the cysteine sulfinic acid pathway in freshwater fish, but not in carnivorous marine fish. However, there are two biosynthetic pathways of taurine: cysteamine pathway and cysteic acid pathway. However, the synthesis via these two pathways has not been investigated by a feeding trial using these taurine precursors. We investigated possible taurine synthesis from these two pathways using freshwater fish (carp) and marine carnivorous fish such as red sea bream and Japanese flounder. As a result, it was found that taurine can be synthesized from the two pathways, and that cysteic acid has a higher potency of taurine accumulation than cysteamine. Also, there is a risk of occurrence of malformations in fish when fed excessive cysteamine in diet. Considering risk of having malformation in fish fed cysteamine, cysteic acid is a better choice as taurine precursor. We also examined effect of taurine supplementation to non-fishmeal diet on distal intestine of juvenile red sea bream. We observed that plant protein-based non-fishmeal diet caused lower growth, pathological changes of the intestine with high expression of cytokine genes of red sea bream but these changes can be ameliorated by taurine. This improvement was observed by 1-2% taurine which is beyond the requirement for red seabream estimated by growth study, suggesting that taurine supplementation is beneficial for fish fed plant-based diet in terms of ameliorating intestinal defects as well as preventing green liver syndrome in marine carnivorous fish. This paper also discusses possible inclusion of cysteamine and cysteic acid in feedstuffs, the possible mechanisms of amelioration of plant ingredient-induced intestinal damage by taurine, the response of taurine synthesizing enzyme gene expression to sulfur amino acid in fish, and new taurine source candidates for aquafeed.

Keywords: *Cysteine, Cysteamine, Cysteic acid, Soybean meal, Low fishmeal diet*

Introduction

Global demand for taurine is 49,000 tones, which is growing at 5-6% annually. There are two sources of taurine: naturally occurring taurine and synthetic taurine. The source of natural taurine includes fish, shellfish (octopus and squid) and cattle bile, but the availability of these ingredients are small, and the price is high. Bovine bile is utilized for isolating ursodeoxycholic acid, which is used for medication for liver, and the price of taurine, which is a by-product of ursodeoxycholic acid production is about one fourth to one fifth compared to that derived from fish and shellfish. However, since bile from 500-550 cattle is needed to produce 1 kg of natural taurine, production efficiency is not high. However, in Japan, only natural taurine is permitted to be used as a source of taurine for food material, it is an important taurine source for powdered milk for human. Taurine is contained in the protein source of the ingredient for fish feed. There are naturally occurring taurine and industrially produced synthetic taurine, and synthetic taurine is cheaper. Industrial manufacturing methods include ethanolamine esterification method and ethylene oxide method. In Japan, Taisho MTC Co. produces taurine by the ethanolamine esterification method, which supplies more than half of the domestic market share of 1200 tons in Japan. The ethylene oxide method has several advantages, but it also has the disadvantage of high cost of plant construction. In the world, Yangnong Pharmaceutical Co Ltd of China, which developed the ethylene oxide method, has a market share of more than 50%.

Taurine is metabolically synthesized from methionine and cysteine, so these compounds are important precursors of taurine. In addition, methionine serves not only a start codon for initiation of gene expression, but taurine also contributes to the stabilization of translation (Asano *et al*., 2018). Taurine is abundant in animal-derived meals, ranging from 90 mg/kg of black soldier fly (BSF) meal to 10 g/kg of fishmeal. In addition, the taurine content of fish meal is 4-10 g/kg, which is about twice higher/lower depending on the type of the fishmeal. On the other hand, taurine is not contained in plant ingredients such as *Spirulina*, soybean meal (SBM) and soy protein concentrate (SPC) at all (Li and Wu, 2020). However, as a sulfur-containing amino acid, *Spirulina* contains 20 g/kg methionine, which is comparable to fish meal (Li and Wu, 2020). In addition, although SBM has a low content of methionine and cysteine, the content of those in SPC is about twice higher, and the sum of methionine and cysteine in the SPC is comparable to that of fish meal (Li and Wu, 2020). Furthermore, methionine is metabolized to glutathione in animals and is thought to contribute to control redox status. Small amount of glutathione is contained in the feed

stuffs, and its levels in the SBM, SPC, and BSF meal exceeds that in the fish meal (Li and Wu, 2020).

As described above, fish meal is rich in taurine, but not in plant ingredients, that are the main alternatives of fishmeal in aquafeed. Therefore, fish species that can synthesize taurine from methionine and cysteine does not require taurine in the diet but when they lack endogenous synthetic ability, it requires taurine in the diet, thus, taurine is considered as a conditional essential nutrient in fish. Taurine involvs in various physiological processes such as growth, vision, reproduction, neurotransmission, osmoregulation, and antioxidant activity (Takeuchi 2014). Taurine is known to control liver function, bile acid conjugation and thus lipid absorption, and improve swimming behavior and early growth in fish (Salze and Davis, 2015). Taurine is not protein bound amino acid but is abundantly found as an extractive component of fish and shellfish.

Taurine requirement in fish

Recently, Li *et al*. (2022) investigated 292 out of 396 published studies and found that taurine requirements are affected by taurine content in the basal diet, and their meta-analysis shows that marine fish requires higher dietary taurine than freshwater fish. It was suggested that the addition of taurine had a high effect of improving growth, and that the average requirement in diet was estimated as 0.79%. This is well agreement with improved carbohydrate synthesis when tilapia fed with 0.8% taurine supplemented diet (Shen et al., 2021). It was suggested that the addition of taurine would improve FCR in all categories of animal species investigated (all fish species, marine and freshwater fish, shrimp, reptiles, animals at low/mid/high trophic levels). Regarding PER, it was suggested that there was no major impact on low trophic level species, shrimp and freshwater fish, but for mid and high trophic level species and marine fish, significant improvement was expected by the addition of taurine.

