ニジマスの成長および免疫能に対する乳酸菌Enterococcus faecalis、マンナンオリゴ糖およびポリ水酸化酪酸の単独および併用効果
Effects of Single and Combined Supplementation of

*Enterococcus faecalis*, Mannan Oligosaccharide and Polyhydroxybutyrate Acid on Growth Performance and Immune Response of Rainbow Trout *Oncorhynchus mykiss*

Uriel RODRIGUEZ - ESTRADA¹, Shuichi SATOH¹,*, Yutaka HAGA¹, Hiroshi FUSHIMI² and John SWEETMAN³

Abstract: This study investigated the single and combined effects of pre- and probiotics supplement on the growth performance and immune response of rainbow trout. Seven diets supplemented with 1% *Enterococcus faecalis* (E), 0.4% mannan oligosaccharides (M) and 1% polyhydroxybutyrate (P) in single (E, M and P diets), double (EM and EP diets) or triple combinations (EMP diet) were fed to juvenile rainbow trout (13.2 ± 0.25 g) for 12 weeks. During the feeding test, the fish were healthy and no mortality occurred. Although weight gain and specific growth rate (SGR) were significantly higher (*P* < 0.05) in the fish fed single supplementation of mannan oligosaccharides, its combined supplementation together with *E. faecalis* showed further improvement of growth as well as feed gain ratio (FGR). On the other hand, single administration of *E. faecalis* could not improve any growth performance parameter. In addition, *E. faecalis* improved the immunological parameters such as hematocrit value, phagocytic index and activity and mucus production when administrated alone or combined with mannan oligosaccharides. After fourteen days challenge test the lowest cumulative mortalities were recorded in the fish fed E, M, EM and EMP diets. A single supplementation with E and M and combination of them improved the growth performance and immune response of rainbow trout.

Key words: *Enterococcus faecalis*; *Oncorhynchus mykiss*; Immune stimulant; Probiotics

Currently safety of fisheries products gathers public concern since it is well known that use of large amounts of medicine and antibiotic were used in fish farming sites (Gatlin III 2002). These medicines could be accumulated in sediments around fish farming sites and potentially threaten biological environment (Gatlin III 2002). Another problem with use of medicines in fish farming enhances the production costs. Dietary manipulation using natural products is expected to be ideal methodology to support fish health, cost effective and environment friendly aquaculture production.

Probiotics are microbial feed supplements that confer health benefits to the host (Fuller 1987). The prebiotics are defined as “non digestible food ingredients that beneficially affects the host by stimulating the growth and / or activity of one or a limited number of bacteria in the colon selectively” (Gibson 2004; Gibson and Robertfroid 1995). It is expected to reduce use of antibiotics and medicines in fish farming by promoting use of probiotics and prebiotics. There are several papers reporting the effects of pre- and probiotics on fish growth and immunity. Kotani et al. (2008) suggested

Received September 5, 2009; Accepted October 21, 2009.

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that Enterococcus faecalis stimulated the vaccination effect of tiger puffer Takifugu rubripes (Temminck and Schlegel) and Japanese flounder Paralichthys olivaceous (Temminck and Schlegel). Effect of mannan oligosaccharides (MOS), a prebiotic, has been investigated in some fish species. Dietary incorporation of 0.4% MOS enhanced growth, immune response and increased resistance to a bacterial infection in sea bass Dicentrarchus labrax (Linnaeus) (Torrecillas et al. 2007). Salze et al. (2008) demonstrated the dietary MOS improved survival of cobia Rachycentron canadum (Linnaeus) larvae exposed to high salinities. In addition, better growth was observed in tilapia Oreochromis niloticus (Linnaeus) which fed diets supplemented 4 and 6 g/kg MOS (Areechon et al. 2008). However, there are no reports on the combined effects of MOS with probiotics. The polyhydroxylbutyrate acid (PHB) also known as (R)-3-hydroxybutyric acid polymerized is another prebiotic included in the group of bio polymers. Although there are no reports of PHB as an enhancer of the fish immune system — numerous reports on other animals suggest positive effects of PHB on immune response (Volova et al. 2003). Therefore, the objective of this research is to determine effect of single or combined supplementation of two prebiotics: MOS and PHB together with the probiotic E. faecalis on the growth performance and immune response of rainbow trout Oncorhynchus mykiss (Walbaum).

