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Effects of dietary raffinose on growth, non-specific immunity, intestinal morphology and microbiome of juvenile hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂)

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ABSTRACT

This study was performed to determine the efficacy of raffinose on the growth, non-specific immunity, intestinal morphology and microbiota of juvenile hybrid sturgeon, (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂). Hybrid sturgeons were divided into 2 groups and each group was fed with diets supplemented with or without raffinose for 56 days. Hybrid sturgeon fed diet supplemented with raffinose had significantly higher final body weight (FBW), specific growth rate (SGR), and weight gain ratio (WGR) than fish fed the control diet ($P < 0.05$). Raffinose in diet had no negative effect on feed intake (FI) and feed conversion ratio (FCR) ($P > 0.05$). Compared with the control diet, the myeloperoxidase (MPO) and respiratory burst (NBT) activitives were significantly higher in sturgeon fed the raffinose supplemented diet ($P < 0.05$). The increasing of intestinal villi area and mucosal folds were observed in intestinal tract of sturgeon when they fed the raffinose supplemented diet. Meanwhile, the residual bait of intestinal tract was relatively lower in sturgeon with raffinose treatment. High-throughput sequencing revealed that majority of reads derived from the sturgeon digesta were constituted by members of Proteobacteria, Firmicutes, Fusobacteria and Actinobacteria. Shannon's diversity index existed significant difference among dietary treatments indicating that the overall microbial community was modified to a large extent by dietary raffinose. In conclusion, supplementation of the diet with raffinose is capable of improving hybrid sturgeon growth performances and intestinal morphology, modifying the intestinal microbial composition.

1. Introduction

As the living fossil, sturgeons are ancient and have relatively low rates of evolution compared with other vertebrates. These unique properties mean sturgeon not only has high economic value, also has valuable academic value. All 27 sturgeon species were already listed in CITES [1–3]. To pursue commercial purposes and restocking human consumption, a lot of research about sturgeon for culture under farmed conditions has been actively explored in the last two decades [4]. China has already become the largest sturgeon culture region since 2000 [5]. Hybrid sturgeons are a dominant culture object due to its rapid growth rate and strong resistance to disease [6,7], and hybrid sturgeon of *Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂ is one of the most common hybrid varieties in China.

With popularizing intensive culture, the disease susceptibility of hybrid sturgeons increased obviously [8]. In consideration of food safety and environmental issues, antibiotics are banned in animal feeds, including fish [9]. Research for alternative nutraceutical products has been a major objective for global fish culture practices [10]. Manipulation of microbial populations associated with the fish host in the rearing environment, as the alternative methods of disease prevention method, can be used to reduce the presence of opportunistic pathogens and simultaneously stimulate the host immunological responses [10]. In this respect, manipulating the microbiota within the gastrointestinal tract (GI) of the fish has given us a more broad understanding of the importance of the intestinal microbiota [11]. This is supported by other investigations which indicate that the GI microbiota of fish play a key role in nutrition and immunity, and it has major influences on growth, health and development of fish [12–16].

Due to the importance of GI microbiota, the related research on fish has received increasing attention [17]. Most researches have mainly focused on evaluating the effect of prebiotics, probiotics and digestive enzymes on fish GI microbiota. In particular, dietary supplementation of prebiotics is given high priority at the present because of it is a novel approach to promote health and growth performance by modifying the intestinal microflora [18]. Prebiotic was a non-digestible food

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ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health [19]. In other words, prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora to enhance health and wellbeing. Indeed, many trials have emphasized the effect of prebiotics for GI microbiota in some kinds of fish, such as tilapia, grass carp, seabass and so on [20–24].

Commonly, β-glucans and chitosan are mainly commercial prebiotics in the fish aquaculture industry to induce and build up protection against a wide range of diseases [25–30]. Raffinose, as a functional oligosaccharide, was widely existed in nature and used as medicine, food and cosmetics additives [31,32]. It has obvious effects on benefit improvement the proliferation of *Bifidobacterium*, healing property, anti-oxidation activity, enhancing the immunity, and so on [33–36]. In addition, raffinose is also popular in animal husbandry. According to Berrocoso et al., raffinose has the potential of enhancing ileum mucosa morphology and improving immunity of broilers [37]. Pacifici et al. studied the effect of raffinose in vivo by utilizing the poultry model (*Gallus gallus*), and the result showed that raffinose beneficially affected the gut microflora, Fe bioavailability, and brush border membrane functionality [38]. And there is research to show that raffinose could improve growth performance and stimulate gut microflora of the chickens [39]. Nevertheless, the use of raffinose to stimulate disease resistance in hybrid sturgeon has received little attention. In order to develop the new type of prebiotic using in the fish aquaculture industry, the present study was to evaluate the effect of raffinose on growth performance, non-specific immunity and gut microbiota of juvenile hybrid sturgeon (*A.* Brandt ♀ × *A. schrenckii* Brandt ♂).

