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Galactooligosaccharide effects as prebiotic on intestinal microbiota of different fish species

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Abstract. Manipulation of the gut microbiota toward potentially beneficial bacteria (probiotics) has beneficial effects on fish physiology and health. The effects of prebiotics on gut microbiota are species specific. The present study aimed at investigation of the effects of galactooligosaccharide (GOS) as prebiotic on intestinal microbiota of Caspian roach and Caspian white fish fingerlings. which are among the most economically valuable species in the Caspian Sea. The study was conducted in a completely randomized design with two set of experiment each of them include three treatments in triplicates in which 0 (control), 1 and 2% GOS were used in diet for 6 weeks. At the end of the period, changes in the intestinal microbiota, including total bacterial count, lactic acid count and lactic acid bacteria (LAB) levels and dominance of LAB in the intestinal microbiota, were measured by culture-based method. Dietary GOS had no significant effect on total bacterial count in both species ($P < 0.05$). The LAB levels in the intestinal microbiota in the treatments fed with prebiotics was significantly higher than the control group ($P < 0.05$). LAB bacteria showed the highest increase and dominance in treatments fed with 2% GOS. Also, the highest ratio of lactic acid bacteria to the total number of viable bacteria was observed in the treatment with 2% GOS treatment ($P < 0.05$). The results of this study indicated the possibility of alterations in the bacterial communities of Caspian roach and Caspian white fish fingerlings gut toward beneficial bacterial communities using GOS as prebiotic.

Key words: prebiotic, Caspian white fish, Caspian roach, galactooligosaccharide, gut microbiota

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Introduction

The study of gut microbiota is of high importance not only regarding disease but also regarding the status of fish physiology and immunity [1]. Establishment of a healthy microbiome in intestine has direct immune-physiological effects on host. It is now well-demonstrated that there is a direct cross talk between gut microbiota and immune response of fish [2, 3]. With the identification of lactic acid bacteria (LAB) in the intestinal microbiota of different fish and shrimp shellfish species in the last decade and determination of their role in the health, welfare and growth performance of the host, the importance of this group of bacteria has become increasingly clear [4, 5]. Although the presence of LAB in the intestinal microbiota of many fish, including Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*), Beluga (*Huso huso*), Persian sturgeon (*Acipenser persicus*) and Arctic charr (*Salvelinus alpinus*) has been proven, they are not the dominant microbiota and constitute a very limited portion of gut microbiota of these species [6]. In addition, it was not possible to isolate LAB bacteria from several fish species [5, 6]. Given this fact, it has been attempted to increase the number of these bacteria through dietary approaches [7, 8]. One of the most important compounds suggested in this regard are prebiotics, which are compounds that are not absorbed by host organism and consumed by potentially beneficial intestinal bacteria (such as LAB) and increase their numbers [9, 10].

Despite recent studies on the effects of prebiotics on fish growth, immunity and physiological indices, many aspects of their potential for alteration of gut microbiota in aquatic and increasing dominance of beneficial bacteria remained unknown [11]. The previous studies revealed that different prebiotics had different effects on LAB levels and also a single prebiotic had different effects on different fish species. Even in some cases, using high levels of more complex prebiotics (higher degree of polymerization) resulted in adverse effects on total bacterial counts and LAB levels [12]. The contradictory of a prebiotic on different host can be due to difference in intestinal microbiota, physiological condition of digestive tract, etc. [13]. Therefore, determination of a prebiotic effect on intestinal microbiota of different species based on comparative studies will help to identify the best prebiotic to change the gut microbiota for that species.

Galactooligosaccharide (GOS) is one of the most promising prebiotic which previous studies revealed that it could exerts positive effects in different fish species [14—16]. In spite of extensive researches on administration of GOS in fish [17—21], to the best of our knowledge there was no published study the effects of GOS on gut microbiota of different fish species using comparative study. Therefore, in the present study we decided to determine the possible effects of GOS on intestinal microbiota of Caspian roach and Caspian white fish.

Materials and methods

Experimental diets

A commercial feed (Dansu, Iran) was used as a control diet (non-supplemented diet). To prepare experimental diet the basal diet was supplemented with two levels of GOS as prebiotic (1 and 2 %). The ingredients were blended thoroughly in a mixer. Then, water was added and made into pellets. The pellets were air-dried, ground and sieved to produce a suitable crumble (ca. 500μm). The experimental diets were stored in plastic bags at −2 °C for further use.

