Effects of Bio-Mos® on Growth and Survival of Channel Catfish Challenged with Edwardsiella ictaluri

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A major problem in the catfish farming industry has been high disease loss to enteric septicemia of catfish (ESC), caused by the bacterium Edwardsiella ictaluri. Methods currently used to control this disease include antibiotic therapy, vaccination, and restricted feeding. Another method that has been examined is the addition of immunostimulants to the diet. Immunostimulants such as glucans with $\beta$-1,3 and $\beta$-1,6 glycosidic linkages ($\beta$-glucans) have been used to improve disease resistance but the results have been inconsistent. Channel catfish injected with yeast glucan responded to subsequent E. ictaluri immunization with higher serum antibody titers and reduced mortality relative to controls (Chen and Ainsworth 1992). Similarly, Aakre et al. (1994) reported that Atlantic salmon injected with a mixture of Aeromonas salmonicida bacterin and yeast glucan showed enhanced antibody responses.

Yeast glucan has also been applied by immersion and oral administration. Raa et al. (1992) demonstrated that oral administration of yeast glucan to Atlantic salmon increased protection against V. anguillarum and V. salmonicida. Tiger shrimp immersed in yeast glucan solution showed enhanced protection against V. vulnificus infection (Sung et al. 1994). Although channel catfish injected with yeast glucan showed increased protection to E. ictaluri (Chen and Ainsworth 1992), oral administration has not shown such an effect (Ainsworth et al. 1994; Duncan and Klesius 1996; Welker et al. 2007).

The addition of products derived from a specific strain of Saccharomyces cerevisiae (Bio-Mos®; Alltech, Inc., Nicholasville, KY, USA), which is composed of the outer cell wall, rich in mannan oligosaccharides, has shown promise in modulating the immune response, improving feed efficiency, and promoting growth in poultry species (Spring et al. 2000; Iji et al. 2001; Hooge 2004; Nollet et al. 2007) and fish species (Staykov et al. 2007; Torrecillas et al. 2007). The use of Bio-Mos® in fish is beginning to emerge with varying growth and immune responses. For example, mortality was reduced and lysozyme and complement activity were increased in rainbow trout fed Bio-Mos® (Staykov et al. 2007). In European sea bass, there was a positive correlation between lysozyme and alternative complement pathway activities in blood and inclusion levels of dietary Bio-Mos® (Torrecillas et al. 2007). In addition, the phagocytic index was increased with the inclusion of Bio-Mos®. Contrary to these studies, Welker et al. (2007) found that lysozyme levels were similar in catfish fed Bio-Mos®.

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In addition, rainbow trout fed a Bio-Mos® supplemented diet showed improved weight gain and feed conversion ratio (FCR) and reduced mortality compared to controls (Staykov et al. 2007). In European sea bass (*Dicentrarchus labrax*), dietary Bio-Mos® enhanced growth but had no effect on FCR (Torrecillas et al. 2007). In another study, channel catfish supplemented with Bio-Mos® did not improve growth performance or resistance to *E. ictaluri* (Welker et al. 2007). In that same catfish study, levels of cortisol were lower after a 30-min low water stress in fish administered Bio-Mos®. The results of the catfish study are hard to interpret as the fish were fed Bio-Mos® for 4 wk and then switched to control diet for 2 wk before being challenged with *E. ictaluri*. In addition, the fish were fed the control diet for 21 d during the disease challenge. It is likely that the effects of Bio-Mos® were diminished because the fish were switched to control feed 2 wk prior to challenge.

In view of the positive effects of Bio-Mos® in other fish species, and the fact that Bio-Mos® was potentially not fed correctly in the catfish study, the objectives of the current studies were to determine if feeding yeast-derived mannans in the form of Bio-Mos® could improve growth, feed efficiency, and improve resistance of channel catfish to *E. ictaluri* challenge.