Rainbow trout, which can synthesize taurine in the body, are fed a fishmeal-free diet supplemented with methionine and reared for 12 weeks and show normal growth that is comparable to that of a fishmeal-based diet (Fig.2; Watanabe *et al*., 1997). In yellowtail, feeding of fishmeal-free diet showed no growth retardation within 8 weeks but sudden stop of growth afterwards (Fig.2; Watanabe *et al*., 1998). Examining taurine requirement of yellowtail by supplementing taurine to low fishmeal diet containing 20-30% fishmeal, it was found that additional taurine was not required for a diet containing 30% fishmeal (Tochino *et al*., 2009). It was suggested that 0.5% taurine should

be supplemented to the diet formulated in a diet containing below 20% fishmeal (Tochino *et al*., 2009). Yellowtail is a relatively fast-growing fish species but it required for more than 8 weeks to observe growth degradation due to taurine deficiency (Fig.2; Watanabe *et al*., 1998). This implies that a 12-week feeding trial is recommended when one studying taurine requirements of marine carnivorous species by evaluating growth performance. It has also been demonstrated that red sea bream requires 0.5% taurine for normal growth by taurine supplementation to the casein-based diet (Matsunari *et al*., 2008). However, ameliorating effect of taurine on soybean meal-induced enteritis has been recognized when surplus level of taurine was supplied which is beyond requirement of red sea bream estimated by growth study (Li *et al*., 2019). With the increasing number of the certification in the aquaculture industry such as Aquaculture Stewardship Council, the use of marine products in aquafeed have been decreasing and the use of terrestrial plant ingredients has been increasing. Thus, further study is needed to clarify taurine requirement which is needed to treat intestinal damage in fish fed plant-based diet.

Taurine synthesis in animals

Taurine is synthesized from methionine and cysteine in animals (Fig.1). Methionine is first converted to cystathionine via homocysteine, and cystathionine is converted to cysteine by cystathionine gamma lyase (CSE)(Fig.1). Taurine is synthesized by either the cysteamine pathway, the cysteine sulfinic acid pathway or the cysteic acid pathway. Cysteine is oxidized to cysteine sulfinic acid by cysteine dioxygenase (CDO) and then decarboxylated to hypotaurine by cysteine sulfinic acid decarboxylase (CSD)(Fig.1). The origin of cysteic acid differs depending on the species, and it is synthesized from cysteine in mollusks but from sulfate in chickens and algae, but details are unclear (Nakamura *et al*., 2021 and references there in). On the other hand, cysteamine is thought to be derived with the degradation of coenzyme A (Dominy *et al*., 2007).

In the cysteine sulfinic acid pathway, the rate-limiting step is where hypotaurine production from cysteine sulfinic acid which is catalyzed by CSD (Fig.1). It is well established that cat cannot synthesized taurine because it lacks CSD activity (Huxtable 1992). So, cat requires dietary taurine as an indispensable nutrient. It was demonstrated that administration of cysteine sulfinic acid, which is a substrate for CSD, into the blood does not increase taurine in cat (Edger *et al*., 1994).

Cysteine is also important for taurine synthesis because it has biological activity as a precursor of cysteine sulfinic acid (Fig.1). Cysteine possesses a thiol group and is highly reactive nature. So, it shows cytotoxicity when it is in excess, and is also known to be converted to coenzyme A and glutathione. CDO requires Fe^{2+} for its maximum activity and plays a role in converting toxic cysteine into more polar compounds such as cysteine sulfinic acid, etc. (Dominy *et al*., 2007). It is assumed that CDO determines the amount of taurine synthesis in rainbow trout, which has relatively high CSD activity and can synthesize taurine from methionine in the body (Wang *et al*., 2016).

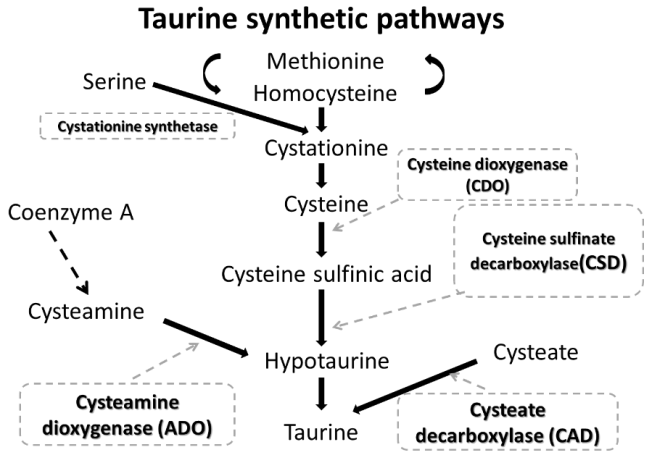

Fig.1.Taurine biosynthesis pathway in animals

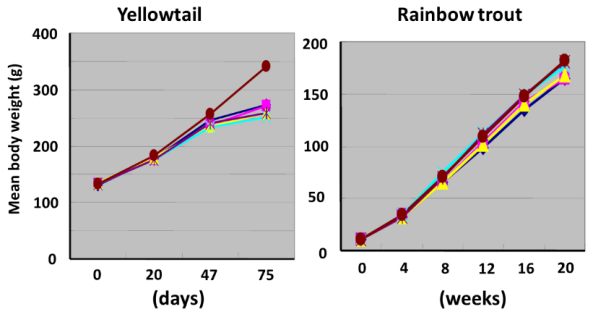

Fig.2. Yellowtail *Seriola quinqueradiata* and rainbow trout *Oncorhynchus mykiss* juveniles fed fishmeal free diets supplemented with crystalline amino acid mixture. Growth retardation of yellowtail was noted after 8 weeks onward when fed fishmeal free diets. Details are described in Watanabe *et al*. (1997 and 1998)