### Materials and Methods

#### Feeding experiment

##### Experimental diets

Seven kinds of Iso-nitrogenous experimental diets were formulated. As probiotic ingredient, E. faecalis (E) (FK-23, lysed preparation of E. faecalis, Nichi Nichi Pharmaceutical Co., LTD, Japan) was used. As prebiotics, two kinds of ingredients were used: MOS (M) (BIO-MOS®, Alltech naturally, Kentucky) and PHB (P) (Azasu, Japan). These ingredients were supplemented to the basal diet at levels of 1% E. faecalis, 0.4% MOS, and 1% PHB; alone and two (probiotic and prebiotic: EM, EP) or three combinations (EMP) (Table 1).

The experimental diets were analyzed for moisture and crude ash by standard method (AOAC 1990). Crude protein was determined by Kjeldahl method. Total lipid contents were determined by Soxhlet method.

#### Table 1. Formulation of experimental diets for rainbow trout (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>C</th>
<th>E</th>
<th>EM</th>
<th>M</th>
<th>EP</th>
<th>P</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Anchovy meal</td>
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<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
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<tr>
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<td>Dextrin</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pregelatinized starch</td>
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<td>10</td>
<td>10</td>
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<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral premixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin premixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline chloride</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin E (50%)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<td>7.4</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<td>0.4</td>
<td>0.4</td>
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</tr>
<tr>
<td>Polyhydroxybutyrate</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Diets**

| TOTALS | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

<sup>a</sup> Composition (g 100 g-1): NaCl (1), MgSO<sub>4</sub>·7H<sub>2</sub>O (15), NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (25), KH<sub>2</sub>PO<sub>4</sub> (52), Ca(H<sub>2</sub>PO<sub>4</sub>)·2H<sub>2</sub>O (20), FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·nH<sub>2</sub>O (2.5), Ca<sub>2</sub>H<sub>2</sub>O·Ca·5H<sub>2</sub>O (1), ZnSO<sub>4</sub>·7H<sub>2</sub>O (1.2), MnSO<sub>4</sub>·5H<sub>2</sub>O (0.6), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.1), CoCl<sub>2</sub>·6H<sub>2</sub>O (0.0038), KI<sub>2</sub> (0.0105) and Cellulose (1.586).

<sup>b</sup> Composition (g 100g-1 premix): Thiamin hydrochloride (0.72), riboflavin (1.21), pyridoxine hydrochloride (0.48), cyanocobalamin (0.06), ascorbic acid (60.40), niacin (4.83), calcium pantothenate (1.21), inositol (24.19), biotin (0.02), Folic acid (0.18), p-aminobenzoic acid (0.09), vitamin A acetate (0.97), vitamin D<sub>3</sub> (0.97), vitamin E<sub>2</sub> (0.90).

<sup>c</sup> Vitamin E as DL-α-tocopheryl acetate, Purity 50%. 
determined gravimetrically after extraction by chloroform and methanol according to Folch et al. (1957). The crude lipids, crude proteins, moisture and crude ash values were 16.6–17.4, 39.9–40.5, 3.4–6.4 and 10.3–11.4 (%) respectively. The proximal composition of experimental diets is shown in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>E</th>
<th>M</th>
<th>P</th>
<th>EM</th>
<th>EP</th>
<th>EMP</th>
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<td>39.9</td>
<td>39.9</td>
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<tr>
<td>Moisture</td>
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<td>4.9</td>
<td>4.8</td>
<td>3.4</td>
<td>6.4</td>
<td>5.1</td>
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<td>10.3</td>
<td>10.5</td>
<td>11.4</td>
<td>10.3</td>
<td>10.3</td>
<td>11.2</td>
</tr>
</tbody>
</table>

**Experimental fish**

Juvenile rainbow trout were obtained from Oizumi Research Station, Tokyo University of Marine Science and Technology (TUMSAT), Yamanashi, Japan. Four hundred twenty fish were kept in a closed-recirculating system at TUMSAT. Water temperature was regulated at 16±2°C. Fish were fed commercial diet (Nippon Formula Feed Mfg Co. Ltd, Yokohama, Japan) until the beginning of the experiment. Thirty fish (mean weight 13.2±0.25 g) were introduced into one of the fourteen 60 l glass tanks and duplicate tanks were randomized to receive one of the seven experimental diets.