2. Materials and methods

2.1. Experimental diets

Two fishmeal based diets were formulated to be isonitrogenous (38.7%), isolipidic (10.0%) and isoenergetic (18.0 MJ Kg^{-1}). The basal diet was used as the control diet. The content of raffinose is 90% and its dose in the experimental diet was recommended by the producer (the recommended dose of raffinose is 0.1% when its purity is 100%), China Leader Biologincal Science and Technology Co., Ltd (Beijing, China). Both experiment and the control diets were made into sinking extruded pellets by an extruder (MY56 × 2A, MUYANG Group, Jiangsu province, China) in different pellet diameters (2.0 mm and 3.0 mm) according to fish size. All diets were air-dried and stored at −20 °C until use. Ingredients and proximate composition of experimental diets are presented in Table 1.

2.2. Experimental animals and husbandry

Juvenile hybrid sturgeons were obtained from a commercial farm (Fangshan, Beijing, China). Fish were acclimatized in the experimental conditions and fed with basal diets for one week. Then, healthy sturgeon (mean initial weight 37.5 ± 0.0 g) were randomly distributed into 8 rearing cubic fiberglass tanks (capacity: 260 L) for the growth trial (30 fish/tank). Each feed diet was randomly assigned into four tanks. Fish were hand-fed to apparent satiation three times (9:00, 13:00 and 17:00) daily for 8 weeks. During the experiment, water temperature was 16.5 ± 1.0 °C and the dissolved oxygen content was approximately 7.8 mg L^{-1}, the photoperiod used was a 12 h light/12 h dark cycle. Feces and uneaten feed were removed by siphon. Dead fish were weighted and mortality was recorded daily.

2.3. Sampling procedures

At the end of the feeding trial, the fish were fasted for 24 h before sampling. 6 fish in each tank were randomly and quickly captured and weighed individually after being anaesthetized by immersion, until sedate, in a 2-phenoxy ethanol (0.30 ml L^{-1} water) solution. Blood samples were taken from the caudal vessels of 3 fish of the total 6 sedated fish using 2 ml disposable syringes in less than 1 min and divided into two portions. The first part was transferred to centrifuge tubes with heparin rinse and used to detect the respiratory burst activity, while the other part was centrifuged (1500 g, 5 min, 4 °C) to obtain serum. The serum samples were stored at −80 °C until applied to determine the antioxidant and non-specific immune indices.

After blood sampling, the gut of these 3 fish were sampled for histological appraisal (scanning electron microscopy, SEM). Each gut was washed in 1% S-carboxymethyl-L-cysteine for 30 s to remove mucus before fixing in 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M pH 7.2) [40,41]. The whole gut was separated into the front, mid- and terminal intestine based on the gut structure and the thickness of the intestinal wall. The SME samples were processed as described elsewhere [41] and screened with a HITACHI S–4800 (HITACHI, Tokyo, Japan) SEM. The SEM images were analyzed to assess the relative abundance of microvilli count and mucosal fold, and to observe the residual diets in intestinal tract.

The whole intestinal tract with faecal matter was removed from the other three sedated fish in each tank. All sampling operations were conducted on the ice, and the whole intestinal tract samples were stored in −80 °C immediately for the following intestinal microbiology analysis.

After all sampling, all rest fish in each tank were weighted and counted. Based on recording the weight of all fish and counting the number of sturgeon, specific growth rate (SGR), feeding intake (FI), feed conversion ratio (FCR) and weight gain rate (WGR) were calculated using the following equations:

$$SGR = 100\% \times (\ln FBW - \ln IBW) / days$$

$$FI = 100\% \times \text{diet consumed} / days / \text{fish number}$$

$$FCR = \text{feed consumed} / (FBW - IBW)$$

$$WGR = 100\% \times (FBW - IBW) / IBW$$

IBW is initial body weight, FBW is final body weight, and days are total duration of experiment.

Table 1
Ingredient composition and proximate composition of experimental diets.[f]

Ingredient (%)	Raffinose	The control
Fish meal[a]	51.0	51.0
Wheat flour[b]	37.48	37.6
Premixes[c]	1.4	1.4
Soy lecithin[d]	2.0	2.0
Anchovy oil[a]	8.0	8.0
Raffinose[e]	0.12	0
Proximate composition		
Dry matter (% DM)	90.26	90.30
Crude protein (% DM)	45.19	45.24
Crude fat (% DM)	9.80	10.16
Ash (% DM)	6.38	6.37
Gross energy (MJ Kg^{-1})	18.05	18.06

[a] Fishmeal and anchovy oil were supplied by TripleNine Fish product Co., Esbjerg, Denmark, and 5g Kg^{-1} BHT (dry diet) was added into anchovy oil to prevent lipid peroxidation.
[b] Guchan Group, Beijing, China.
[c] Beijing Enhalor Biotech Ltd. Co. Beijing, China.
[d] YiHai Kerry Investment Company Limited, Shandong, China.
[e] China Cotton-unis Bioscience Co.,Ltd, Beijing, China.
[f] All diets were produced at National aquafeed safety evaluation station, Beijing, China, as extruded pellets.