Aquaculture 486 (2018) 98–106

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary stachyose altered the intestinal microbiota profile and improved the intestinal mucosal barrier function of juvenile turbot, *Scophthalmus maximus* L.

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ARTICLE INFO

Keywords:
Stachyose
Intestinal microbiota
Intestinal tight junction
Turbot

ABSTRACT

Recent studies have revealed the beneficial effects of stachyose on intestinal histology and digestive function of fish. However, a comprehensive understanding of stachyose's impact on intestinal health of fish remains unclear, limiting its use in aqua-feed. In the present study, a 12-week feeding trial was conducted to investigate the effects of dietary stachyose on intestinal microbiota and mucosal barrier function of turbot (*S. maximus* L). Three isonitrogenous and isolipidic experimental diets were formulated to contain 0%, 1.25% and 5% stachyose, respectively. Sequencing of bacterial 16s rRNA V_4 region indicated that dietary stachyose altered the intestinal adherent microbiota profile, which was supported by the diet-cluster of PCA, PCoA, beta diversity heatmap and phylogenetic tree. LEfSe and MetaStat analysis indicated that both 1.25% and 5% dietary stachyose significantly elevated the abundance of intestinal cellulose-degrading bacteria. However, the higher level of stachyose (5%) increased the abundance of intestinal beneficial bacteria as well as that of potential pathogenic bacteria. Moreover, 1.25% dietary stachyose significantly up-regulated the genes expression of occludin, claudin-3, and ZO-1, and down-regulated the gene expression of claudin-like in the intestine ($P < 0.05$). Dietary stachyose at 5% significantly increased mucin-2 secretion and the gene expression of ZO-1, while significantly decreased the gene expression of claudin-like in the intestine ($P < 0.05$). Collectively, our study showed that dietary stachyose supplementation could favorably modulate the profile of intestinal microbiota and enhance the intestinal mucosal barrier function in juvenile turbot. Stachyose showed promising potential of being used as prebiotic in diet for enhancing the intestinal health of turbot.

1. Introduction

As an oligosaccharide, stachyose has been used as prebiotics in human and mammal food industry, and it has been reported to be able to promote the growth of specific gut bacteria species and improve the intestinal health (Mussatto and Mancilha, 2007; Li et al., 2013; Li, T., et al., 2017; Pacifici et al., 2017). Studies concerning the effects of stachyose on fish performances are limited. In some cases, contradictory results even existed (Cai, 2006; Cai et al., 2012; Hu et al., 2015). Some recent studies in fish have suggested the potential prebiotic properties of stachyose. Moderate levels of stachyose in diets improved the intestinal development and digestive capability (Mi et al., 2011; Sørensen et al., 2011; Hu et al., 2015). Nevertheless, these studies mainly focused on the gut histological structures and digestive function, whereas more expanded effects of stachyose on intestinal health-related properties is less investigated in fish.

Both the homeostasis of intestinal bacteria and the integrity of intestinal mucosal barrier function are important components of gut health (Maloy and Powrie, 2011). In the healthy state, the intestinal bacteria can exert a series of beneficial effects on the intestinal health of the host, such as providing nutrients and energy to the host via the fermentation of non-digestible dietary components, influencing the development of intestinal mucosal and protecting the gut from invasive pathogens (Sekirov et al., 2010; Flint et al., 2012; de Medina et al., 2014; Liu et al., 2016). Meanwhile, the intact intestinal mucosa provides an essential barrier against harmful substances and pathogens

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https://doi.org/10.1016/j.aquaculture.2017.12.014
Received 17 August 2017; Received in revised form 17 November 2017; Accepted 10 December 2017
Available online 12 December 2017
0044-8486/

from the external environment. Its function is built mainly upon the epithelial layer which constitutes a physical barrier (de Medina et al., 2014; König et al., 2016), and mucus secreted from epithelial goblet cells provides protection for the epithelium layer(Maloy and Powrie, 2011; Sahlmann et al., 2013). Tight junctions (TJs) proteins between intestinal epithelial cells which include members of the Claudin family, Occludin family and Zonula occludens 1 (ZO-1) are also indispensable in the maintenance of barrier integrity and function (Turner, 2009; Jutfelt, 2011). Among the various factors that regulate intestinal bacterial community and barrier function, dietary ingredients were found to play an vital role in influencing gut microbial composition and modifying expression and localization of TJ proteins (Clemente et al., 2003; Drago et al., 2006; Ulluwishewa et al., 2011; Luo et al., 2014; Ringø et al., 2016; Schmidt et al., 2016; Huyben et al., 2017; Li, Y., et al., 2017; Zhou et al., 2017).However, no information has been reported about the effects of dietary stachyose on these two aspects of intestinal health in fish.

To further investigate the expanded effects of dietary stachyose on fish intestinal health, the present study evaluated the effects of a low (1.25%) or high (5%) dose of stachyose on the intestinal microbiota profile and mucosal barrier integrity in turbot, which are both an important fish species for aquaculture industry and a good model marine fish for academic research. The results will gain novel insight into the nutritive property of stachyose.