At the end of the feeding experiment, four fish from each tank (eight fish/treatment) were taken for blood samples after a 24 h starvation period. Blood was obtained from the caudal vein of individual fish after anesthetized with 300 ppm 2-phenoxyethanol (Wako Pure Chemical Industries, Ltd. Tokyo, Japan). Approximately 2 ml of blood samples were taken by using heparinized syringes and needles.

To collect leucocytes from head kidney, the organ was aseptically removed from the fish after partial decapitation to expose the trunk kidney area. The leukocytes were prepared and enriched according to the techniques of Chung and Secombes (1988).

The skin mucus was taken by following the method described by Staykov et al. (2007). Briefly, the fish skin surface was scraped by following a straight line path at 10 cm, from the base of the operculum by a slide glass (2×5 cm).

The growth performance was determined based on weight gain, daily food consumption, specific growth rate (SGR) and feed gain ratio (FGR).

\[ \text{WG} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of rearing days}} \times 100 \]

The specific growth rate (SGR) was calculated by the formula described in Steffens (1989).

\[ \text{SGR} = \frac{\ln(\text{Final body weight}) - \ln(\text{Initial body weight})}{\text{Number of rearing days}} \times 100 \]

Feed gain ratio is defined as the feed intake as dry matter divided by the fish weight gain of fish (Steffens 1989).

\[ \text{FGR} = \frac{\text{Feed intake as dry matter (g)}}{\text{Weight gain (g)}} \]

In order to perform a pathogen challenge test, two hundred eighty fish from the feeding experiment were randomly selected and distributed among fourteen 60 l glass tanks in the closed recirculating system of the TUMSAT, Shinagawa campus. Each group of fish were fed the same diet that they were fed during the feeding experiment. Before the commencement of the test, four fish per experimental group were randomly selected in order to make a bacterial plating (TSA salt 1.5% at 15°C during 14 hours to ensure that the obtained colonies were actual Vibrio anguillarum colonies) and to ensure that the fish were not infected by any pathogenic bacteria. At the beginning of the test, all the fish were intraperitoneally injected with 1 ml of 10^5 cfu ml\(^{-1}\) of V. anguillarum strain 775. Water temperature was 21°C and photoperiod was adjusted to 12L:12D. The mortality was recorded in a daily basis in a fourteen days period. In order to confirm deaths and presence or absence of V. anguillarum in survivors, samples from liver, spleen and head kidney were taken and plated in TSA 1.5% salt (15°C, 14 hours). To confirm the internal damage caused by the pathogen, all death fish were submitted to a necropsy and internal vibriosis symptoms were compared to the descriptions of Bruno and Poppe (1996).
**Immunological analyses**

Phagocytic activity of leucocytes was determined according to Puangkaew et al. (2004) with minor modifications. Following collection, the head kidneys of 8 fish (0.5 g approximately) per treatment were homogenized and filtered through a nylon membrane (100 μm mesh sized) and diluted in L-15 medium (SIGMA, sterile filtered with glutamine). The obtained head kidney cells were centrifuged twice at 250 × g for 5 min. This new solution was centrifuged at 400 × g for 5 min. The resulting pellet was diluted with L-15 medium which was layered onto percoll. The obtained head kidneys of 8 fish (0.5 g approximately) were homogenized and filtered with glutamine. The obtained head kidneys were centrifuged at 400 × g for 5 min. The resulting pellet was diluted with L-15 medium (SIGMA, 100% and stained by the Giemsa staining method (Clark 1973).

Immediately after collection blood samples were stored in 1.5 ml Eppendorf tubes. In the commencement of the assay, blood was directly taken from the tubes by using heparinised capillary tubes (length: 75 mm, Diameter: 1.45–1.65 mm) (Shibuya Co, Tokyo, Japan) and centrifuging at 250 × g during 5 minutes by using a TOMY high speed centrifuge MC-150. The hematocrit value was measured with a TOMY hematocrit scale. The collected mucus was weighed by using an analytical balance.

**Statistical analysis**

One way analysis of variance (ANOVA) was done by STATISTICA (Stat Soft, 6.0 software, USA). Differences between means were analyzed by Duncan's test.

