Effects of probiotics dietary supplementation on growth performance, innate immunity and digestive enzymes of silver pomfret, *Pampus argenteus*

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**ABSTRACT**

The effects of dietary probiotics on growth performance, innate immunity and digestive enzymes of silver pomfret were investigated. Compared to the control, diets supplemented with *Bacillus subtilis*, *Lactobacillus plantarum* and *Clostridium butyricum* caused significant improvements of growth performance, including final weight, weight gain, specific growth rate and feed conversion rate. A better growth performance among probiotic treatments was obtained in fish fed *B. subtilis*. To reveal the effects of these three probiotics on innate immunity of silver pomfret, lysozyme activity, SOD activity and IgM concentrations were examined. Results showed that silver pomfret fed diets containing *C. butyricum*, *L. plantarum* and *B. subtilis* had led to an enhanced innate response, notably higher lysozyme activity, SOD activity and IgM concentrations, as recorded after 30 and 60 days of feeding. In addition, study of different digestive enzymes *viz* lipase, protease and amylase activities showed significant higher values in fish fed the probiotic diets in comparison to the untreated group. These findings demonstrated that administration of *C. butyricum*, *L. plantarum* and *B. subtilis* can improve growth performance through enhanced immune response and digestive enzyme activity in silver pomfret. Furthermore, the *B. subtilis* is highly recommended as dietary probiotics in silver pomfret.

**Key words:** Digestive enzymes, Growth performance, Immune response, Probiotics, Silver pomfret.

**INTRODUCTION**

Aquatic products contribute a significant share to the world economy and provide a valuable food source. Fish is one of the richest sources of animal protein. People obtain a quarter of animal protein from fish and shellfish (Naylor et al., 2000). Because of an increasing demand for seafood and overfishing of the world’s waters, the importance of aquaculture product is set to increasing rapidly. Aquaculture has become an important economic activity globally. In aquaculture industry, aquatic animals are usually cultured at high densities and a consequence is the increased probability of exposure to stressful conditions. Thus various diseases often occur and result in serious economic losses (Balcázar et al., 2006). In order to prevent and control diseases, the antibiotics has been used extensively. Considering that extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria, the utilities of antibiotics as preventive drugs and feed additives have been questioned. Therefore, it is necessary to find an alternative to antibiotics and the contribution of probiotics may be considerable.

Probiotics are becoming an alternate viable therapeutic modality to overcome the adverse effects of antibiotics. To date, a number of probiotic strains including gram-positive bacteria and gram-negative bacteria have been evaluated in aquaculture to replace antibiotics. The use of microbial probiotics in aquaculture is now widely accepted (Cha et al., 2013; Wang, 2007). The involvement of probiotics in better growth, digestion, immunity, and disease resistance of the host in fish though modulating the composition of the gut microbiota has been proven beyond any doubt (Nayak, 2010; Xing et al., 2014). Thus probiotics open a new era in aquaculture. *Bacillus subtilis*, *Lactobacillus plantarum* and *Clostridium butyricum* are most commonly used probiotics, the beneficial roles of which in the aquaculture field are well established (Ai et al., 2011; Giri et al., 2013; Song et al., 2006). However, to the best of our knowledge, no reports are available to describe the beneficial effects of these probiotics on the silver pomfret. Therefore, the aim of this study was to examine the efficacy of these probiotics, as dietary supplements on growth performance, immune response and digestive enzymes of silver pomfret.

**MATERIALS AND METHODS**

**Experimental fish:** The fishes used in this study are silver pomfret juveniles bred by artificial fertilization with an average body weight of 4.84 ± 1.05 g.