Fish husbandry

The present study was conducted at the Gharasu Fisheries Research Station. The Caspian white (*Rutilus kutum*) fish and Caspian roach (*Rutilus caspicus*) fingerlings were supplied by Sijowal Caspian Sea Teleost Fish Propagation & Cultivation Centre (Golestan province, Iran). Fish with mean weight of 1.3 g were stocked in nine separate tanks for each species (totally 18 tanks) at density of 30 fish per tank. The fish were acclimated to lab condition for 2 weeks and then feeding with experimental diets were started. During acclimation, fish were fed with control diet. The culture system water was closed with constant aeration. To maintain water quality every 2 days 50 % of water was exchanged. The water quality parameters were controlled and maintained at optimum levels.

Prebiotic

The prebiotic used in the presents study was GOS that was kindly supplied by Friesland Foods Domo Company (Zwolle, The Netherlands). The commercial product name was Vivinal-GOS® and obtained through the enzymatic conversion of lactose and mainly consists of galactose and glucose molecules.

Evaluation of gut microbiota

Total viable autochthonous heterotrophic aerobic bacteria and LAB levels were determined at the start of trial from 15 specimens from the initial pool of fish. Also, at the end of the feeding trial (week 8) microbiological studies were performed. Fish were starved for 24 h to study the autochthonous microbiota. Three specimen were randomly selected from each tank (i.e. $n = 9$ per treatment).The intestine of fish were assessed and prepared for bacteria culture as we described on previous study [12]. Briefly, the surface bacteria were killed before dissection using 0.1 % benzalkonium chloride. With utmost care to be aseptic, the intestine of samples obtained, washed with sterile saline and homogenized using tissue homogenizer (*Potter-Elvehjem, USA)*. The homogenized intestine was serially diluted to 10^{-7} by using sterile saline (0.85 % NaCl). Then, to dermine the level of total bacteria and LAB a portion of the diluates (100 μL) was spread onto plate count agar (PCA) (Merck, Germany) and de Man, Rogosa and Sharpe (MRS) agar (Merck, Germany), respectively. The seeded plates were incubated at room temperature (25ºC) for 5 days [22]. Thereafter, the colony forming units (CFU) g^{-1} were counted from statistically viable plates (i.e. plates containing 30–300 colonies)[23].

Statistical analysis

Prior to statistical analysis, the normality of data and homogeneity of variance were checked and confirmed. Then, the statistically significant difference (at $P < 0.05$) between treatments was checked using One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (36). All statistical analysis were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The figures were drawn using Excel software (Micorsoft Office ver. 2016).

Results and discussion

The total viable autochthonous heterotrophic aerobic bacteria (THAB) level (Log CFU/g) in the intestine of Caspian roach and Caspian white fish fingerlings fed with different levels of galactaligosaccharide (GOS) as prebiotics is shown in Figure 1. At the beginning of the feeding trial, the THAB of intestinal microbiota was 5.10 ± 0.24 log CFU/g. As shown in Figure 1 A, dietary administration of 1 or 2% GOS in diet had no significant effect on THAB counts in the gut microbiota of Caspian roach ($P > 0.05$). Similar result was noticed in case of the gut microbiota of Caspian white fish ($P > 0.05$) (Figure 1 B).