**Results**

**Growth performance**

The experimental fish was healthy and no mortality was observed during the feeding period.

After 12 weeks of feeding, fish fed with diets EM and EMP recorded significantly higher (P < 0.05) weight gain (119.9 and 121.6 g, respectively) and SGR (2.8) than those of the rest experimental groups. Concerning to the single supplemented groups (E, M, P), the fish fed M diet recorded significantly higher (P < 0.05) weight gain (107.1 g) and SGR values (2.6). No any single supplementation of *E. faecalis* or MOS and PHB could positively affect the FGR. However, the combined supplementation of *E. faecalis* and MOS improved this parameter (0.8) (Table 3).

**Whole body proximate composition**

Moisture, crude ash, crude lipids and crude protein contents in the whole body were 68.2–70, 1.19–1.89, 10.5–13.4 and 16.0–16.9%, respectively (Table 4). There were no significant differences among all the experimental groups.

<table>
<thead>
<tr>
<th>Table 3. Growth performance of rainbow trout fed different diets containing MOS, <em>E. faecalis</em> or PHB (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>M</td>
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<tr>
<td>P</td>
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<tr>
<td>EM</td>
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<tr>
<td>EP</td>
</tr>
<tr>
<td>EMP</td>
</tr>
</tbody>
</table>

*D dry matter basis.

Different superscript letters in a line denotes significant differences (P < 0.05).
Hematocrit value

Significantly higher hematocrit value was recorded in the EM and EMP groups (53.3 and 54.3%, respectively) than the C, E, M, P and EP groups (less than 50% each) (Fig. 1, P<0.05). Significantly higher hematocrit value was recorded in the E and M groups (45.7 and 44.6%, respectively) than the C and P groups (26.9 and 37.0%, respectively) (Fig. 1, P<0.05).

Phagocytic activity and index

Phagocytic activities and indices in the EM and EMP groups were significantly higher (53.8 and 57.6%, respectively) than those of the C, E, M, P and EP groups (less than 50% each) (Fig. 2, P<0.05). Phagocytic activity in the M group (46.6%) was significantly higher than those of the C, P and EP groups (34.5, 38.7 and 40.13, respectively) (Fig. 2, P<0.05). Phagocytic indices in the E and M groups (6 and 5.6) were significantly higher than that of the C group (4.13) (Fig. 3, P<0.05).

Table 4. Whole body proximal composition (% wet basis) of rainbow trout fed experimental diets

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Initial fish</th>
<th>C</th>
<th>E</th>
<th>M</th>
<th>P</th>
<th>EM</th>
<th>EP</th>
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<tbody>
<tr>
<td>Crude lipids</td>
<td>Crude proteins</td>
<td>Moisture</td>
<td>Crude ash</td>
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<tr>
<td></td>
<td>3.3±0.0</td>
<td>3.6±2.2</td>
<td>78.7±0.2</td>
<td>2.2±0.1</td>
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<tr>
<td></td>
<td>12.3±0.1</td>
<td>16.9±0.2</td>
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<tr>
<td></td>
<td>11.9±0.5</td>
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<td>69.4±0.8</td>
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<tr>
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<tr>
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Fig. 1. Hematocrit value of final fish (feeding test: 3 months), fed different diets containing MOS, E. faecalis or PHB in a single (E, M, P diets), a two (Probiotic-Prebiotic: EM, EP diets) or a three combination (probiotic and two prebiotics: EMP diet), (mean ± SD, n=8). Different letters in a line denotes significant differences (P<0.05).

Fig. 2. Phagocytic activity of final fish (feeding test: 3 months), fed different diets containing MOS, E. faecalis or PHB in a single (E, M, P diets), a two (Probiotic-Prebiotic: EM, EP diets) or a three combination (probiotic and two prebiotics: EMP diet), (mean ± SD, n=8). Different letters in a line denotes significant differences (P<0.05).

Fig. 3. Phagocytic index of fish, fed diets during three months, containing MOS, E. faecalis or PHB. (mean ± SD, n=8). Different letters in a line denotes significant differences (P<0.05).