2. Materials and methods

2.1. Ethics statement

Procedures for animal care and handling in the present study were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

2.2. Experimental diets design

The present experiment is an extension of our precious study (Hu et al., 2015), which was designed to evaluate the effects of dietary stachyose, an oligosaccharide often regarded as an anti-nutritional factor (ANF), on the growth performance and intestinal health of turbot. Among all oligosaccharides, stachyose is one of the most predominant ANFs in plant ingredients, and the content of stachyose in soybean meal is around 5%. In our previous experimental design, five isonitrogenous and isolipidic diets were formulated to contain 0, 0.625%, 1.25%, 2.5% and 5% stachyose respectively, which is equal to the content of stachyose in fish diets when 12.5%, 25%, 50% and 100% soybean meal was used in the diet of turbot. Based on the results our precious study, three diets were chosen in the present experiment to contain 0% (FM) (As the Control group), 1.25% (S-1.25) and 5% (S-5) stachyose, respectively (Table 1).

The feed was made, packed and stored following the standard procedures of our lab. Briefly, dietary ingredients were ground into fine powder through 320 μm mesh. All ingredients were thoroughly mixed with fish oil, then water was added to produce stiff dough. The dough was then pelleted with an experimental single-screw feed mill. After being pelleted, the feeds were dried in a ventilated oven at 45 °C for about 12 h and then stored in a freezer at − 20 °C (Xu et al., 2010).

2.3. Feeding trial

Juvenile turbot (*Scophthalmus maximus* L.) were obtained from a commercial farm in Laizhou, China. Prior to the start of the experiment, fish were acclimated to a commercial diet (Great Seven Bio-Tech Co. Ltd., Qingdao, China) for two weeks. Then the fish were fasted for 24 h and weighed. A total of 270 fish (initial weight 4.63 ± 0.01 g) were randomly distributed to 9 cylindrical fiberglass tanks (200L) in an indoor rearing system with flowing sea water. Each experimental diet was randomly assigned to three tanks. The feeding trial lasted for 12 weeks. During the feeding period, fish were slowly hand-fed to apparent satiation twice daily (7:30 and 19:30). The rearing conditions followed those in our previous studies (Hu et al., 2015).

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients (%)	FM	S-1.25	S-5
Menhaden fish meal[a]	67.00	67.00	67.00
α-Starch[a]	16.00	16.00	16.00
Menhaden fish oil[a]	3.50	3.50	3.50
Soybean lecithin[a]	0.50	0.50	0.50
Choline chloride	0.30	0.30	0.30
Vitamin premix[b]	1.00	1.00	1.00
Mineral premix[c]	0.50	0.50	0.50
$Ca(H_2PO_3)_2$	0.50	0.50	0.50
Y_2O_3	0.05	0.05	0.05
Stachyose[d]	0	1.37	5.47
Microcrystalline cellulose	10.65	9.28	5.18
Analyzed nutrients compositions (dry matter basis)			
Crude protein	48.45	48.33	47.99
Crude lipid	9.40	9.02	9.14
Ash	8.41	8.23	8.64

Abbreviations: FM, fish meal diet; S-1.25: 1.25% stachyose diet; S-5: 5% stachyose diet.

[a] Menhaden fish meal, α-starch, menhaden fish oil and soybean lecithin were obtained from Great Seven Bio-tech (Shandong, China). Menhaden fish meal: crude protein 74% dry matter, crude lipid 9.7% dry matter.

[b] Vitamin premix (mg/kg diet): thiamin, 25; riboflavin (80%), 45; pyridoxine HCl, 20; vitamin B_{12}, 10; vitamin K_3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin (2%), 60; retinyl acetate, 32; cholecalciferol, 5; α-tocopherol, 240; ethoxyquin 3; ascorbic acid 2000; Microcrystalline Cellulose, 6470.

[c] Mineral premix (mg/kg diet): $MgSO_4{\cdot}7H_2O$, 1200; $CuSO_4{\cdot}5H_2O$, 10; $FeSO_4{\cdot}H_2O$, 80; $ZnSO_4{\cdot}H_2O$, 50; $MnSO_4{\cdot}H_2O$, 45; $CoCl_2{\cdot}6H_2O$ (1%), 50; $Ca(IO_3)_2$ (1%), 60; Na_2SeO_3 (1%), 20; Zeolite, 3485.

[d] 91.45% stachyose, Xi'an Rongsheng Bio Technology Co., Ltd.

2.4. Sample collection

At the end of the feeding trial, six hours after the last feeding, fish were anesthetized with eugenol (1:10,000) (purity 99%, Shanghai Reagent Corp, Shanghai, China), and then counted and weighed. For the analysis of intestinal microbiota, the surface of one randomly selected fish per tank (three fish per treatment) was sterilized by tampon with 70% alcohol, then the abdominal cavity of fish was opened; the whole intestine was removed, opened, and cleared of intestinal content carefully, by using sterile scissors and bistoury, around alcohol flame. The whole intestinal mucosa layer of these three fish per treatment were carefully scraped from foregut region to hindgut region using sterile rubber spatula, then transferred to 2 mL sterile tubes (Axygen, America). To analyze intestinal gene expression the whole intestinal mucosa layer from other six randomly selected fish per tank were collected following the procedure described above, then transferred to 1.5 mL RNase-free tubes (Axygen, America). All the above mentioned samples were immediately stored at liquid nitrogen before further processing.

2.5. Intestinal microbiota DNA extraction and sequencing

Genomic DNA sample was extracted from the whole intestinal mucosa layer of one fish per tank using a QiAamp DNA stool Mini Kit (Qiagen, Germany) on super clean bench around alcohol flame with some modifications as we previous described. Briefly, the intestinal mucosa layer samples were transferred from − 80 °C to ice for a short while, then carefully transferred to a 5 mL sterile tube containing sufficient InhibitEX buffer (proportional to the tissue weight). The tube was subjected to vortex at maximum speed for 1 min, and 1 mL of the homogenate was used for the downstream DNA extraction according to