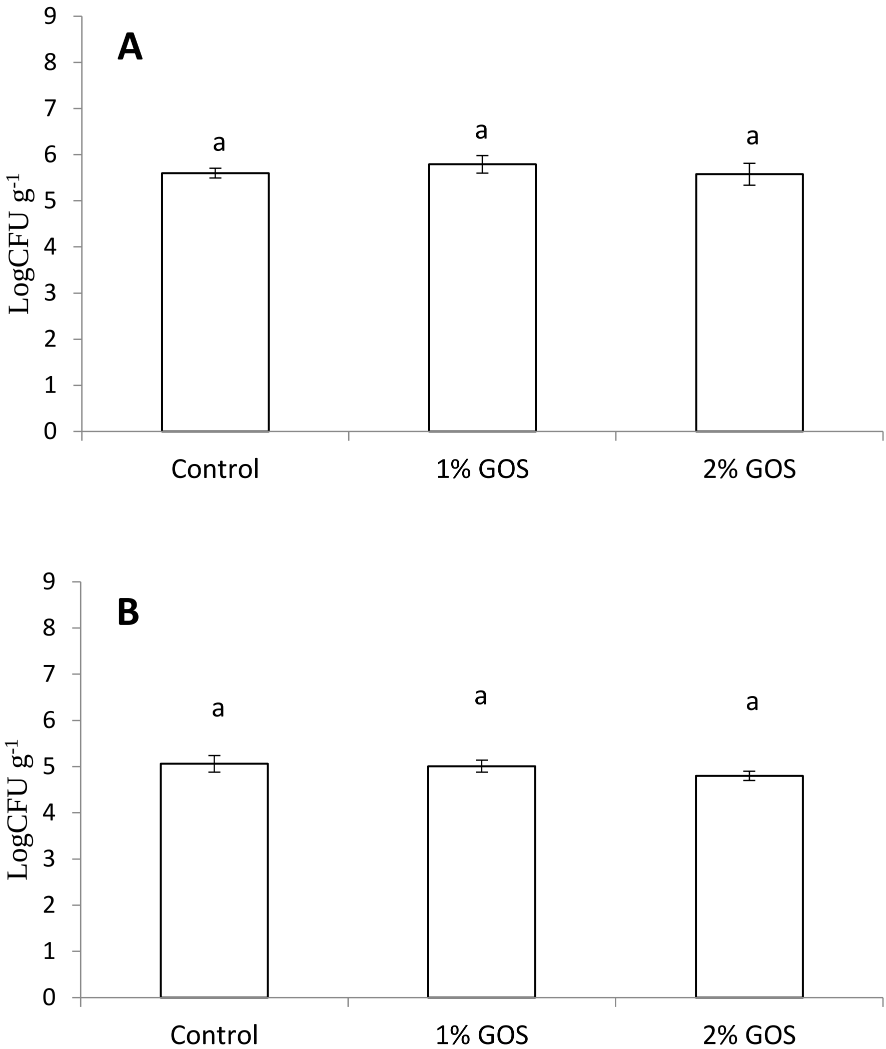

Fig. 1. The effects of different levels of galactoaligosaccharide (GOS) as prebiotic on total bacterial counts (log CFU/g) in Caspian roach (A) and Caspian white fish (B) fingerlings.
The bars (mean ±SD) assigned similar letters indicate no significant difference ($P > 0.05$)

The effects of different levels of GOS prebiotics on the level of LAB of lactic acid bacteria (Log CFU/g) in the gut microbiota of Caspian roach and Caspian white fish fingerling sare summarized in Figure 2. At the beginning of the period, no lactic acid bacteria were isolated from the gut microbiota of both fish species. Indeed, the number of LAB in the intestinal microbiota were statistically too few to count (TFTC; lower than 30 colonies in the first dilution). Similarly, at the end of trial in case of both fish species the LAB levels were TFTC in the control treatment. While, feeding with GOS caused significant increase of LAB level in gut microbiota of the Caspian roach and Caspian white fish fingerlings. In both species, the highest LAB level was noticed in gut microbiota of fish fed with 2% GOS. There were significant difference between 1% GOS and 2% GOS treatment in case of gut microbiota LAB level in Caspian roach ($P < 0.05$). However, no significant difference was noticed in case of 1 and 2% GOS in Caspian white fish ($P > 0.05$).