On the other hand, Japanese flounder lacks CSD activity but has high CDO activity (Wang *et al*., 2016). Thus, it seems that the ability to synthesize cysteine sulfinic acid is high in this species. However, flounder cannot synthesize taurine from the cysteine sulfinic acid pathway due to its low CSD activity (Goto *et al*., 2002; Haga *et al*., 2015; Wang *et al*., 2016). In previous reports, bluegill has the highest CSD activity in fish, followed by red tilapia and tiger puffer (Goto *et al*., 2002; Divakaran *et al*., 1992). It has also been suggested that seabass also has high CSD activity (Zhang *et al*., 2019). Other reports have shown no CSD activity in higher predators such as chub mackerel, yellowtail, tuna, bonito, and dolphin fish *Coryphaena hippurus* (Divakaran *et al*., 1992; Jacobsen and Smith 1968; Yokoyama *et al*., 2001). From the above, it was hypothesized that carnivorous fish species lacks the ability to synthesize taurine by the cysteine sulfinic acid pathway.

Fan *et al*. (2018) reported CDO activities in two species of mollusks, two species of crustaceans, and three teleosts. Xhang *et al*. (2019) reported CSD activities in five mollusks, one squid, and three teleosts. Unfortunately, unit of activity is unclear and thus hinders comparison of the activities reported in other studies. Also, only common name in Chinese was provided but not scientific names of the species examined in their study. However, these studies suggested presence of cysteine sulfinic acid pathway in seabass. Also, all species in mollusks and crustacean such as *Litopenaeus vannamei* examined, CDO activities are found in muscle but not in intestine and midgut gland/liver (Fan *et al*., 2018). Also, among five mollusks examined, no species have CSD activity in intestine, gill and midgut gland/liver but three of them have the activity in the muscle (Xhang *et al*., 2019). Likewise, very limited study has been done for invertebrates but CDO and CSD activities in mollusks are tended to be found in muscle but not in the midgut gland. Although invertebrates have a high taurine content, research has not progressed, but CSD activity has recently been reported from two species of squids such as *Heteroloigo bleekeri* and *Uroteuthis edulis*, suggesting that at least these squids can synthesize taurine (Matsumoto *et al*., 2021).

Taurine content in fish administered taurine precursor

0.25 mM, 0.5 mM and 1 mM cysteamine or L-cysteine were injected intraperitoneally to carp at 1 mL / 100 g body weight and the taurine content in plasma and hepatopancreas after 2-24 hours was examined (Gonzales, 2018). As a result, in the L-cysteine injection group, the taurine level in the blood reached the highest level 2 or 4 hours after the injection, and then decreased. On the other hand, in the cysteamine injection group, the taurine level in the blood reached the highest

level after 6-12 hours and then decreased. The taurine content in the hepatopancreas did not increase in the L-cysteine injection group, but in the cysteamine injection group, the taurine content in the hepatopancreas increased 24 hours after the injection (Gonzales, 2018). In addition, when cysteamine HCL, methionine, and cysteine-supplemented diet were given to carp, the taurine content in fish body increased, and the level was higher in fish fed the cysteamine HCL supplemented diet than those fed the other precursors supplemented diets. These results suggest that carp can synthesize taurine by the cysteamine pathway and that cysteamine has a higher potency as a taurine precursor than cysteine (Gonzales-Plasus *et al*., 20019). Our study also showed that taurine could be synthesized by the cysteine sulfinic acid pathway in carp since serum taurine level increased in fish injected with cysteine, but the synthesized taurine was not accumulated in the hepatopancreas, suggesting that the mode of taurine accumulation differs depending on the synthetic pathway (Table 1).

In addition, when Japanese flounder was fed a taurine-deficient diet containing 0.25, 0.5, and 1.0% cysteic acid for 30 days, the percent weight gain and specific growth rate in the 0.25-0.5% cysteic acid supplemented groups were significantly higher than those in the control group (P<0.05, Nakamura *et al*., 2021). With this increased growth, feed efficiency was tended to be improved in fish fed cysteic acid supplemented diet (Nakamura *et al*., 2021). In addition, the expression level of *igf-1* in the liver was significantly higher in the 1.0% cysteic acid supplemented group than the control group (P<0.05, Nakamura *et al*., 2021). In addition, the taurine content of the liver and whole fish increased linearly as supplemental cysteic acid level increased in the diets (Nakamura *et al*., 2021), strongly suggesting that taurine is synthesized from cysteic acid in Japanese flounder (Table 1).

CSD have also been reported to involved in the cysteic acid pathway in red sea bream (Goto *et al*., 2003). However, these reports were based on a short-term study using cultured hepatopancreas, and there was no study examining how much taurine was accumulated by feeding diets supplemented with cysteic acid and cysteamine to red sea bream. Therefore, we examined the accumulation of taurine in the red sea bream fed 0.5% cysteic acid, and cysteamine to red sea bream (Itoh *et al.*, 2019). As a result, the growth of the fish fed with the diet supplemented with cysteic acid was higher and an increased taurine content was observed (Itoh *et al*., 2019). Interestingly, increased taurine level in serum was observed in red sea bream injected cysteamine (Itoh *et al*., 2018), no taurine deposition was observed in red sea bream fed 0.5% cyteamine supplemented diet

(Itoh *et al*., 2019). These reports suggest that fish can synthesize taurine from cysteamine and cysteic acid pathways but taurine deposition in liver is different depending on the synthetic pathways (Table 1).