**Materials and Methods**

**Study I**

Juvenile catfish (USDA103 strain) were obtained from natural pond spawns at the USDA Catfish Genetics Research Unit, Stoneville, MS, USA. One hundred catfish (45.8 ± 1.2 g) were randomly assigned to two treatments with five replicates each: (1) Con-Sink (36% crude protein [CP] catfish diet, control) and (2) Bio-Mos®-Sink (36% CP catfish diet with Bio-Mos® supplemented at 2 g/kg). The fish were stocked into 76-L tanks (10 fish/tank) and allowed to acclimate for 10 d. During the acclimation period, the fish were fed the control diet. The feed (Land O’Lakes, Shoreview, MN, USA) was ground using a hammer mill, water added, mixed with a Hobart mixer, and repelleted using a Hobart grinder (Hobart, Troy, OH, USA). The Bio-Mos®-sink diet was made as described above except Bio-Mos® (Alltech, Inc.) was included at 2 g/kg of diet.

After the acclimation period, the fish were anesthetized with 0.1 g/L tricainemethane sulfonate (MS-222; Western Chemical Inc., Ferndale, WA, USA) and weighed to the nearest 0.1 g. The fish were fed their respective diets once a day to apparent satiation. Fish were maintained in 26.7 ± 0.2 C flow-through well water and a 14 h L:10 h D photoperiod. Water quality (pH ∼8.5 and dissolved oxygen levels >5.0 mg/L) and flow rates (7.6 L/min) were similar between tanks. The fish were maintained for 6 wk and the amount of feed provided was recorded weekly. No mortalities were observed throughout the growth study. The fish were also weighed on d 21 and 42 as previously described.

At the end of the 6-wk growth study, the fish were transferred to a quarantine facility into 76-L aquaria and allowed to acclimate for 10 d before they were challenged with *E. ictaluri*. Water quality (pH ∼8.5 and dissolved oxygen levels >5.0 mg/L), flow rates (7.6 L/min), and temperature 26.6 ± 0.1 C flow were similar between tanks. An *E. ictaluri* isolate from a natural outbreak (confirmed by the Fish Diagnostic Laboratory at the Delta Research and Extension Center, Stoneville, MS, USA) was used for the challenge. Fish were challenged with virulent *E. ictaluri* (1.9 × 109 cfu/mL) by an *in situ* bath immersion for 30 min (Wolters and Johnson 1994). Mortality was recorded daily for 21 d.

The fish were fed their respective diets during the acclimation period and during the challenge. Fish were taken off feed 1 d prior to challenge and the day of the challenge. Prior to challenge, two fish per tank were bled from the caudal vasculature into syringes coated with heparin and removed from the study. The plasma was separated and frozen at −20 C. On the last day of the challenge (Day 21), two surviving fish per tank were bled as described above. The plasma was used to measure lysozyme activity.
Levels of lysozyme activity were determined using the EnzChek Lysozyme Assay Kit (Invitrogen, Carlsbad, CA, USA). Briefly, 25 μL of plasma was diluted with 25 μL reaction buffer (0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5) and incubated with 50 μL fluorescein labeled Micrococcus lysodeikticus (50 μg/mL) for 30 min at 37 °C. The fluorescence was measured in a fluorescence microplate reader using excitation/emission wavelengths of 485/535 nm. Background fluorescence, determined for a no-enzyme control, was subtracted from each value. The lysozyme activity of the samples was calculated from a standard curve prepared with lysozyme from chicken egg white. All samples were run in duplicate.

Study II

Two hundred and forty catfish (19.3 ± 0.3 g) were randomly assigned to two treatments with eight replicates each: (1) Con-Float (32% CP catfish diet) and (2) Bio-Mos®-Float (32% CP catfish diet with Bio-Mos® supplemented at 2 g/kg). A 32% CP commercial floating catfish feed (Delta Western Feed Co., Indianola, MS, USA) was used in the study. Delta Western also made a 32% CP commercial floating catfish feed with Bio-Mos® (Alltech, Inc.) included at 2 g/kg. A 36% CP was not utilized in the experiment because Delta Western would only make a 28 or 32% diet.