**Probiotic bacteria:** The *Bacillus subtilis*, *Lactobacillus plantarum* and *Clostridium butyricum* are all recommended for animal and fish culture as probiotics in China. The

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Experimental diets: A basal experimental diet which was obtained from Hayashikane Sangyo Co., Ltd (Japan), was prepared and regarded as a control and three other diets were prepared by supplementing diets with three probiotic bacteria (C. butyricum, L. plantarum and B. subtilis) respectively. The bacteria were first cultured in medium for about 36 h at 37°C in an anoxic environment, and centrifugated at 3000×g for 5 min and washed twice with sterile saline, and then re-centrifugated and suspended at 1×10^8 CFU ml^-1 in sterile saline. The probiotic diets were prepared by gently spraying the bacterial suspension on the basal diet followed by mixing thoroughly to obtain a final probiotic concentration of 1×10^8 CFU g^-1.

Experimental design: The experiment was conducted at the Shanghai Fisheries Research Institute, China. The feeding experiment was carried out in about 1.5-m-diameter circular tanks. Each tank was randomly stocked with 20 fish, and three tanks of fish were assigned to each treatment. Food was provided in excess for 2 h (08:30-09:30 and 14:00-15:00) daily for 60 days. Uneaten feed were collected and counted to estimate feed intake. About one-thirds of the water in all tanks was exchanged with fresh seawater daily. Water temperature was maintained at 19±3°C, salinity 28-30 and pH 7.4-7.9, and dissolved oxygen was kept at more than 5 mg/L. Dead fish were removed and counted every day, and survival was calculated as the percentage of survivors.

Sample collection: Three silver pomfret juveniles in each tank were sampled at day 30 and 60 after probiotic feeding.

Table 1: Effects of dietary C. butyricum, L. plantarum and B. subtilis on the final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR) and survival.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C. butyricum</th>
<th>L. plantarum</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>4.99±0.08</td>
<td>5.07±0.09</td>
<td>5.14±0.04</td>
<td>4.98±0.05</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>11.88±0.14a</td>
<td>13.48±0.34b</td>
<td>13.61±0.27bc</td>
<td>14.40±0.20c</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>6.89±0.15a</td>
<td>8.41±0.42b</td>
<td>8.47±0.23b</td>
<td>9.42±0.25c</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>2.89±0.06a</td>
<td>3.26±0.14b</td>
<td>3.25±0.04b</td>
<td>3.54±0.08b</td>
</tr>
<tr>
<td>Feed conversion rate</td>
<td>1.28±0.03a</td>
<td>1.18±0.02b</td>
<td>1.17±0.03b</td>
<td>1.16±0.03b</td>
</tr>
<tr>
<td>Survival</td>
<td>0.97±0.02</td>
<td>0.98±0.02</td>
<td>0.95±0.00</td>
<td>0.98±0.02</td>
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0-60 d

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<td>4.98±0.05</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>19.4±0.52a</td>
<td>23.46±0.66b</td>
<td>22.35±0.36b</td>
<td>23.71±0.43b</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>14.41±0.50a</td>
<td>18.39±0.72b</td>
<td>17.21±0.35b</td>
<td>18.73±0.47b</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>2.26±0.05a</td>
<td>2.55±0.07b</td>
<td>2.45±0.03b</td>
<td>2.60±0.05b</td>
</tr>
<tr>
<td>Feed conversion rate</td>
<td>1.48±0.05a</td>
<td>1.36±0.05ab</td>
<td>1.38±0.04ab</td>
<td>1.30±0.04b</td>
</tr>
<tr>
<td>Survival</td>
<td>0.95±0.03</td>
<td>0.95±0.03</td>
<td>0.93±0.02</td>
<td>0.97±0.02</td>
</tr>
</tbody>
</table>

Values (mean±SE) are from three groups of fish (n=3), with different superscripts in rows denoting significantly different (p < 0.05).
administration compared with the fish fed the control diet ($p < 0.05$). These probiotics did not affect survival (0.95-0.98).