Table 1. Taurine content in fish administered taurine precursors

Table 1. Taurine content in fish administered taurine precursors					
Compounds	Delivery method	Speceis	Feeding habits	Taurine content	References
Cysteamine-HCL	Feeding	Carp	Omni/hervivorous	Increased in liver	Gonzales-Plasus et al. 2019
Cysteamine	Feeding	Carp	Omni/hervivorous	Increased in liver	Gonzales-Plasus et al. 2019
L-cysteine	Intraperitoneal injection	Carp	Omni/hervivorous	Increased in serum	Gonzales MMG. 2018
Cysteamine	Intraperitoneal injection	Carp	Omni/hervivorous	Increased in liver and serum	Gonzales MMG. 2018
Cysteic acid	Feeding	Japanese flounder	Carnivorous	Increased in liver and whole body	Nakamura et al. 2021
Cysteic acid	Feeding	Red sea bream	Carnivorous	Increased in liver	Itoh et al. 2019
Cysteamine	Feeding	Red sea bream	Carnivorous		Itoh et al. 2019
L-cysteine	Feeding	Red sea bream	Carnivorous		Itoh et al. 2019
Cysteic acid	Intraperitoneal injection	Red sea bream	Carnivorous	Increased in liver and serur	Itoh et al. 2018
Cysteamine	Intraperitoneal injection	Red sea bream	Carnivorous	Increased in serum	Itoh et al. 2018
L-cysteine	Intraperitoneal injection	Red sea bream	Carnivorous		Itoh et al. 2018

It has been reported that in rats, dietary keratin can be source of cysteic acid. It was then converted into taurine in liver of the rat fed casein and keratin mixture (Wolber *et al*., 2016). It is well known that cats cannot synthesize taurine from the cysteine sulfinic acid pathway (Huxtable, 1992). However, it has been suggested that taurine cannot be synthesized by cysteine sulfinic acid in cat, an obligate carnivore, but it can convert cysteic acid into taurine (Edger *et al*., 1998). Considering that red sea bream and Japanese flounder are carnivorous fish, it may be possible to synthesize taurine to some extent by the cysteic acid pathway in carnivorous fish which are located at higher trophic level in the food web. We also observed that toxicity of excessive cysteamine such as hemorrhage of base of caudal fin and dorsal vending in common carp and abnormal gill cover in Japanese flounder (Gonzales-Plasus *et al*., 2019; Nakamura *et al*., 2019), suggesting toxicity of cysteamine for fish. In contrast, we did not observe such kind of morphological abnormality in fish fed cysteic acid supplemented diet. This probably due to high water solubility of cysteic acid. Thus, considering low risk of toxocity of cysteic acid, it can be better source of taurine.

Other taurine sources

Our studies have shown that cysteamine and cysteic acid can be sources of taurine. It will be of interest to the reader how much of these compounds are contained in the feed material. As for cysteamine, cysteamine preparations are sold for chickens, pigs, and fish feeds because they can be expected to have an enhancing effect by inhibiting the action of somatostatin, which suppresses growth hormone. On the other hand, cysteamine is a thiol compound, and when it becomes excessive due to its high reactivity, it causes a physiological disorder characterized by damage to the digestive tract. Therefore, although it is also commercially used as a fish additive, its use in animal feed is prohibited in the United States, Canada, Malaysia and Thailand (Huynh *et al*. 2018). Cysteamine, seems to be used as a premix with other additives for aquafeed, has also been suggested to react with vitamin K in the premix (Huynh *et al*. 2018). In Vietnam, about 30 mg/kg of cysteamine is mixed in two types of commercially available supplements, and 23-51 mg/kg of cysteamine is also detected in three types of commercially available feed (Huynh *et al*. 2018). On the other hand, cysteamine is also produced during fishmeal processing, and higher cysteamine was detected in the meal produced from fish preserved for 7 days than that from fresh fish (Tozawa and Kawabata 1987). It is a matter of concern whether these cysteamines become

taurine precursors and meet the taurine requirements of fish. It is reported that increased taurine was observed in carp fed a diet with 10 g/kg cysteamine. Compared to this value, cysteamine generated in the fishmeal manufacturing process and detected in Vietnamese feed are about one-thousandth and one fortieth to one twentieth lower. Therefore, it is unlikely that trace amounts of cysteamine contained in such commercial feed items and feed ingredients are a source of taurine.

Marine products have higher sulfur-containing amino acids than terrestrial animals, and both cysteamine and cysteic acid can be produced by oxidation of cysteine. In addition, cysteine is abundant in skin keratin and is abundant in feather meal. In fact, it has also been reported that lanthionine contained in feather meals is decomposed into cysteic acid by acid hydrolysis (Latshaw 1990). Then, is there a possibility that cysteic acid etc. will be generated due to oxidation during storage of these feed ingredients? So we looked at the cysteic acid content in feather meal, fishmeal and SBM. As a result, cysteic acid was the highest in fish meal, the lowest in SBM, and feather meal was an intermediate value, which was considered to reflect the content of sulfur-containing amino acids in feed ingredients and their susceptibility to oxidation. The content was 0.25 g/kg (fish meal), 0.1 g/kg or less for defatted soybean meal, and 0.15 g/kg or less for feather meal. In red sea bream, 1 g/kg of cysteic acid promoted growth (Itoh *et al*., 2019), and in Japanese flounder, 2.5-5.0 g/kg of cysteic acid promoted growth (Nakamura *et al*., 2021), the content was about 1/10, and it was found that it is unlikely that existing feed ingredients would supply enough cysteic acid to promote growth.