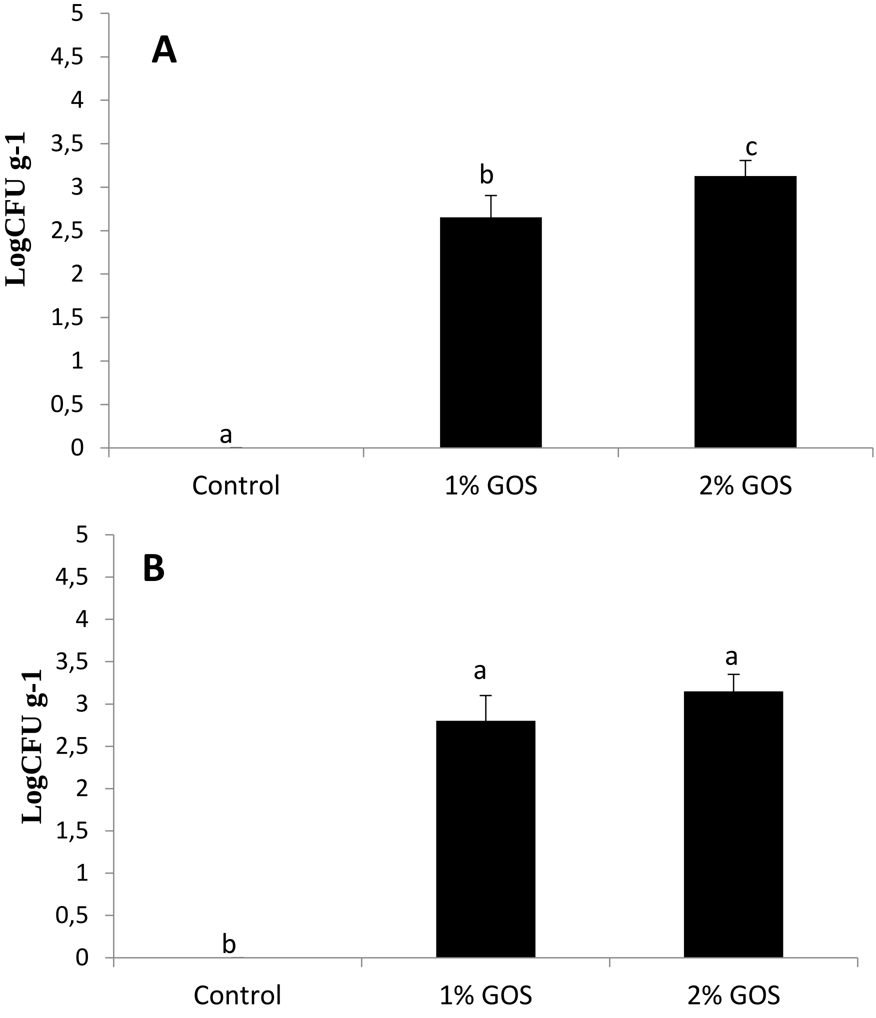

Fig. 2. The effects of different levels of galactoaligosaccharide (GOS) as prebiotic on Lactic acid bacteria levels (log CFU/g) in Caspian roach (A) and Caspian white fish (B) fingerlings. The bars (mean ±SD) assigned with different letters indicate significant difference ($P < 0.05$)

In addition, we calculated the ratio of LAB (potentially useful probiotic bacteria) to THAB in the gut microbiota of both species to see the alteration in the dominance of LAB in gut microbiota (Table 1). The obtained results showed that the ratio of LAB to THAB in all prebiotic treatments was significantly higher than the control treatment ($P < 0.05$). The highest increase in the ratio of lactic acid bacteria to the total number of viable bacteria was observed in the 2% GOS treatment ($P < 0.05$). Although the addition of GOS to Caspian white fish diet significantly increased the ratio of lactic acid bacteria, this increment was not dose dependent; there was no significant difference between 1 and 2 % levels ($P < 0.05$).

Table 1

The ratio (%) of lactic acid bacteria to the total viable bacteria in the gut microbiota of Caspian roach and Caspian white fish fingerlings fed with different levels of GOS as prebiotic. The data in a row (mean ± SD) assigned with different letters indicate significant difference ($P < 0.05$)

Fish species	Treatments		
	Control	1% GOS	2% GOS
Caspian roach	TFTC^{c}	1.70±0.34^{b}	4.84 ± 0.81^{a}
Caspian white fish	TFTC^{b}	4.45±0.16^{a}	4.12 ± 0.41^{a}

The intestinal microbiota of the fish includes a complex and diverse community of aerobic and anaerobic bacteria. One of the group of bacteria in the gut microbiota are LAB that are of great importance nowadays as probiotics [24]. Although isolation of lactic acid bacteria from the gut microbiota of various species of fish has been reported, these bacteria are not among the predominant bacterial communities in the gut and are present in low abundance [5]. Lactic acid bacteria are capable of inhibiting the growth of pathogenic bacteria through excretion of bacteriocins and thereby can pose positive effects on the health status and disease resistance of fish [25]. Although identification of the gut microbiota of fish and its manipulating is complex and is not fully understood, providing knowledge regarding possible alternative for modulation of gut microbiota toward beneficial populations is of high importance and can be a promising strategy for enhancing immunity and disease resistance [26—28]. This strategy can help to reduce utilization of antibiotics in aquaculture which per results in sustainable aquaculture [11]. One of the proposed methods for modulation of the intestinal microbiota composition is the use of dietary supplements such as prebiotics [2, 7—9, 29]. To date, many studies have been conducted on the beneficial effects of prebiotics on humans and pets, and in recent years, the use of these supplements in the diet of fish and other aquatic animals has been considered. The efficacy and efficacy of prebiotics have been shown to be influenced by the degree of polymerization, fermentabulity, host species, resident gut microbiota [8]. Therefore, considering the inter-species variation, in order to ensure the beneficial effect of the prebiotic used in the diet and the optimal prebiotic selection, comparative studies should be done.