After 60 days of feeding, fish fed with diet supplemented with *C. butyricum*, *L. plantarum* and *B. subtilis* showed significantly higher FW, WG and SGR compared to fish fed the control diet. The FCR among dietary treatments ranged from 1.30 to 1.36 g with significant decreasing ($p < 0.05$) compared to the control (1.48). Survival rate was not significantly affected (0.93-0.97). For growth performance, the highest significant value was obtained in fish fed with the *B. subtilis* followed by *C. butyricum* and *L. plantarum*.

**Immune parameters:** The lysozyme activity in all probiotic treatments sharply increased ($p < 0.05$) with the highest value in *B. subtilis* at day 30 and 60 compared to the control after feeding (Fig.1). However, there were no statistically significant differences between the probiotic groups at day 30 and 60 ($p > 0.05$).

SOD activities of silver pomfret were significantly increased ($p < 0.05$) in probiotic groups at day 30 and 60 compared with the control. A sharp increase in SOD activity with the highest value at day 30 was observed in fish fed *B. subtilis*, which was remarkably higher than that fed *C. butyricum* and *L. plantarum* ($p < 0.05$). However, there were no significant differences between probiotic groups at day 60 ($p > 0.05$) (Fig.2).

IgM concentrations showed significant increases in the probiotic groups compared to the control after 30 days of feeding ($p < 0.05$), while no significant increase was observed between the probiotic treatments ($p > 0.05$). After 60 days of feeding, IgM concentrations of the probiotic treatments were significantly higher than that of control, and the values of *C. butyricum* treatment were the highest among the experimental groups which were significantly higher ($p < 0.05$) than the *L. plantarum* treatment (Fig.3).

**Digestive enzyme activity:** As can be seen from Fig.4, higher significant ($p < 0.05$) protease activity of silver pomfret after 30 and 60 days of feeding with different probiotic supplemented diets when compared to the control. Fish group fed diet supplemented with *B. subtilis* exhibited the highest protease activity.
Results of lipase activities of fish measured per treatment are presented in Fig. 5. The lipase activity increased significantly (p < 0.05) in C. butyricum, L. plantarum and B. subtilis dietary groups compared to the control. After 30 days, the highest lipase activity was recorded for the intestine of fish fed C. butyricum followed by L. plantarum and B. subtilis, respectively. After 60 days, the lipase activity of fish fed L. plantarum was higher than that of fish fed C. butyricum and B. subtilis. However, the increment was not statistically significant (p > 0.05) between probiotic treatments.

There was a significant increase (p > 0.05) in amylase activity of the silver pomfret fed C. butyricum, L. plantarum and B. subtilis compared to those fed basal diet both at day 30 and 60, and the values of B. subtilis group were the highest among the experimental groups, followed by L. plantarum and C. butyricum (Fig. 6). Clearly, these probiotics showed their ability to increase digestive enzyme activities and improve the growth performance.

Silver pomfret, Pampus argenteus, are one of the most important marine fish in China for its excellent biological characteristics such as popular taste and high economic. However, overfishing for many years has caused silver pomfret stocks to decline. The wild species is now under threat. Owing to the loss of wild stock in the sea and the increased market demand, technological research for development of silver pomfret aquaculture was initiated in China in 2004 by the East China Sea Fisheries Research Institute. Despite recent significant advances in the techniques of breeding silver pomfret (Shi et al., 2008; Yin et al., 2011; Peng et al., 2012a, 2012b and 2013), the production of high quality juveniles is still a bottleneck.

Extensive studies on probiotics have demonstrated that they are not only an efficient replacement for antibiotics in aquaculture but also possess numerous positive effects such as enhancement of fish growth, improvement of immune response and inhibition of pathogens (Arndt et al., 2007; Merrifield et al., 2010). Due to these benefits, the aim of this study was to apply probiotics to increase the growth performance and survival rate of the silver pomfret juveniles.