Red algae are also rich in taurine. In red algae, *Gelidiaceae* and *Gracilaria* are widely produced as natural agar source. Aquaculture production of aquatic products is increasing worldwide, but red algae are second highest at 18 million tons, and their production has been increasing rapidly since 2000. Red algae contain phycoerythrin, which is a red pigment not found in green algae and land plants, so that green light in water can be used for photosynthesis. Gelidiaceae is a popular natural source of agar, and in Japan, so-called nori (*Porphyra yezoensis*) is also one of the very popular food (Fig.3). Among the seaweeds, the taurine content of *Colpomenia sinuosa* and *Gliopeltis* is as high as about 650 mg/100 g, and the content fluctuates from 90 to 350 mg/100 g in *Gliopeltis* depending on the place of collection (Kawasaki *et al*., 2017). The physiological significance of taurine in red algae is unknown, but it could be used for osmoregulation since it grows a little deeper water than green algae. It was suggested that inclusion of taurine-rich red algae has a beneficial effect on Atlantic salmon (Lozano *et al*., 2016).

Since taurine is rarely detected in processed products from red algae-derived agar, agar cannot be used as a source of taurine (Kawasaki *et al*., 2017). Since the natural agar source such as gelidiaceae is repeatedly washed with running water until the color changes, it is presumed that water-soluble taurine is lost in these washing steps (Kawasaki *et al*., 2017).

Nopal catus is widely distributed in the warm arid zones of Central and South America, South Africa, and the Mediterranean coast, and the fruit of the cactus contains 15 g of taurine per 100 g of protein (El-Mostafa *et al*., 2014; Fig.3). It is suggested that the taurine content varies depending on the color of the fruit and is abundant in the orange one (Horibe *et al*., 2020). When the above taurine content was calculated per dry weight, it was about 7 g per kg, which was comparable to fish meal. Nopal cactus fruit is rich in polysaccharides in addition to taurine, and its addition effect is also expected. In addition, nopal cactus is a CAM plant adapted to the harsh habitat of arid zones and accumulates malic acid at night. Therefore, it seems that it has a pathway to metabolize this malic acid to taurine like the above-mentioned zebrafish (Yang *et al*., 2020). So far, there is no information on taurine synthesis from malic acid except case of zebrafish, but it is expected that the stems of nopal cactus could be a source of malic acid for fish species that can convert it into taurine. It is also expected that processed residues from cactus fruits could be used as a source of taurine for aquafeed.

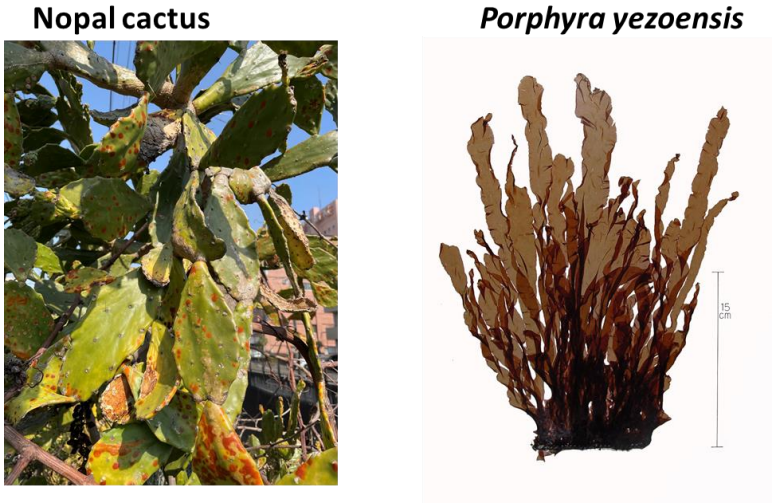

Fig. 3 New taurine source candidates for aquafeed. Nopal cactus contains high taurine in its fruit. Red algae also known to contain high taurine. A picture of *Porphyra* is kindly provided by Dr. Kyosuke Niwa, Tokyo University of Marine Science and Technology.

The plant-based protein source is undoubtedly a promising ingredient for fish diet because of its high production volume and low price, but it contains almost no taurine. Soybean meal and soy saponin flatten the intestinal mucosal fold of the distal intestine of Atlantic salmon and trout, less absorptive vacuoles, expantion of lamina propria, with neutrophil infiltration (Baeverfjord and Krogdahl, 1996; Bakke-McKellep *et al*., 2000, 2007; Li *et al*., 2019). In recent years, the effects of soybean meal on the intestinal tract of marine carnivorous fish such as red sea bream and Japanese flounder have also been reported. The effectiveness of taurine on liver function and green liver disease in red sea bream has been investigated so far, but most of the studies focus on liver fucntion but few of them sudied effect of taurine on distal intestine. Therefore, we studied effect of taurine supplementation on non-fishmeal diet formulated with plant protein sources and examine morhological changes of intestine of red sea bream.

