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Regulation of dietary glutamine on the growth, intestinal function, immunity and antioxidant capacity of sea cucumber *Apostichopus japonicus* (Selenka)

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ABSTRACT

The present study examined the effects of dietary glutamine (Gln) on the growth, intestinal function, immunity and antioxidant capacity of sea cucumber *Apostichopus japonicus* (Selenka). The specific growth rate, intestinal morphology, activity of digestive enzymes, activity and gene expression of lysozyme and antioxidative enzymes of the sea cucumbers were determined after feeding 5 experimental diets with additions of increasing levels of Gln (at 0%, 0.4%, 0.8%,1.2% and 1.6%, respectively) for 60 days. We discovered that the specific growth rate of the sea cucumbers in 0.4%, 0.8% and 1.2% groups increased 35.3%, 27.3% and 24.1%, respectively, compared to the control (0%) group with significant differences. Dietary Gln can improve the intestinal function of the sea cucumbers by increasing the activities of trypsin and lipase in the intestine and the villus height and villus density of the intestine, eventhough significant differences were not observed in some groups. 0.4%–0.8% of dietary Gln can significantly increase the activity of lysozyme (LSZ) in the coelomic fluid of the sea cucumbers. Significant improvements were observed on the SOD activity in coelomic fluid of the sea cucumbers fed diets supplemented with 0.4%–1.6% of Gln compared to the control group. Similarly, the CAT activity in coelomic fluid of the sea cucumbers significantly increased in 0.8%, 1.2% and 1.6% groups compared to the control and 0.4% groups. Change pattern of the activity of CAT was consistent with the change pattern of the expression of CAT gene, indicating the dietary Gln can up-regulate the expression of CAT gene and consequently promote the secretion of CAT. However, the down-regulation of the expression of SOD gene by dietary Gln were observed in almost all of the treatment groups, which is in contrast with the change pattern of the activity of SOD, indicating the negative feedback regulation of the secretion of SOD on the expression of SOD gene. In summary, the suitable supplementation levels of Gln in diets of sea cucumber *A. japonicus* are 0.4%–0.8%, based on the effectiveness of dietary Gln on the growth, intestinal function, immunity and antioxidant capacity of the sea cucumbers.

1. Introduction

Glutamine (Gln, 2-amino-4-carboxamide butyric acid) is one of the most abundantly free α-amino acids in the body fluids, and there has been a growing interest in Gln as a functional amino acid in nutrition and health in recent years [1,2]. Previous studies have indicated that Gln is the main source of energy for enterocytes and is vital in maintaining the integrity and function of the small intestine by regulating gene expression, cell proliferation, protein turnover and immune function [3–5]. More positive effects of Gln on aquatic animals have been discovered. Some of these positive effects include that dietary Gln plays a crucial role in the immune response of fish [6,7], improves the growth performance of juvenile Jian carp (*Cyprinus carpio* var. Jian) [8] and half-smooth tongue sole (*Cynoglossus semilaevis* Günther) [9], increases the intestine fold height in carp [8,10] and promotes the proliferation of the enterocytes of the fish [11], and Gln is important for protecting cells against H_2O_2 induced oxidative stress in the carp's intestinal

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epithelium [12].

As a commercially important species for mariculture, the sea cucumber *Apostichopus japonicus* has been widely cultured in Asia due to its nutritional and economic value in recent years [13–15]. The terrestrial plants such as corn meal and soybean meal with the advantages of low price and stable market supply have been increasingly used in the formulation diets of sea cucumbers as the replacement of the traditional feed ingredient *Sargassum thunbergii* of which price has dramatically increased due to the rapidly expanded aquaculture of the sea cucumber [16–19]. High levels of terrestrial plants in diets, however, generally lead to various negative effects on aquatic animals, such as damage of the intestinal structure and intestinal inflammation due to the anti-nutritional factors [20–22]. Yu et al. [19,23] discovered that replacing the *S. thunbergii* with supplements of corn meal and soybean meal in the diets of the sea cucumbers reduced the growth performance, ingestion rate and the feed utilization efficiency of sea cucumbers. It is still not known if dietary glutamine can improve the growth performance and intestinal function of sea cucumbers, especially when high levels of terrestrial plants are added in the diets.

Rapid expansion and high farming density have resulted in the occurrence of serious diseases in sea cucumber farming, and it has negatively affected the development of this industry due to the huge economic losses [24–28]. The use of antibiotics in preventing these diseases was not suitable for the sustainable development of aquaculture due to the experimental pollution and drug resistance. Dietary modulation using amino acid, in recent times, has received increased attention as potential alternatives to the antibiotic therapy that is used to control the disease, due to the positive effects of amino acids on the immune response of animals [1]. However, no previous study has been conducted to determine the modulation of the immunity and antioxidant capacity of the sea cucumbers, which is, induced by dietary Gln, otherwise, the role of dietary Gln as modulators of the expression of certain genes involved in the immunity and antioxidant capacity of sea cucumbers, would have been known.

The objective of the present study was to evaluate the effects of dietary Gln on the growth, intestinal function, immunity and the antioxidant capacity of the sea cucumber *Apostichopus japonicus*. It was also aimed at determining the suitable addition levels of Gln in the diets of sea cucumbers and understanding the role of dietary Gln as modulators of the intestinal function, immunity and the antioxidant capacity of sea cucumber *Apostichopus japonicus* (Selenka).