The fish were stocked into 76-L tanks (10 fish/tank) and allowed to acclimate for 10 d. During the acclimation period, the fish were fed the control floating diet. Other procedures were the same as described for Study I. The fish were also weighed on Days 21 and 42 as previously described. At the end of the 6-wk growth study, the fish were transferred to the disease room facility.

Studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, United States Department of Agriculture/Agriculture Research Service Catfish Genetics Research Unit.

Statistical Analysis

Statistical analyses were conducted using the mixed procedure of the Statistical Analysis System (SAS, Version 9.1 software) followed by a Duncan’s multiple range test. Weight gain, specific growth rate (SGR), FCR, plasma lysozyme activity and survival were subjected to one-way analyses of variance (ANOVA) with treatment as a fixed effect and tank within treatment as a random effect. Tank served as the experimental unit for each variable measured. Differences among treatments were considered significantly different at \( P < 0.05 \).

Results

In Study I, catfish were fed a 36% CP sinking diet with or without Bio-Mos®. At the end of the 6-wk growth study, weight gain, SGR, and FCR were similar between treatments (Table 1). Survival after E. ictaluri challenge was higher (\( P < 0.05 \)) in fish fed Bio-Mos® compared to Controls (90 ± 7.3% vs. 55 ± 6.4%). Plasma levels of lysozyme activity were similar between treatments prior to post-challenge with E. ictaluri.

In Study II, catfish were fed a 32% CP floating diet with or without Bio-Mos®. At the end of the 4-wk growth study, weight gain, SGR, and FCR were similar between treatments
Table 1. Weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and percent survival of channel catfish fed BioMos® or Control diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g/fish)</th>
<th>SGR</th>
<th>FCR</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con-Sink</td>
<td>59.5</td>
<td>2.0</td>
<td>1.40</td>
<td>52.5y</td>
</tr>
<tr>
<td>Bio-Mos®-Sink</td>
<td>54.9</td>
<td>1.9</td>
<td>1.49</td>
<td>90.0z</td>
</tr>
<tr>
<td>SE</td>
<td>4.0</td>
<td>0.1</td>
<td>0.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con-Float</td>
<td>30.8</td>
<td>3.4</td>
<td>1.01</td>
<td>61.7</td>
</tr>
<tr>
<td>Bio-Mos®-Float</td>
<td>30.1</td>
<td>3.4</td>
<td>1.03</td>
<td>65.8</td>
</tr>
<tr>
<td>SE</td>
<td>1.6</td>
<td>0.01</td>
<td>0.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*Mean initial weight was 45.8 ± 1.2 and 19.3 ± 0.3 g/fish in Studies I and II, respectively.

bSGRs were calculated from the formula \( \frac{ln(BW_2) - ln(BW_1)}{t} \times 100 \) where \( BW_1 \) and \( BW_2 \) are initial and final weights, respectively, and \( t \) is feeding period (days).

cFCRs were calculated as ingested food (g)/ weight gain (g).

dPercent survival is the percentage of fish that survived after challenge with Edwardsiella ictaluri.
eSE is the pooled standard error of the mean.

y, zWithin columns, values with different letters are different (\( P < 0.05 \)).

Table 2. Weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and percent survival of channel catfish fed Bio-Mos® or Control diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g/fish)</th>
<th>SGR</th>
<th>FCR</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-Sink</td>
<td>40.5(^e)</td>
<td>3.8(^e)</td>
<td>1.17</td>
<td>51.4(^y)</td>
</tr>
<tr>
<td>Bio-Mos®-Sink</td>
<td>38.9(^e)</td>
<td>3.8(^e)</td>
<td>1.26</td>
<td>66.7(^z)</td>
</tr>
<tr>
<td>Con-Float</td>
<td>24.3(^y)</td>
<td>3.1(^y)</td>
<td>1.29</td>
<td>45.0(^y)</td>
</tr>
<tr>
<td>Bio-Mos®-Float</td>
<td>24.2(^y)</td>
<td>3.1(^y)</td>
<td>1.32</td>
<td>44.5(^y)</td>
</tr>
<tr>
<td>SE</td>
<td>0.9</td>
<td>0.06</td>
<td>0.01</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\(^a\)Mean initial weight was 9.3 ± 0.2 g/fish.