1-2% of taurine was added to a diet in which fish meal was replaced with plant ingredients, and the growth performance, the tissue morphology of the intestine, and the expression of cytokine genes were examined in red sea bream. As a result, the weight gain was significantly lower in fish fed the fishmeal-free diet than in the control diet (P <0.05), but it tended to improve with increasing supplemental taurine in diet (Li *et al*., 2019). Similarly, the specific growth rate was improved by taurine supplementation. Further, 2% taurine improved the feed conversion efficiency to the extent that there was no significant difference from the control group (Li *et al*., 2019). The apparent digestibility coefficient of crude protein and fat was significantly inferior to that of the control group when supplemented 1% taurine in non-fishmeal diet but was not significantly different from that of the control group when more than 1.5% of taurine was supplemented (Li *et al*., 2019). In the 1% supplemented group, infiltration of neutrophils was observed in the submucosal layer together with inflammation in the intestine, but it was ameliorated by further increasing taurine supplementation level in the det, and the expression level of cytokine gene was also significantly reduced in fish with the addition of taurine (Li *et al*., 2019). In this study, it was speculated that more than 1% of taurine in the diet was required to ameliorate intestinal damage caused by soybean meal, which exceeds the requirement estimated based on the growth of red sea bream (Matsunari *et al*., 2008). Presumably, recovery from the pathological symptom of intestinal injury requires more taurine than that required to support normal fish growth.

The mechanism by which taurine ameliorates intestinal damage caused by soybean meal is unknown, but taurine regulates the osmotic pressure of intestinal cells as an osmotic substance

and improves the apparent digestive absorption rate by assisting the absorption of nutrients. May have prompted. In addition, improving the suppression of lipid absorption due to taurine deficiency by promoting bile acid synthesis is also considered to be one of the factors contributing to the improvement of the digestive absorption rate of lipids. Recently, taurine has been reported to improve bacterial mortality by promoting nitric oxide and reactive oxygen species synthesis in the intestine of zebrafish (Yang *et al*. 2020). Furthermore, it has been suggested that when bile acid synthesis is promoted in mammals, taurine in bile acids secreted into the intestinal tract is decomposed by intestinal bacteria, and the resulting toxicity of sulfate gas reduces intestinal pathogens. (Stacey *et al*. 2021). It has also been reported that this effect can be reproduced by oral administration of taurine. In fish as well, it is worthwhile to compare the intestinal bacterial flora of fish fed the plant-based diet with or without taurine and examine whether it can be improved by taurine addition.

Gene expression of taurine metabolizing enzymes

The expression of the taurine synthase gene (CSD, ADO, CDO) in Asian sea bass is suppressed by the taurine content in the feed (Poppi *et al*., 2020). It has also been suggested that this species is affected not by sulfur-containing amino acids but by food intake itself (Poppi *et al*., 2019). Expression of the taurine synthase gene (CSD, and CDO) in tiger puffer is suppressed by the taurine content in the diet (Wei *et al*., 2020). However, the taurine content in plasma, muscle, and liver did not significantly decrease in taurine deficiency, suggesting that this species was able to synthesize taurine (Wei *et al*., 2020). This is consistent with the highest CSD activity of tiger puffer fish examined in Yokoyama *et al*. 2001. In rainbow trout, tiger puffer, and Asian sea bass, CSD activity is high, and when the feed contains sufficient methionine and cysteine, taurine is synthesized by the cysteine sulfinic acid pathway. Ingestion of taurine in these fish species seems to reduce the expression of *cdo*, reduce the amount of taurine synthesis in the body, and maintain the amount of taurine in the body. On the other hand, although the reason is unknown, ingestion of taurine reduces the activity of CSD in fish with originally low CSD activity such as Japanese flounder and turbot. In Japanese flounder, the expression level of *cdo* is twice as high as that of rainbow trout, and the ability to produce cysteine sulfinic acid is high (Wang *et al*., 2016). Considering that cysteic acid can be synthesized in animals by oxidation of cysteine sulfinic acid, it can be said that high CDO activity has a high ability to synthesize a precursor of cysteic acid in

the cysteic acid pathway. The high ability to synthesize cysteic acid precursors may be indirect evidence that flounder makes effective use of the cysteic acid pathway.

Measurement of enzyme activity

Goto *et al.* (2021) reevaluated the optimum conditions for CDO activity in the liver of bluegill, and found that the optimum condition for bluegill CDO was slightly different from that reported in mammals, such as temperature, the type of buffer solution and the concentration of Fe ions, etc. They also suggested that optimizing conditions enabled to detect approximately twice higher CDO activity than those measured under optimal conditions for mammalian CDO (Goto *et al*., 2021). However, most of the enzyme activity of taurine synthesis in fish reported seemed to be measured under optimal condition established for mammals. For example, it was considered that the CSD activity of rainbow trout was about 1/3 of that of mice, but CSD activity of rainbow trout should be re-evaluated under optimal condition for fish enzyme.

Since an analytical kit using the ELIZA method for examining the activity of CSD in fish are commercially available in China, this is a great advantage for studying taurine synthesizing enzyme activity in fishes. Currently, relatively less effort was paid for studying taurine synthetic ability in omni-herbivorous freshwater fish. Although we have the largest production of freshwater fishes among fisheries production in the world, dietary taurine requirement of freshwater fishes seems to be lower than that of marine species, which is presumed to reduce the motivation to study taurine requirement of freshwater fishes. Even in the results of the meta-analysis by Li *et al*. (2022), there is a wide range of taurine requirement of shrimp and freshwater fish species, and it has to be studied in more detail. On the other hand, the production of capture fisheries is mainly occupied by marine fish, and it is presumed that many of them require taurine. Thus, further study is needed to determine taurine requirement of these target species of marine aquaculture.

References

Asano K, Suzuki T, Saito A, Wei F-Y, Ikeuchi Y, Numata T, Tanaka R, Yamane Y, Yamamoto T, Goto T, Kishita Y, Murayama K, Ohtake A, Okazaki Y, Tomizawa T, Sakaguchi Y, Suzuki S. 2018. Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease. Nucleic Acids Research 46, 1565–1583, https://doi.org/10.1093/nar/gky068

Baeverfjord G, Krogdahl Å. 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. Journal of the Fish Diseases 19, 375-387.