2. Materials and methods

2.1. Feed ingredients and diets formulation

Ingredients and nutrient composition of the experimental diets are presented in Table 1. Five isoproteic (19.6% crude protein) and isolipidic (3.48% crude lipid) diets were formulated, with the diets adding graded levels of glutamine (Gln) (0%, 0.4%, 0.8%, 1.2% and 1.6% of dry diet), and the 0% Gln group was the control group. The glycine that has no functions of glutamine was used to balance the crude protein levels among the different diets [29]. Glutamine and glycine were obtained from Fulin Biotech. Co., Ltd (Qingdao, China), and the purity were over 99%. All ingredients were ground into fine powder through 200 mm rate and thoroughly blended. Pellets (2.0 × 2.0 mm) were made automatically using the pellet-making machine (Weihai, Shandong province, China) and dried in a ventilated oven at 40 °C for about 10 h. Then, the feeds were packed in double plastic bags and stored at −20 °C until use.

Table 1
Proximate composition of trial diets for sea cucumbers (Dry matter basis).

	Glutamine (Gln) levels (%)				
	0	0.4	0.8	1.2	1.6
Ingredients (%)					
Fish meal[a]	8	8.0	8.0	8.0	8.0
Soybean meal[a]	10	10.0	10.0	10.0	10.0
Corn meal[a]	20	20.0	20.0	20.0	20.0
Sargassum thunbergii[a]	30	30.0	30.0	30.0	30.0
Glutamine[a]	0.0	0.4	0.8	1.2	1.6
Glycine[a]	1.6	1.2	0.8	0.4	0.0
Vitamin premix[b]	0.5	0.5	0.5	0.5	0.5
Mineral premix[c]	0.5	0.5	0.5	0.5	0.5
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
Fish oil[a]	0.5	0.5	0.5	0.5	0.5
Soybean oil[a]	0.5	0.5	0.5	0.5	0.5
Sea mud[a]	27.9	27.9	27.9	27.9	27.9
Proximate composition (%)					
Moisture	10.5	10.5	10.5	10.5	10.5
Crude protein	19.6	19.6	19.6	19.6	19.6
Crude lipid	3.48	3.48	3.48	3.48	3.48
Ash	36.5	36.5	36.5	36.5	36.5

[a] Soybean meal (dry matter, %): crude protein 50.3, crude lipid 1.79; corn meal (dry matter, %): protein 10.7, crude lipid 4.54; *Sargassum thunbergii* (dry matter, %): crude protein 19.4, crude lipid 2.00; fish meal (dry matter, %): protein 70.1, crude lipid 8.06; sea mud (dry matter, %): protein 2.74, crude lipid 0.90; These ingredients were obtained from Great seven Bio-Tech (Qingdao, China).

[b] Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1}premix): L-ascorbic acid, 100; DL-a-tocopheryl acetate, 2; thiamin hydrochloride, 8; riboflavin, 10; pyridoxine hydrochloride, 15; niacin, 45; Ca-D-pantothenate, 18; myo-inositol, 80; D-biotin, 0.3; folic acid, 1.5; menadione, 4; retinyl acetate, 3.2; cholecalciferol, 1; cyanocobalamin, 0.004; ethoxyquin 16.

[c] Mineral premix contained the following ingredients which were diluted in zeolite (g kg^{-1} premix): $MgSO_4$ $7H_2O$, 80.5; Ferric citrate, 16; $ZnSO_4.H_2O$, 9; $CuSO_4$ $5H_2O$, 3; $AlCl_3$ $6H_2O$, 6; KIO_3, 0.04; $MnSO_4$ H_2O, 2; $CoCl_2$ $6H_2O$, 0.04.

2.2. Experimental procedures and sample collection

The experimental sea cucumbers were collected from a local sea cucumber farm in Qingdao, China. Prior to the start of the experiment, the sea cucumbers were reared in plastic tanks and fed the control diet for two weeks for adapting to the experimental feeds and laboratory conditions. After acclimation, the sea cucumbers were fasted for 24 h, and 120 individuals of the sea cucumbers with similar sizes (5.00 ± 0.05 g) were selected and randomly distributed into 20 aquariums (30 × 40 × 50 cm). For each of the 5 experimental diets, 4 tanks of sea cucumbers were cultured as triplicates. The sea cucumbers were fed with a ration of 5% of the body weight once a day at 4:00 pm. During the experiment, aeration was continuously provided and half of the water in each aquarium was exchanged every day at 10:00. Seawater temperature was kept at 17 ± 0.5 °C using an air conditioner, a salinity of 29–31, pH of 8.0–8.4, and dissolved oxygen >6.0 mg L^{-1}.

The experiment lasted for 60 days. At the termination of the experiment, the sea cucumbers were fasted for 24 h, and then, the total number and body weight of the sea cucumbers in each aquarium were measured. After biomass weighing, all individuals of the sea cucumber in each aquarium were dissected on ice, and coelomic fluid were obtained from the coelom with 1 ml syringes and put into 1.5 ml eddffe tubes for enzymatic assay. The separated intestinal tracts and the inspiration tree which were used for gene expression were thoroughly put into 1.5 ml eddffe tubes (RNAase-Free; Axygen), and the separated body walls were put into plastic bags. All the samples were frozen in liquid nitrogen and stored at −80 °C immediately for future analysis. Samples from each tank were pooled. The dissection tools used for the sample treatment were pretreated at 180 °C for 4 h in order to eliminate RNAase.