\(^b\)SGRs were calculated from the formula \( \frac{ln(BW_2) - ln(BW_1)}{t} \times 100 \) where \( BW_1 \) and \( BW_2 \) are initial and final weights, respectively, and \( t \) is feeding period (days).

cFCRs were calculated as ingested food (g)/ weight gain (g).

dPercent survival is the percentage of fish that survived after challenge with Edwardsiella ictaluri.
eSE is the pooled standard error of the mean.

\(^y, z\)Within columns, values with different letters are different (\( P < 0.01 \)).

(Table 1). Survival after \( E. ictaluri \) challenge was also similar between fish fed Bio-Mos® and Controls diet.

In Study III, catfish were fed either a 32% CP floating diet or a 36% CP sinking diet with or without Bio-Mos®. At the end of the 6-wk growth study, weight gain and SGR were higher (\( P < 0.001 \)) in fish fed the 36% CP sinking diet with or without Bio-Mos® compared to the other treatments (Table 2). Weight gain and SGR were similar between the Control and Bio-Mos® 36% CP sinking diets. FCR was similar among all treatments. Survival after \( E. ictaluri \) challenge was higher (\( P < 0.01 \)) for fish fed Bio-Mos®-Sink compared to the other treatments.

Discussion

Immunostimulants (β-glucans) have been explored as a possible method to improve growth and disease resistance but the results have been inconsistent. For example, feeding of β-glucans for 14 wk did not improve growth in Nile tilapia (Whittington et al. 2005). Conversely, feeding β-glucans increased growth of snapper, Pagrus auratus, after 56 and 84 d of feeding during suboptimal growth (Cook et al. 2003). In this same study, growth was not improved during the summer months of optimal growth temperature (Cook et al. 2003). In another study, Misra et al. (2006) observed increases in growth of Labeo rohita fed β-glucans for 56 d.
The results of using β-glucans to improve disease resistance have also been variable. For example, Raa et al. (1992) demonstrated that oral administration of yeast glucan to Atlantic salmon increased protection against *V. anguillarum* and *V. salmonicida*. Tiger shrimp immersed in yeast glucan solution showed enhanced protection against *V. vulnificus* infection (Sung et al. 1994). However, oral administration of yeast glucan to channel catfish has not shown such an effect (Ainsworth et al. 1994; Duncan and Klesius 1996; Welker et al. 2007). The differences in responses to β-glucan administration are not clear but may be confounded by differences in species, source of β-glucan, and length of time the β-glucan was administered.

The results of current studies show that the addition of Bio-Mos® at 2 g/kg diet to catfish does not improve weight gain or FCR when fed for 6 wk, but may improve resistance to *E. ictaluri*. In the first study, catfish were fed either a sinking 36% CP commercial diet or a 36% CP diet supplemented with Bio-Mos® for 6 wk followed by a 21-d *E. ictaluri* challenge. Weight gain and FCR was similar between treatments. However, survival after *E. ictaluri* challenge was approximately 42% higher in fish fed Bio-Mos® compared to Controls. Lysozyme activity in pre- and post-challenge was similar.

In the second study, catfish were fed a 32% floating diet with or without the addition of Bio-Mos® for 4 wk followed by a 21-d *E. ictaluri* challenge. The idea behind the second study was to include Bio-Mos® into a catfish diet that would typically be fed to catfish raised in ponds. A 28–32% CP diet is typically fed to catfish raised in commercial ponds. Bio-Mos® was included into the diet at 2 g/kg diet and was prepared by extrusion technology at the Delta Western Feed Mill, Indianola, MS, USA. Similar to what was observed in the first study, there was no difference in weight gain or FCR between the two treatments. During the 21-d bacterial challenge, survival was also similar between treatments. The lack of difference in survival in Bio-Mos® fed catfish was unexpected because survival was significantly improved in Study I.

