Effect of a Short Chain Fructooligosaccharide (scFOS) Prebiotic on the Growth, Survival and Immune Response of the Pacific White Shrimp *Litopenaeus vannamei*

Brandon C. Klim, Addison L. Lawrence, Joe M. Fox, Bart R. Dunsford, Susmita Patnaik, David A. McKee

Texas A & M University - Corpus Christi
Mariculture Department
Texas AgriLife Mariculture Research Laboratory
Port Aransas, TX
What is a Prebiotic?

- A non-digestible ingredient selectively fermented by certain bacteria already present in the gastrointestinal tract.
- Allows for changes in the microflora of the gut producing benefits to host well-being and health. (Roberfroid 2007)
3 Criteria Defining a Prebiotic

1) Non-digestible by the gut of the host
2) Fermented by intestinal microorganisms
3) Selectively stimulates the growth or activity of beneficial microorganisms in the gut

Currently only 3 types of compounds meet criteria:

a) Fructooligosaccharides
b) Galactooligosaccharides
c) Lactulose

(Roberfroid 2007)
How Do Prebiotics Work?

- Some bacteria (e.g., lactobacilli and bifidobacteria) can enzymatically digest prebiotics and utilize them as a nutrient.
- Other bacteria cannot
- Results in gut proliferation of beneficial bacteria, competitive inhibition of non-desirable species (e.g., Gram-negative)
- Beneficial bacteria enhance digestion, absorption of nutrients, immune response and general gut health.
- Enhances overall health of the animal.
General Structure of the scFOS Prebiotic

Glucosyl $\alpha (1\rightarrow 2)$ fructose (sucrose)

$(\text{fructosyl})_n \beta (2\rightarrow 1)$ fructose

(Sc-FOS : $n = 1$ or $3$)

$GF_2$

1-kestose

$GF_3$

nystose

$GF_4$

fructosyl-nystose

Figure 1. Short-chain fructo-oligosaccharide structure.

(Bornet 2002)
Uses in Agriculture and Aquaculture

- Prebiotics are beneficial to humans, terrestrial animals and some fish species (Bornet 2002, Correa-Matos 2003 and Mahious 2006).
- Currently incorporated in poultry, livestock and pet foods.
- Aquaculture use followed success of terrestrial use.
- Does the scFOS prebiotic produce the same growth and health benefits seen in terrestrial animals for shrimp?
Objectives

• Determine if 0.08% scFOS prebiotic present in a commercial shrimp feed has an effect on growth, survival, feed conversion and immune response of *Litopenaeus vannamei* in a clear water environment.

• Identify optimum feed rates.

NOTE: Initially conducted outdoors under primary productivity (as in abstract), but was cut short by Hurricane Ike
Experimental Design

- **5 × 2 factorial:**
  - **5 Feed Rates** – 0.9, 1.2, 1.6, 2.1 and 2.7 grams of feed/shrimp/week.
  - **2 Feed Types** - WITH 0.08% scFOS or WITHOUT

- Ten feed rate/type treatment combinations
- n = 5 replication

Experimental System
Experimental Protocol

- Initial average weight of shrimp 5.23g
- Stocking density: 50 shrimp per 0.35 m² (bottom surface area) tank or 0.8 kg/m²
- Temperature: 29 ± 1 C
- Salinity: 30 ± 2ppt
- Photoperiod: 12L:12D
- Dissolved oxygen: 5.50 ± 0.5mg/L
- Water exchange rate: 960%/day
Experimental Protocol

- Shrimp fed 15-times daily using wheel-type feeders
- Daily removal of molts, feces and dead shrimp
- Weekly monitoring of ammonia, nitrite, nitrate, pH and turbidity
- Shrimp harvested after 43 d for growth and survival data and returned to respective tanks
Immune Response Analyses

After shrimp were returned to their tanks they were allowed to reacclimate for 5d before performing the following analyses (n = 2 shrimp/tank):

1) **Total Hemocyte Count** - number of hemocytes per g of hemolymph.

2) **Hemocyte Respiratory Burst** - superoxide anion ($O_2^-$) present in hemolymph, a measure of phagocytic activity (Hernandez-Lopez 1996).