Dietary administration of C. butyricum, L. plantarum and B. subtilis significantly improved FW, WG and SGR silver pomfret. It is clear that these probiotics lead to improved health status without causing any significant
negative physiological effect. Therefore, these probiotics could be used as dietary additives for silver pomfret. However, not all probiotics influence the growth performance in a same manner as recorded in this study, but demonstrate different efficacy. A better growth performance was obtained in fish fed B. subtilis. In agree with our results, the advantages of using B. subtilis in fish aquaculture were recently demonstrated by numerous studies (Ai et al., 2011; Telli et al., 2014; Zhang et al., 2014). Thus, the B. subtilis was highly recommended for silver pomfret culture.

It is well known that probiotics play important roles in the host immune responses, which is considered an immediate and key effect of dietary probiotic supplementation. They are able to augment fish innate and adaptive immune responses in the gut to protect the body toward microbial intruders. However, at present, information on the probiotic effects on the immunity of silver pomfret is limited. In this regard, the serum lysozyme, SOD activities and IgM concentrations of silver pomfret were tested in the present study. Lysozyme is a cationic enzyme that lyases certain Gram-positive bacteria, and in conjunction with complement, even some Gram-negative bacteria by splitting glycosidic linkages in the peptidoglycan layers (Alexander and Ingram, 1992). Our results showed that the C. butyricum, L. plantarum and B. subtilis could enhance serum lysozyme activity. In addition, no significant difference in serum lysozyme was observed between probiotic treatments.

In the present study, serum SOD activity in the probiotic-fed groups was higher significantly than that in the control group. It has been well demonstrated that SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. It can alternately catalyze the dismutation of the toxic superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. There are reports that dietary supplementation with probiotics is effective for promoting SOD activity in fish or shrimp (Tseng et al., 2009; Sun et al., 2010). It was hypothesized that the increased SOD activity occurs to decrease the toxic superoxide anion level or to transform it to singlet oxygen or hydrogen peroxide to increase bactericidal capacity of living cells of silver pomfret.

In addition to the serum lysozyme and SOD activities, the IgM concentration was also tested in this study. The predominant antibody type in fish is high molecular weight Ig. IgM is the main immunoglobulin and by far the physically largest antibody in circulatory system. Stimulation of immunoglobulin level after dietary probiotic supplementation has been previous reported in various fish (Sun et al., 2014; Panigrahi et al., 2005). In the present study, the serum IgM concentrations of fish fed probiotics supplemented diets were significantly higher compared to the control group after 30 days of feeding which is line with previous studies (Sun et al., 2014; Giri et al., 2013). Previous works revealed that the simulation of immunoglobulin levels was a short-term phenomenon attributable to probiotics (Sun et al., 2010; Giri et al., 2013). In contrast, our results showed that the probiotic bacteria could enhance expression of IgM after 60 days of feeding. The differences may be explained by different fish species and growth environments.

Another possible explanation for the improvement of the fish and silvfish growth by probiotics may be due to the enhancement of digestive enzyme activity, including lipase, protease and amylase (Zokaeifar et al., 2012). Fish feeding on probiotics manipulate microbiota in the gastrointestinal tract, which produce exoenzymes that can increase nutrient digestibility and promote better health conditions (Zhang et al., 2010). Probiotic bacteria secret a variety of digestive enzymes that help fully digest feed, which beneficially affect nutritional status (Sharifuzzaman et al., 2014). In this study, the higher level of total digestive enzyme activity was recorded in fish fed probiotic diets where the better growth performances were observed compared to the control. The increased growth performance of silver pomfret fed probiotics could be due to better utilization of the diets through enhancement of digestive enzyme activity.

In conclusion, the present results indicate that the three strains were beneficial for silver pomfret in terms of the growth performance, immune response and digestive enzyme. A better growth performance was obtained in fish fed B. subtilis that is highly recommended for silver pomfret. However, some intestinal autochthonous bacteria may be good candidate strains for screening probiotics, and need further investigations in the future.

Competing interests
The authors declare that they have no competing interests.

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REFERENCES


