DIETARY INCLUSION OF A COMMERCIAL PROBIOTIC DURING RAINBOW TROUT REARING AT A PRODUCTION HATCHERY: A PILOT STUDY

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ABSTRACT

Natufermen, a commercial probiotic containing Bacillis subtilus and Aspergillus oryzae, was included in juvenile rainbow trout Oncorhynchus mykiss diets at 0.0% (control), 0.125% or 0.25%, with three tanks per dietary treatment. At the end of the 21 day rearing period, there were no significant differences in growth, feed conversion ratio (FCR), or mortality among treatment fish fed any of the diets. Further investigation concerning appropriate concentrations and the possible probiotic effects of Natufermen is recommended.

INTRODUCTION

Probiotics are simple or complex bacterial assemblages that can promote health and may stimulate the growth of other organisms (Lilley and Stillwell 1965). When added as a dietary supplement to human, terrestrial, and aquatic animal diets, probiotic microorganisms have been found to increase overall immune response, aid in digestion, and reduce the pH in the gastrointestinal tract to better prevent colonization of many microbial pathogens (Fuller 1989; Salminen et al., 1999; Mombelli and Gismondo 2000; Klewicki and Klewicka 2004). This study focused on the addition of a commercial probiotic containing Bacillis subtilus and Aspergillus oryzae to a commercial salmonid diet. B. subtilus is a rod shaped, gram positive, catalase positive, obligate aerobe bacteria. A. oryzae is a filamentous, asexual, ascomycetous fungus.

Aquaculture diets containing B. subtilus have led to an increase in fish size and an improvement in feed conversion ratio when compared to control or non-supplemented feeds (Aubin et al. 2005; Bagerhi et al. 2008; Merrifield et al. 2010b). For example, Bairagi et al. (2004) found when B. subtilus was added to the diet of rohu Labeo rohita, growth and feed conversion were significantly improved compared to fish fed control diets, likely due to extracellular cellulol-
lytic and amylolytic enzyme production from the introduced probiotic bacteria. Likewise, Ai et al. (2011) noted that a dietary supplement containing *B. subtilis* increased growth rate and feed efficiency of juvenile large yellow croaker *Larimichthys crocea*.

Dietary probiotics containing *B. subtilis* may help reduce disease outbreaks and increase innate immune responses (Aly et al. 2008; Cerezuale et al. 2012) particularly during immunosuppressive events that routinely occur during hatchery rearing (Wood 1979; Decostere and Haesebrouck 2000; Madetoja et al. 2002; Taylor 2004; Cipriano and Holt 2005). However, Aly et al. (2008) found that feed supplemented with only *B. subtilis* did not significantly improve the survival of Nile tilapia *Oreochromis niloticus*.

Ample research has been conducted using *A. oryzae* as a probiotic mixed into cattle feed to heighten both lactation and growth of masticating ruminants (Kellems et al. 1990; Wiedmeier et al. 1987). However, no research has used *A. oryzae* either alone as a probiotic or as a symbiotic feed supplement with *B. subtilis* in aquaculture.

This study used a commercial probiotic containing both *B. subtilis* and *A. oryzae* included at two different concentrations in the hatchery diet of an Arlee strain of rainbow trout *Oncorhynchus mykiss* as part of normal hatchery production. The current study focus was on growth and survival.

**METHODS**

This study took place at Giant Springs State Fish Hatchery, Great Falls, Montana. Spring water at a temperature of 11.9 °C (7.6 pH, 9 mg/L dissolved oxygen) was used for both trials. A commercial probiotic (Natufermen, Nutraflo, North Sioux City, South Dakota, USA), was included in a commercial trout diet (Silvercup, Nelson and Sons, Murray, Utah) at either 0.125% or 0.25%. Table 1 shows proximate analysis of the Silver Cup (Skretting) Salmon Fry diet used in this study. All data were analyzed using one-way analysis of variance with SPSS software with a predetermined significance of \( P \leq 0.05 \).

The trial began on January 3, 2012, and ended 21 days later. Nine indoor tanks (1.7 m³) each received 17,000 fish (40.64 ± 0.22mm and 0.71 ± 0.02; mean ± SE), and flows were set at 75.6 L/min. The control group was fed 1.0mm extruded pellets while the experimental groups received the same diet with either 0.125% or 0.25% Natufermen as a top coating. All diets were fed using a hatchery constant of 7.9 (Buterbaugh and Willoughby 1967). Three tanks were used for each treatment.

At the start of the study, 20 randomly selected fish from each of the nine raceways were individually weighed to the nearest 0.1 g and measured to the nearest 1.0 mm. Mortality was recorded daily. Twenty fish per tank were again weighed and measured at the end of the trial.