The results of the present study revealed no significant alteration in THAB in the gut microbiota of both Caspian roach and Caspian white fish (Figure 1). In line with the findings of the present study, dietary administration of inactive yeast (*Saccharomyces cerevisiae*) and prebiotic fructoaligosaccharide (FOS) had no significant effects on THAB in the intestinal microbiota of Beluga sturgeon (*Huso huso*) [12, 30]. Similarly, feeding turbot with FOS supplemented diet exerts no significant effect on THAB [31]. On the other hand, negative effects on THAB level was reported in beluga fed with inulin [32]. The inability of dietary prebiotic to alter the THAB seems to be due to the limited binding sites in the gut [1, 33]. Indeed, the previous studies revealed that prebiotics seems to change the balance of gut microbiota by providing energy source for the beneficial bacteria rather than increasing the THAB. Therefore, the THAB cannot be altered very much due to the limited binding sites.

Concerning the effects of the tested prebiotic (GOS) on potentially useful intestinal bacteria, the results indicated a significant increase in the number of LAB in the intestinal microbiota of both Caspian white fish and roach compared to the control treatment. The highest increase was observed in fish fed with 2% GOS. Previous studies on the aquatic gut microbiota revealed despite the limited number of LAB in the gut microbiota, these potentially useful (probiotic) bacteria can be increased through administration of optimum prebiotics and become dominant bacterial communities [8]. Although there is no comparative study regarding the effects of prebiotics on the composition of the gut microbiota of Caspian white fish and roach, the results of this study are consistent with those of Hoseinifar, Mirvaghefi, Amoozegar, Merrifield, Ringø [34] that showed the use of GOS (as a prebiotic) in the diet significantly increased the number of LAB in the gut microbiota of rainbow trout. In addition, the use of FOS and yeast prebiotics significantly increased the number of LAB in the gut microbiota of Beluga [12, 30]. Similar results have been observed regarding the effects of FOS on the levels of probiotic bacteria in the intestinal microbiota of Turbot [31]. However, in contrast with these finding, inulin had no significant effect on LAB levels in the intestinal microbiota of the Beluga [35]. Despite the several reports on the prebiotic effects of GOS on physiological and health indices of fish, there are limited reports on the prebiotic effect on the intestinal microbiota composition of fish. According to the results of the present study, GOS is an effective prebiotic for modulation of gut microbiota of both species. The observed differences regarding the dose can be due to differences in the physiological characteristics of the gut, the prebiotic type and the microbiota composition of the gut of these species.

In conclusion, the results of this study showed that the use of GOS can be taken into account as an effective prebiotic in Caspian roach and Caspian white fish diet, aimed at modulation of the balance of gut microbiota toward beneficial bacteria. However, determining the possible effects on physiological parameters as well as mode of action needs further investigation.

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Научная статья

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Влияние галактоолигосахаридов в виде пребиотика на микрофлору кишечника различных видов рыб

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Аннотация. Обогащение кишечной микробиоты потенциально полезными бактериями (пробиотиками) оказывает благотворное влияние на физиологические процессы и здоровье рыб. Однако, воздействие пребиотиков на микрофлору кишечника является видоспецифичным. Настоящее исследование направлено на изучение влияния галактоолигосахаридов в качестве пребиотика на кишечную микробиоту каспийской плотвы и мальков каспийского кутума, являющихся одними из наиболее экономически ценных видов рыб, обитающих в Каспийском море. Исследование проводилось в течение 6 недель по полной рандомизированной схеме, в двух повторениях, каждое из которых включало три варианта обработки — 0 (контроль), 1 и 2 % ГОС, в трехкратной повторности. После этого с помощью культурального метода были изучены изменения в микробиоте кишечника рыб, включая общее количество бактерий, количество молочной кислоты и молочнокислых бактерий, а также влияние молочнокислых бактерий на микрофлору кишечника. Диетические галактоолигосахариды не оказали значительного влияния на общее количество бактерий у обоих видов ($P < 0.05$). Уровень молочнокислых бактерий в кишечнике был значительно выше при лечении пребиотиками, чем в контрольной группе ($P < 0.05$). Значительное увеличение количества молочнокислых бактерий и их преобладание было отмечено в варианте с использованием 2 % галактоолигосахаридов. Кроме того, самое высокое количество молочнокислых бактерий по отношению к общему количеству жизнеспособных бактерий наблюдалось в варианте с использованием 2 % галактоолигосахаридов ($P < 0.05$). Результаты данного исследования доказывают возможность и эффективность использования галактоолигосахаридов в качестве пребиотика для обогащения кишечной бактериальной микрофлоры каспийской плотвы и мальков каспийского кутума.

Ключевые слова: пребиотик, каспийский кутум, каспийская плотва, галактоолигосахарид, кишечная микробиота

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