Bakke-McKellep CM, Baeverfjord PG, Krogdahl Å, Landsverk T. 2000. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. Journal of the Fish Diseases 23, 115-127.

Divakaran S, Ramanathan S, Ostrowski AC. 1992. Endogenous production of taurine in two teleost fsh: *Coryphaena hippurus* and red hybrid tilapia. Comparative Biochemistry and Physiology 101B, 321–322.

Dominy JE, Simmons CR, Hirschberger LH, Hwang J, Coloso RM, Stipanuk MH. 2007. Discovery and characterization of a second mammalian thiol dioxygenase, cysteamine dioxygenase. Journal of the Biological Chemistry 282, 25189-25198.

Edgar SE, Hickman MA, Marsden MM, Morris JG, Rogers QR 1994. Dietary cysteic acid serves as a precursor of taurine for cats. Journal of Nutrition 124, 103–109.

Edgar SE, Kirk CA, Rogers QR, Morris JG. 1998. Taurine status in cats is not maintained by dietary cysteinesulfnic acid. Journal of Nutrition 128, 751–757.

El-Mostafa K, El Kharrassi Y, Badreddine A, Andreoletti P, Vamecq J, El Kebbaj MS, Latruffe N, Lizard G, Nasser B, Cherkaoui-Malki M. 2014. Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. Molecules 19, 14879-14901.

Fan Z, Hu J, Jiang R, Yang X, Jin X. 2018. Evaluation of cysteine dioxygenase activity in marine organisms by high performance liquid chromatography. Food Science 39, 174-178 (in Chinese with English abstract).

Gonzales MMG. 2018. Basic studies on taurine synthesizing ability and its synthetic pathways in Japanese flounder *Paralichthys olivaceus* and common carp *Cyprinus carpio*. Ph D thesis, Tokyo University of Marine Science and Technology.

Gonzales-Plasus MM, Kondo H, Hirono I, Satoh S, Haga Y. 2019. Cysteamine dioxygenase as enzymes for taurine synthesis and the negative effect of high dietary cysteamine on growth and body shape of the common carp, *Cyprinus carpio*. Aquaculture Science 67, 95-108.

Goto T, Matsumoto T, Murakami S, Takagi S, Hasumi F. 2003. Conversion of cysteate into taurine in liver of fsh. Fisheries Science 69, 216–218.

Goto T, Mochizuki A, Hasumi F. 2002. Distribution and activities of enzymes involved in taurine biosynthesis in liver of fsh. Aquaculture Science 50, 443–449.

Goto T, Ooya K, Yamamoto S, Onoda K, Kobayashi T, Imai K. 2021. Characterization of cysteine dioxygenase in the liver of bluegill *Lepois macrochirus* for the determination of enzyme activity. Aquaculture Science 69, 299-305.

Haga Y, Kondo H, Kumagai A, Satoh N, Hirono I, Satoh S .2015. Isolation, molecular characterization of cysteine sulfnic acid decarboxylase (CSD) of red sea bream *Pagrus major* and yellowtail *Seriola quinqueradiata* and expression analysis of CSD from several marine fsh species. Aquaculture 449, 8–17.

Horibe, T., Sumi, H., Teranobu, R. 2020. Zinc biofortification of the edible cactus nopalea cochenillifera grown under hydroponic conditions. Environmental Control in Biology 58, 43-47.

Huxtable RJ .1992. Physiological actions of taurine. Physiological Review 72. 101–163.

Huynh PTN, Nguyen PH, Nguyen MA. 2018. Determination of cysteamine in animal feeds by high performance liquid chromatography with diode-array detection. Science & Technology Development Journal, 21, 37-43.

Ito T, Cho JH, Nakamura K, Masuda R, Haga Y, Satoh S .2019. Sulfur amino acid metabolism and growth of juvenile red sea bream. Japanese Journal of Taurine Research 5, 32–33 (in Japanese).

Itoh T, Haga Y, Kurihara A, Kondo H, Hirono I, Satoh S. Effects of intraperitoneal injection of sulfur compounds on taurine synthesis in juvenile red sea bream (*Pagrus major*). International Symposium on Fish Nutrition and Feeding 2018, Las Palmas, Spain.

Jacobsen JG, Smith LH .1968. Biochemistry and physiology of taurine and taurine derivatives. Physiological Review 48, 424–511.

Kawasaki A, Ono A, Mizuta S, Kamiya M, Takenaga T, Murakami S. 2017. The taurine content of Japanese seaweed. Advances in Experimental Medicine and Biology 975, 1105-1112.

Latshaw JD. 1990. Quality of feather meal as affected by feather processing conditions. Poultry Science 69, 953-958.

Li F, Haga Y, Kondo H, Hirono I, Satoh S. 2019. Effect of graded levels of taurine supplementation to non fishmeal diet on growth, nutrient digestibility, intestinal morphology and cytokine gene expression of juvenile red seabream *Pagrus major*. Aquaculture Science 67, 333-346. https://doi.org/10.11233/aquaculturesci.65.239

Li L, Liu H-Y, Xie S-Q, Zhang P-Y, Yang Z-C. 2022. Effects of taurine supplementation on growth performance and feed utilization in aquatic animals: A meta-analysis. Aquaculture 551, 737896, https://doi.org/10.1016/j.aquaculture.2022.737896.

