Synbiotic (Biomim imbo) alters gut bacterial microflora of Russian sturgeon, *Acipenser guldenstadti* (Brandt & Ratzeburg, 1833) in a time-dependent pattern

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Abstract We investigated the efficiency of Synbiotic (Biomim imbo) in alternation and stabilization of gut microflora of Russian sturgeon, *Acipenser guldenstadti*. For this purpose, five experimental treatments and one control group with three replicates were considered. In treatment groups, fish were fed with diet containing 1 (T_1), 1.5 (T_2), 2 (T_3), 2.5 (T_4) and 3 (T_5) g Synbiotic/kg diet. In control group, fish were fed without supplement of Synbiotic. Sampling for bacterial studies was conducted at days 20, 40 and 60 after the beginning of the experiment. According to results, there were no significant differences in total bacterial count between Synbiotic treatments and also in comparison with control group 20 days after the beginning of the experiment ($P > 0.05$). At days 40 and 60 of experiment, the highest total bacterial counts were observed in control and T_1 group respectively. At day 20, the lactic acid bacteria count was higher significantly in Synbiotic treatments compared to control group ($P < 0.05$). In all sampling times (i.e. days 20, 40 and 60), the highest lactic acid bacteria count was observed in fish fed with 3 g Synbiotic/kg diet i.e. treatment 5 ($P < 0.05$). In each experimental group, total bacteria and lactic acid bacteria counts increased significantly as the duration of experiment expanded. In this regard, these parameters were significantly higher at day 60 compared to day 40 and 20 ($P < 0.05$). In conclusion, our results showed that Synbiotic (Biomim imbo) alters and stabilizes efficiently the

microflora of Russian sturgeon gut towards lactic acid bacteria in a time-dependent pattern.

Keywords Synbiotic (Biomim imbo) · Gut microflora · Lactic acid bacteria · Russian sturgeon

Introduction

Natural microflora of fish gut includes a various community of bacterial strains which increase growth parameters through improvement of digestion and absorption of nutrients (Tannock 1988; Walker and Duffy 1998; Fooks et al. 1999; Vershuere et al. 2000; Mishra et al. 2001; Irianto and Austin 2002; Roberfroid 2007; Sahu et al. 2008; Yousefan and Amiri 2009; Ringø et al. 2010; Rombout et al. 2010). In recent years, there has been a growing interest in use of probiotics and prebiotics in aquaculture. Probiotics are live microbial adjuncts which affect the host by modifying the microbial community of gastrointestinal tract and subsequently improve feed utilization, nutritional value and resistance against pathogens (Irianto and Austin 2002, Vershuere et al. 2000). Also, prebiotics are attributed to substrates that promote selectively the growth one or number of bacterial community in gut (Yazawa et al. 1978; Gibson and Roberfroid 1995, Mahious and Ollevier 2005; Dimitroglou et al. 2011). It was suggested that simultaneous use of probiotics and prebiotics under general title of Synbiotics can be more efficient than applying them alone (Chou et al. 1991; Hagi and Hoshino 2009; Lauzon et al. 2010a, b). Synbiotics exert almost their beneficial effects via various patterns including additivity, synergism and potentiation (Cerezuela et al. 2011). In additive pattern, the effect of two ingredients used together is equal to the sum of the individual ingredient effects. In synergism pattern, the combined effect

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of the two ingredients is significantly more than the sum of the effects of each agent applied alone. In the potentiation pattern, the effects are observed when two parts be used just concurrently (Fuller 1989; Vershuere et al. 2000). One of the main problems in application of probiotics is their instability in gut so that lost usually their dominance during long periods of time. It was recognized that using probiotics in combination with prebiotics or namely as synbiotics can alter gut microflora towards beneficial bacteria which this alternation is sustainable during Long periods of time. Sturgeons including Russian sturgeon, *Acipenser guldensadti* are of the economically and culturally important fishes in Caspian Sea basin and world. Due to the considerable reduction in natural resources, Russian sturgeon was listed in the IUCN Red List of Threatened Species as a critically endangered anadromous fish species. Aquaculture of sturgeons has been considered recently as an alternative way to restock their natural resources and for other aquaculture purposes. During last decade, probiotics have been used for sturgeons to improve growth and also resistance to diseases and cultural condition. Despite numerous studies evaluating the efficacy of probiotics and prebiotics in fish, there is rare information about their efficacy when used in combination (synbiotics) especially in sturgeons. Therefore, the present study was conducted to evaluate the efficiency of Synbiotic (Biomimbo) on alternation of gut microflora of Russian sturgeon.