Fig. 4. Mucus weight of final fish (feeding test: 3 months), fed different diets containing MOS, E. faecalis or PHB (mean ± SD, n=8). Different letters in a line denotes significant differences (P<0.05).
**Mucus weight**

Significantly higher mucus weight was recorded in fish fed EM and EMP diets (23 and 22 mg/cm²) than those fed C, E, M, P and EP diets (9, 16, 17, 12 and 13 mg/cm²) (Fig. 4). Significantly higher mucus weight was observed in fish fed E and M diets (16 and 17) than those fed C, P and EP diets (9, 12, 13) (Fig. 4, P<0.05).

**Challenge test**

All the experimental fish showed the typical symptoms of vibriosis, throughout the experimental period (lethargy and a loss of appetite). From the third day post infection (dpi), the skin of infected survivors, become discoloured and some areas showed necrotic and reddish appearance. In some cases, bloody blocks (erythema) appeared around the fins and mouth. Despite no mortality were recorded during the first two dpi, massive mortalities (due to the endotoxic hyper-guade shock produce by *V. anguillarum* extracellular products) were occurred at third and fourth dpi. Mortality in the C group cessed at day 12, while it did at 8, 9 and 11 dpi (respectively) in M, E and P groups. On the other hand mortalities recorded in EM, EMP and EP groups cessed at 7, 9, 12 dpi (respectively). Mortality continued to be observed until the first 14 dpi when it cessed (Figure 5) and the survival fish recovered the appetite and did not show any symptom of the disease.

After 14 dpi the EM and EMP groups showed significantly lower mortality (10.4 and 18.7%, respectively) (Fig. 6) than those of C, E, M, P and EP groups (showing between 22.9 and 62.5%) (Fig. 6, P<0.05). E and M groups showed significantly lower cumulative mortality (22.9 and 27.0%, respectively) than C, P and EP groups (62.5, 50.0 and 45.8%, respectively) (Fig. 6, P<0.05).

*V. anguillarum* colonies were obtained from head kidney of all death fish however after the fourteen days challenge test period, the percentage of survivors showing the presence of this pathogen was higher in the fish fed C, P and EP diets. In contrast, less than 10% of the fish fed E, M, EM and EMP recorded presence of *V. anguillarum* (Fig. 7).

![Fig. 5.](image5.png) **Fig. 5.** Mortality curves in a day by day basis of rainbow trout challenged with *Vibrio anguillarum* strain 775 for 2 weeks.

![Fig. 6.](image6.png) **Fig. 6.** Mortality of rainbow trout challenged with *Vibrio anguillarum* strain 775 for 2 weeks (mean ± SD, n=40). Different letters within a line denotes significant differences (P<0.05).

![Fig. 7.](image7.png) **Fig. 7.** Presence of *Vibrio anguillarum* on head kidney of rainbow trout after a 2 weeks challenge test (mean ± SD, n=variable number). Different letters within a line denotes significant differences (P<0.05).

**Discussion**

In this experiment, significant enhancement of weight gain, SGR and FGR in M, EM and EMP groups in which fish were fed diets supplemented with MOS. These results suggest that dietary MOS significantly improved growth performance and nutrient utilization of fish. This
idea is supported by the fact that faster growth was observed in rainbow trout fed MOS supplemented diets (Staykov et al. 2007; Grisdalle-Helland et al. 2008).

Although there is no difference in growth of fish fed the control, E and P diets, fish fed the EP diet showed significantly better growth, SGR and FGR. These findings suggest that combination of *E. faecalis* and PHB slightly improved growth performance of rainbow trout.

Non-specific immune function was significantly improved in fish fed *E. faecalis* and MOS, suggesting that these supplements stimulate immune function of rainbow trout. Administration of the 1 and 2% Grobiotic AE® to hybrid striped bass and tilapia showed significant higher protection against *Streptococcus iniae* (Li and Gatlin III 2003) and with *Mycobacterium marinum* (Li and Gatlin III 2005). Peterson et al. (2009) and Sang et al. (2009) also demonstrated the immune stimulant capacity of MOS and its conferred protection against pathogens. It has been suggested that lactic acid bacteria improved immunocompetence in several fish species (Gildberg and Mikkelsen 1998; Panigrahi et al. 2004; Nikoskelainen et al. 2003). Improvement of immunocompetence was well demonstrated by much lower mortalities in fish fed diets supplemented with *E. faecalis* and MOS or triple combination of *E. faecalis*, MOS and PHB. Modulation of non specific immune system of fish by lactic acid bacteria (Kotani et al. 2008) was reported in marine fish species including Japanese flounder, tiger puffer and fresh water species rainbow trout (Panigrahi et al. 2004; Panigrahi et al. 2005) and common carp *Cyprinus carpio* (Linnaeus) (Kumar et al. 2006). Similarly, positive effects of MOS were clearly demonstrated in rainbow trout (Dimitroglou et al. 2008) and tilapia (He et al. 2009). These studies, demonstrated that MOS can improve the microvilli of the fish gut, improving the absorption of nutrients with a consequent growth performance improvement.