Li P, Wu, G. 2020. Composition of amino acids and related nitrogenous nutrients in feedstufs for animal diets. Amino Acids 52, 523–542, https://doi.org/10.1007/s00726-020-02833-4

Lozano I, Wacyk JM, Carrasco J, Cortez-San Martín MA. 2016. Red macroalgae *Pyropia columbina* and *Gracilaria chilensis*: sustainable feed additive in the *Salmo salar* diet and the evaluation of potential antiviral activity against infectious salmon anemia virus. Journal of the Applied Phycology 28, 1343–1351. https://doi.org/10.1007/s10811-015-0648-8

Matsumoto T, Akita M, Ogawa M, Goto T. 2021. Evaluation f taurine biosynthesis in the livers of the spear squid *Heteroloigo bleekeri* and the swodtip squid *Uroteuthis edulis*. Fisheries Science 87, 717-725.

Matsunari H, Furuita H, Yamamoto T, Kim S-K, Sakakura Y, Takeuchi T. 2008. Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*. Aquaculture 274, 142-147.

Nakamura K, Gozanles-Plasus MM, Itoh T, Masuda R, Haga Y, Satoh S. 2019. Growth and amino acid content in Japanese flounder ingested cysteamine. Japanese Journa of Taurine Research 5, 34-35.

Nakamura K, Gozanles-Plasus MM, Ushigusa-Itoh T, Masuda R, Kabeya N, Kondo H, Hirono I, Satoh S, Haga Y. 2021. Taurine synthesis via the cysteic acid pathway: efect of dietary cysteic acid on growth, body taurine content, and gene expression of taurine-synthesizing enzymes, growth hormone, and insulin-like growth factor 1 in Japanese founder *Paralichthys olivaceus*. Fisheries Science 87, 353–363, https://doi.org/10.1007/s12562-021-01500-1

Poppi DA, Moore SS, Wade NM, Glencross BD. 2019. Postprandial plasma free amino acid profile and hepatic gene expression in juvenile barramundi (Lates calcarifer) is more responsive to feed consumption than to dietary methionine inclusion. Aquaculture 501, 345-358. doi: 10.1016/j.aquaculture.2018.11.044

Poppi DA, Moore SS, Wade NM, Glencross BD. 2020.Adequate supply of dietary taurine stimulates expression of molecular markers of growth and protein turnover in juvenile barramundi (*Lates calcarifer*). Fish Physiology and Biochemistry 46, 953-969. doi: 10.1007/s10695-020-00762-3

Salze GP, Davis DA. 2015. Taurine: a critical nutrient for future fsh feeds. Aquaculture 437, 215–229.

Shen GP, Ding ZN, Dai T, Feng JH, Dong JY, Xia F, Xu JJ, Ye JD. 2021. Effect of dietary taurine supplementation on metabolome variation in plasma of Nile tilapia. Animal 15, 100167, https://doi.org/10.1016/j.animal.2020.100167.

Stacy A, Andrade-Oliveira V, McCulloch JA, Hild B, Oh J-H, Perez-Chaparro PJ, Sim CK, Lim AI, Link VM, Enamorado M, Trinchieri G, Segre JA, Rehermann B, Belkaid Y. 2021. Infection trains the host for microbiota-enhanced resistance to pathogens. Cell 184, 1-13.

Takeuchi T. 2014. Progress on larval and juvenile nutrition to improve the quality and health of seawater fish: a review. Fisheries Science 80, 389–403.

Tochino M, Nagano T, Satoh S, Shiratori M, Ueta Y. 2009. Effect of taurine supplement to low fish meal feed on yellowtail *Seriola quinqueradiata* in a practical net cage. Aquaculture Science 57, 595-600.

Tozawa H, Kawabata T. 1987. Mechanism of N-nitrosothiazolidine formation at the stage of fish meal preparation. Nippon Suisan Gakkaishi 53, 2209-2216.

Wang Q, He G, Wang X, Mai K, Xu W, Zhou H. 2014. Dietary sulfur amino acid modulations of taurine biosynthesis in juvenile turbot (*Psetta maxima*). Aquaculture 422–423, 141–145. https://doi.org/10.1016/j.aquaculture.2013.12.014

Wang X, He G, Mai K, Xu W, Zhou H. 2016. Differential regulation of taurine biosynthesis in rainbow trout and Japanese founder. Scientific Reports 6:article no. 21231

Watanabe T, Aoki H, Shimamoto K, Hadzuma M, Maita M, Yamagata Y, Kiron V, Satoh S. 1998. A trial to culture yellowtail with nonfshmeal diets. Fisheries Science 64, 505–512.

Watanabe T, Verakunpiriya V, Watanabe K, Kiron V, Satoh S. 1997. Feeding of rainbow trout with non-fish meal diets. 1997. Fisheries Science 63, 258-266.

Wei Y, Zhang Q, Xu H, Liang MQ (2020). Taurine requirement and metabolism response of tiger puffer *Takifugu rubripes* to graded taurine supplementation. Aquaculture 524, p.735237.

Wolber FM, McGrath M, Jackson F, Wylie K, Broomfeld A. 2016. Cysteic acid in dietary keratin is metabolized to glutathione and liver taurine in a rat model of human digestion. Nutrients 8, 104. https://doi.org/10.3390/nu8020104

Yang M-J, Xu D, Yang D-X, Li L, Peng X-X, Chen Z-G, Li H. 2020. Malate enhances survival of zebrafish against *Vibrio alginolyticus* infection in the same manner as taurine. Virulence 11, 349-364.

Yokoyama M, Takeuchi T, Park G-S, Nakazoe J. 2001. Hepatic cysteinesulphinate decarboxylase activity in fsh. Aquaculture Reserach 32, 216–222.

Zhang Q-W, Xu H-P, Mao L-S, Jin H-X. 2019. Evaluation of cysteine sulfinate decarboxylase activity in marine life by high performance liquid chromatography. Journal of the Anhui Agricultural Science 47, 212-215. (in Chinese with English abstract).