Materials and methods

540 Persian sturgeon juveniles (264.4 ± 31.4 g) were obtained from the Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center, Iran, Rasht. After 10 days adaptation, fish were distributed into the 18 fiberglass tanks containing 500 l of dechlorinated and gentle aerated water at density of 30 fish per tank. Totally, 5 experimental groups and one control group with three replicates were considered for experiment. In experimental treatments, fish were fed three times a day and at the rate of 3 % body weight with a commercial dry feed (manufacturing Company: Chineh, Iran) containing various concentrations of Synbiotic (Biomimbo) (Table 1). Also, same diet without Synbiotic supplement was used for control group (Table 1). During 60 days experiment, the some water quality parameters including total unionized ammonia, nitrate and nitrite were monitored weekly and others such as pH, dissolved oxygen and temperature measured daily. Also, the water exchange was done entirely every 24 h by water with same temperature.

Sample preparation for bacterial study

Gut sampling for bacterial study was conducted at days 20, 40 and 60 after the beginning of the experiment. For this

Table 1 Experimental treatments and fish groups

Experimental groups	
Treatment 1 (T ₁)	Fish fed with diet containing 1 g Synbiotic/kg diet
Treatment 2 (T ₂)	Fish fed with diet containing 1.5 g Synbiotic/kg diet
Treatment 3 (T ₃)	Fish fed with diet containing 2 g Synbiotic/kg diet
Treatment 4 (T ₄)	Fish fed with diet containing 2.5 g Synbiotic/kg diet
Treatment 5 (T ₅)	Fish fed with diet containing 3 g Synbiotic/kg diet
Control group	Fish fed without supplement of Synbiotic

purpose, in each sampling, 10 fish were scarified and the contents of intestine removed by washing it with 0.9 % physiological serum. To same manner, the contents of intestine were washed three times and the obtained suspension diluted 7 times according to Pollock et al. (2002). Then, 100 µl of each solution was added separately into sterile Petri dish containing Tryptic Soy Agar (TSA) and then incubated at 30 °C for 96 h. At the end, bacterial colonies (CFU/g) were counted using a colony counter. Also, the strain of bacteria was identified from the color and shape of the colonies according to Ampofo and Clerk (2010).

Statistics

The SPSS software was used to analyse data. Data normality was investigated according to Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which groups were different.

Results

According to ANOVA outputs, the various concentrations of Synbiotic (Biomimbo) affected total bacteria and lactic acid bacteria counts at days 40 and 60 after the beginning of the experiment ($P < 0.05$) (Tables 2, 3). At day 20, there were no significant differences in total bacterial count between Synbiotic treatments and also in comparison with control group ($P > 0.05$) (Table 2). At days 40 and 60, the highest total bacterial count was observed in control and treatment 1 (Fish fed with diet containing 1 g Synbiotic/kg diet) group respectively (Table 2). At day 20, the lactic acid bacteria count was higher significantly in Synbiotic treatments compared to control group ($P < 0.05$) (Table 3). In all sampling times

Table 2 Total number of bacterial colonies (mean $\pm$ SD) in experimental treatments and control group

Experimental groups	Mean $\pm$ SD		
	Day 20	Day 40	Day 60
Control group	5.33 $\pm$ 0.29 ^{aA}	5.81 $\pm$ 0.04 ^{cB}	5.59 $\pm$ 0.06 ^{cAB}
Treatment 1 (T ₁)	5.14 $\pm$ 0.05 ^{aA}	5.27 $\pm$ 0.01 ^{bB}	5.9 $\pm$ 0.04 ^{dC}
Treatment 2 (T ₂)	5.09 $\pm$ 0.12 ^{aA}	5.17 $\pm$ 0.02 ^{bA}	5.18 $\pm$ 0.15 ^{bA}
Treatment 3 (T ₃)	4.79 $\pm$ 0.56 ^{aA}	5.10 $\pm$ 0.1 ^{bA}	4.47 $\pm$ 0.13 ^{aA}
Treatment 4 (T ₄)	5.08 $\pm$ 0.04 ^{aB}	4.84 $\pm$ 0.24 ^{aAB}	4.62 $\pm$ 0.09 ^{aA}
Treatment 5 (T ₅)	4.69 $\pm$ 0.57 ^{aA}	5.87 $\pm$ 0.04 ^{aA}	4.66 $\pm$ 0.2 ^{aA}