3) **Phenoloxidase Activity** - phenoloxidase present in hemolymph, terminal enzyme in proPhenoloxidase activating system in response to presence of a foreign body (e.g., fungi, viruses or bacteria) (Liu 2004).
Statistical Analyses

- Mean final weight, survival, harvest biomass, and immune response data were analyzed among treatments ($\alpha = 0.05$) using SPSS 15.0 statistical software (SPSS Inc. Chicago, Il).
- Independent sample t-test for significant differences in immune response between scFOS/NO scFOS treatments within each feed rate.
- One-way ANOVA used to determine significant differences in growth, survival and biomass among treatment means.
Results - Immune Response

- No significant difference in immune response variables among treatments.

<table>
<thead>
<tr>
<th>Feed Rate (g/shrimp/wk)</th>
<th>Total Hemocyte Count</th>
<th>Hemocyte Resp. Burst</th>
<th>Phenoloxidase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>0.573</td>
<td>0.491</td>
<td>0.443</td>
</tr>
<tr>
<td>1.6</td>
<td>0.670</td>
<td>0.282</td>
<td>0.451</td>
</tr>
<tr>
<td>1.2</td>
<td>0.825</td>
<td>0.238</td>
<td>0.282</td>
</tr>
<tr>
<td>0.9</td>
<td>0.210</td>
<td>0.869</td>
<td>0.722</td>
</tr>
</tbody>
</table>

Table 1 - P-values for immune response parameters between scFOS/no scFOS treatments at each feed rate

Independent sample t-test, $\alpha = 0.05$. n=10
Fig. 1 Mean final wt. - FOS vs. NO FOS

- scFOS Prebiotic
- Without Prebiotic

One-Way ANOVA
α = 0.05  n=5

Mean final weight (g)
Feed rate (g/shrimp/wk)

a, ab, ab, ab, bc, bc, d, cd, e, e
Fig. 2 Mean survival - FOS vs. NO FOS

![Graph showing survival percentage vs. feed rate (g/shrimp/wk). The graph compares scFOS Prebiotic with a control group. The feed rates are 2.7, 2.1, 1.6, 1.2, and 0.9 g/shrimp/wk. The survival percentages are indicated by different letters (a, ab, abc, bcd, cd, d), indicating statistical differences at α = 0.05 with n=5.](image-url)
Fig. 3. Mean final biomass - FOS vs NO FOS

One-Way ANOVA
\( \alpha = 0.05 \)  \( n=5 \)

Final biomass (g)

Feed rate (g/shrimp/wk)

- scFOS Prebiotic
- Without Prebiotic

p = .035
Results – Final wt., Survival and Biomass

- Mean final wt. and survival were not significantly different between scFOS / no scFOS treatments at the same feed rates.

- Total biomass (final wt. x survival) was significantly greater in treatments with scFOS compared to no scFOS at the 2.7g/shrimp/wk feed rate.
Discussion

• Immune response data varied widely between individuals.

• Appears to be inherent variability in these parameters in healthy shrimp.
• Perhaps immune response analysis would be better suited in a challenge study.

• Previous studies have indicated increased immune response at higher levels of scFOS (Li et. al. 2007)
• This is thought to be an excess burden on the shrimp, so lower levels were used in this study and immune response did not appear to be stimulated.
Total harvested biomass was significantly higher in tanks fed scFOS.

- Recent studies have shown that the microbial community composition of the gut of shrimp fed a diet consisting of 0.1% scFOS was significantly different than those not fed scFOS (Li et. al. 2007).

- This suggests that scFOS may be capable of acting selectively on the microbial community of the gut in such a way that confers growth and survival benefits to the shrimp.
Conclusions

- No effect of scFOS on immune response of shrimp at the dietary inclusion level used.
- Addition of scFOS to diets appeared to have a significant beneficial affect on total biomass and beneficial trends in growth and survival at an optimal feed rate of 2.7g/shrimp/wk in a clean water system.
- Evaluation is required in the presence of natural productivity to assess scFOS prebiotic effects under practical conditions.
Acknowledgements

- GTC Nutrition
- Committee Members
- Texas A&M - Corpus Christi - College of Science and Technology
- Texas AgriLife Research Mariculture Laboratory at Port Aransas, Texas A&M System. Research funded in part by a grant from the US Dept. of Commerce US Marine Shrimp Farming Program USDA/CSREES Grant No. 2002-38808-01345, Project R-9005, Gulf Coast Shrimp Project
Literature Cited


Liu CH, Yeh ST, Cheng SY, Chen JC. The immune response of the white shrimp Litopenaeus vannamei and its susceptibility to Vibrio infection in relation with the moult cycle. Fish and Shellfish Immunology, 2004; 151-161.