Until now, most of the research using pre- and probiotics have been focused on the symbiotic effects in the gut (Hai and Fotedar 2009) where the prebiotic is fermented by the ingested probiotic conferring health benefits to the host (Gibson 2004). In the present study, the combine diet supplemented with MOS and lysed cells of *E. faecalis* might have had a different mechanism of action. The combination of these two ingredients triggered a significant better growth performance and an enhancement of immune system. This can be explained, by the potential capacity of *E. faecalis*, to improve the permeability of the intestinal epithelial cells (IECs) facilitating its absorption by transcytosis in the microfold cells (Shida and Nanno, 2008) located in the already improved microvilli morphology by MOS (Dimitroglou et al. 2008). Such positive permeable conditions also favour the absorption of MOS and its consequent improvement of the nutrient utilization.

Once improved the absorption conditions, *E. faecalis* could affect IECs to secrete an array of cytokines which modulate the functions of dendritic cells (DCs), T cells and B cells in the gut associated lymphoid tissue. MOS can stimulate the mannose receptors (involved in antigen recognition and in the binding process of antigen presenting cells) (Engering et al. 1997) and the mannose binding lectin (MBL) by liver secretion triggering a complete cascade stimulating the non specific immune system (Janeway 1993) of the fish.

Another possible mutual mechanism of action (between *E. faecalis* and MOS) by which growth performance and immune system are improved, can be explained by the fact that, while MOS stimulate the growth of the naturally occurring probiotic bacteria in the rainbow trout gut, excluding pathogenic organisms (Rastall and Maitin 2002) *E. faecalis* permeate the IECs. Hence, these beneficial bacteria are easily transported from microfolds to the intestinal mucosa where the stimulation of cytokines initiates a cascade of biochemical reactions, initiating the cellular and humoral immunity.

Functional diets are complete feeds, stimulating not only the growth performance but also the immune system of the farmed organism (Vega-Villasante et al. 2004). This study suggested that lysed cells of *E. faecalis* and the
prebiotic MOS are potential ingredients of functional diets for fish.

In summary, these results showed that dietary incorporation of MOS in a single or a combined inclusion with *E. faecalis*, enhances not only the rainbow trout growth but also activates its immune system. Further studies must be conducted to clarify the mechanism of interaction of MOS and *E. faecalis* to stimulate the immune system of rainbow trout.

**Acknowledgements**

U.R.E. would like to greatly appreciate the partial economical support for this research by the Ministry of Education, Culture, Sports, Science and Technology (MONBUKAGAKUSHO) of the Japanese government.

**References**


Immune Stimulants and Growth Promoters in Fish


ニジマスの成長および免疫能に対する乳酸菌 *Enterococcus faecalis*, マンナンオリゴ糖およびポリ水酸化酪酸の単独および併用効果

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ニジマスの成長および免疫能に対する各種プロバイオティクスおよびプレバイオティクスの添加効果を検討した。平均体重13.2 g のニジマスに乳酸菌 *Enterococcus faecalis* (E) 1％、マンナンオリゴ糖 (M) 0.4％、およびポリ水酸化酪酸 (P) 1％を単独または組み合わせて添加した7 種類の飼料を与え12週間飼育した。

M および E を添加した飼料区で成長および増肉係数が有意に優れるとともに、ヘマトクリット値、マクロファージ活性、単位面積当たりの皮膚の粘液量の有意な増加が見られた (P<0.05)。さらに、*Vibrio anguillarum* による攻撃試験後の生存率は、E および M を添加した飼料区で有意に高かった。以上より、ニジマスの成長および免疫能に及ぼす E および M 添加の有効性が示唆された。