Different lower case and capital letters indicate respectively the dose and time-dependent differences among groups at $P < 0.05$

Table 3 Total number of lactic acid bacteria colonies (mean $\pm$ SD) in experimental treatments and control group

Experimental groups	Mean $\pm$ SD		
	Day 20	Day 40	Day 60
Control group	1.49 $\pm$ 1.32 ^{aA}	2.01 $\pm$ 1.74 ^{aA}	3.12 $\pm$ 0.34 ^{aA}
Treatment 1 (T ₁)	2.81 $\pm$ 0.47 ^{bA}	3.93 $\pm$ 0.1 ^{bB}	3.80 $\pm$ 0.14 ^{bB}
Treatment 2 (T ₂)	3.41 $\pm$ 0.04 ^{bA}	4.04 $\pm$ 0.04 ^{bC}	3.88 $\pm$ 0.12 ^{bcB}
Treatment 3 (T ₃)	3.66 $\pm$ 0.08 ^{bA}	4.01 $\pm$ 0.12 ^{bB}	3.95 $\pm$ 0.11 ^{bcB}
Treatment 4 (T ₄)	3.51 $\pm$ 0.03 ^{bA}	4.28 $\pm$ 0.14 ^{bB}	4.15 $\pm$ 0.13 ^{cdB}
Treatment 5 (T ₅)	3.64 $\pm$ 0.07 ^{bA}	4.44 $\pm$ 0.07 ^{bB}	4.35 $\pm$ 0.06 ^{dB}

Different lower case and capital letters indicate respectively the dose and time-dependent differences among groups at $P < 0.05$

(i.e. days 20, 40 and 60), the highest lactic acid bacteria count was observed in fish fed with 3 % Synbiotic i.e. treatment 5 ($P < 0.05$) (Table 3). In each experimental group, total bacteria and lactic acid bacteria counts increased significantly as the duration of experiment expanded. In this regard, these parameters were significantly higher at day 60 compared to day 40 and 20 ($P < 0.05$) (Tables 2, 3).

Discussion

Increasing the fish production and reducing the production costs are of the main goals of developed aquaculture. To meet these goals, recently the simultaneous use of probiotics and prebiotics or Synbiotics has obtained special regards in aquaculture. Examinations of Synbiotics on fish have showed that they improve growth parameters by influence on gut microflora, digestion enzymes, appetite and immune system (Irianto and Austin 2002; Merrifield et al. 2009; Panigrahi et al. 2010; Montajami et al. 2012; Nekoubin et al. 2012). Nevertheless, rare information are available about the efficacy of Synbiotics on fish in particular sturgeons. In the present study, we examined the efficacy of Synbiotic (Biomin imbo) in alternation of gut microflora of Russian sturgeon. It was found that Synbiotic

(Biomin imbo) increase the number of total bacteria colonies and lactic acid bacteria 40 days after the beginning of the experiment. This result shows that Synbiotic needs more than 20 days for alternation of gut microflora of Russian sturgeon. Likely, this period of time is necessary for overcoming and loading of lactic acid bacteria incorporated through dietary Synbiotic in Russian sturgeon gut. Strains of *Lactobacillus* are the most common microbes employed as probiotics. lactic acid bacteria such as *Lactobacillus* strains are known to produce compounds that inhibit the growth of Gram-positive and Gram-negative fish pathogens (Ringø and Gatesoupe 1998; Vershuere et al. 2000). Also, it seems that the alternations induced by Synbiotic are time-dependent since the values of total bacteria colonies and lactic acid bacteria increased continually as the duration of experiment expanded. The highest values of theses parameters were observed 60 days after the beginning of the experiment. This result shows that the efficiency of Synbiotic in alternation and stabilization of gut microflora towards lactic acid bacteria increases with the passing of the time. In our study, high concentrations of Synbiotic in diet (i.e. 3 g Synbiotic/kg diet) had more efficiency in alternation and stabilization of gut lactic acid bacteria. This suggests that colonization and stabilization of target microflora through Synbiotic depends on applying dosage in addition to duration of usage.

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