At the end of the 21d study period individual lengths (1.0mm), weight (0.1g), condition factor (K factor: weight (g)/length (mm)^3), gain, feed conversion (FCR), total tank weights and percent survival were collected and analyzed.
RESULTS

There were no significant differences in total tank weights (total Kg of fish per rearing unit), gain, feed conversion ratio, or mortality among all three diet groups (Table 2). There were also no significant differences among mean fish length, weight, or condition factor variables (Table 3).

### Table 1. Proximate analysis of Silver Cup Salmon diet (Skretting) fed to Rainbow trout during the study.

<table>
<thead>
<tr>
<th>Salmon diet</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Fiber (%)</th>
<th>Phosphorus (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>19</td>
<td>3</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

### Table 2. Mean (+ SE) total rearing unit data from indoor tanks of rainbow trout receiving one of three diets containing different concentrations (%) of Natufermen (N = 3).

<table>
<thead>
<tr>
<th>% Natufermen</th>
<th>0.0</th>
<th>0.125</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start weight (kg)</td>
<td>12.6 ± 0.1</td>
<td>11.8 ± 0.1</td>
<td>11.6 ± 0.1</td>
</tr>
<tr>
<td>End weight (kg)</td>
<td>24.0 ± 0.1</td>
<td>22.3 ± 0.1</td>
<td>23.8 ± 0.1</td>
</tr>
<tr>
<td>Gain (kg)</td>
<td>11.4 ± 1.1</td>
<td>10.5 ± 0.8</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>0.90 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.82 ± 0.02</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0.10 ± 0.06</td>
<td>0.16 ± 0.09</td>
<td>0.13 ± 0.08</td>
</tr>
</tbody>
</table>

### Table 3. Mean (+ SE) individual fish data from rainbow trout in indoor tanks receiving one of three diets with varying amounts of Natufermen (N = 60).

<table>
<thead>
<tr>
<th>% Natufermen</th>
<th>0.0</th>
<th>0.125</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start length (mm)</td>
<td>41.1 ± 0.5</td>
<td>40.5 ± 0.4</td>
<td>40.3 ± 0.4</td>
</tr>
<tr>
<td>Start weight (g)</td>
<td>0.74 ± 0.02</td>
<td>0.69 ± 0.02</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td>End length (mm)</td>
<td>52.9 ± 0.6</td>
<td>53.1 ± 0.6</td>
<td>53.4 ± 0.4</td>
</tr>
<tr>
<td>End weight (g)</td>
<td>1.41 ± 0.04</td>
<td>1.31 ± 0.04</td>
<td>1.40 ± 0.03</td>
</tr>
<tr>
<td>Condition factor (K)*</td>
<td>0.94 ± 0.02</td>
<td>0.87 ± 0.01</td>
<td>0.91 ± 0.01</td>
</tr>
</tbody>
</table>

*Condition factor (K)* = 10^5 x [weight/(length^3)]
DISCUSSION

The lack of difference in total tank weight or individual fish size at the end of the trial might have been due to the short trial period of only 21 days. Other studies have seen significant changes in length and weight with trials lasting 28 to 150 days (Aubin et al. 2005; Bagerhi et al. 2008; Merrifield et al. 2010a and 2010b). Ghosh et al. (2008) found feeding *B. subtilis* to ornamental fish (million fish *Poecilia reticulate*, short-finned molly *Poecilia sphenops*, green swordtail *Xiphophorus helleri*, and southern platyfish *Xiphophorus maculates*) had a significant effect on overall feed conversion. On the contrary, Merrifield et al. (2010a) found no significant effect on length, weight, or survival when feeding *B. subtilis* to rainbow trout for a period of 70 days.

Although *A. oryzae* appeared to have no noticeable effect on trout rearing performance in this study, results have been mixed in other vertebrates. In poultry receiving 0.1% *A. oryzae*, Grimes et al. (1997) observed an increase in egg size, but found no effects on feed conversion or egg production. In other studies, *A. oryzae* has had significant effects on ruminants and poultry (Varel and Kreikemeir 1994; Lee et al. 2006).

Although there was no significance difference in survival, therapeutic probiotic treatments have been shown to reduce salmonid mortality during pathogen outbreaks (Nikoskelainen et al. 2001; Austin et al. 1995). Merrifield et al. (2010b) found that rainbow trout fed *B. subtilis* had elevated levels of leucocytes, which may increase phagocytosis and decrease mortality during infectious outbreaks (Panigrahi et al. 2004). Gildberg and Mikkelsen (1998) also noted that dietary probiotic bacterial supplementation delayed the onset of mortality during infection trials.

The shortcomings of these trials make it difficult to ascertain the effects of Naturfermen during rainbow trout rearing. However, the potential influence of Naturfermen supplement warrants further investigation. Future studies should be conducted in longer-term trials particularly during disease outbreaks to determine if probiotics can lower mortality rates.

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LITERATURE CITED


*Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient
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