The third study was conducted to validate the results of the prior experiments. Fish fed both 36% CP diets gained more weight compared to fish that consumed 32% CP diets. Protein concentration affecting maximum weight gain of channel catfish has ranged from 24 to 40% (Reis et al. 1989; Li and Lovell 1992; Robinson and Li 1998; Li et al. 2004). The large variation in these reported protein requirements might be caused by fish size, differences in environmental conditions, feeding practices, protein quality, and energy content of the diet (Lovell 1988). In the current study, the 36% CP diet contained 5.4% fat (average for both diets) while the 32% CP diet contained 3.3% fat (average for both diets). It is likely the higher levels of fat in the 36% CP diets contributed to higher intake and thus weight gain.

Survival after *E. ictaluri* challenge was significantly higher in fish fed the 36% CP sinking diet supplemented with Bio-Mos® compared to the other treatments. These results are similar to what was observed in the first and second study. It is not clear why the 32% CP diet supplemented with Bio-Mos® that had undergone extrusion at a commercial feed mill did not perform similarly to the 36% CP sinking diet. The extrusion process has not been shown to damage or degrade Bio-Mos® activity in poultry diets.

There was no improvement in weight gain or FCR in catfish supplemented with Bio-Mos®. In agreement with the results of our study, Welker et al. (2007) reported that Bio-Mos® supplementation (2 g/kg) did not affect total weight gain or FCR in a 4-wk catfish study. In another study, Bio-Mos® supplementation improved weight gain and FCR in rainbow trout grown in both net cages and raceways (Staykov et al. 2007). In European sea bass, dietary Bio-Mos® also enhanced growth but had no effect on FCR (Torrecillas et al. 2007). Growth rate and feed efficiency were also unaffected in juvenile red drum, *Sciaenops ocellatus*, fed brewers yeast (Li et al. 2005) while growth performance was improved in hybrid striped bass, *Morone chrysops* female
M. saxatilis male fed brewers yeast (Li and Gatlin 2004). There is considerable variation in the effects of dietary yeast-derived mannans on growth and feed efficiency, which is likely dependent on fish species, feeding duration and concentration of supplement, and type of yeast-derived mannans used in the study.

The primary benefit of feeding Bio-Mos® to channel catfish in the present research was the improvement in survival after E. ictaluri challenge. These results are different from Welker et al. (2007) who fed channel catfish Bio-Mos® for 4 wk followed by a 2-wk period of control feed before E. ictaluri challenge. The fish were also fed the control diet for 21 d during the disease challenge. Welker et al. (2007) did not observe any improvement in survival. It is likely that the effects of Bio-Mos® were diminished because the fish were switched to control feed 2 wk prior to challenge. Bio-Mos® is intended to be fed on a continuous basis (John Sweetman, Alltech, Inc., pers. comm.); while short-duration feeding of immunostimulants, followed by control feeding, has been shown to be an effective method of enhancing the immune system and disease resistance (Chen and Ainsworth 1992; Bridle et al. 2005). In commercial catfish production, withdrawing Bio-Mos® 2 wk prior to disease outbreaks in catfish ponds would not be practical because it is difficult to predict when a disease breaks in a pond.

Studies have demonstrated improvement in disease resistance and improvement of indicators of immune status when fish were administered Bio-Mos®. In rainbow trout, mortality was reduced and lysozyme and complement activity were increased in fish fed Bio-Mos® (Staykov et al. 2007). In European sea bass, there was a positive correlation between lysozyme and alternative complement pathway activities in blood and inclusion levels of dietary Bio-Mos® (Torrecillas et al. 2007). In addition, the phagocytic index was increased with the inclusion of Bio-Mos® at 4 g/kg. We observed an increase in resistance to ESC during the bacterial challenge, but lysozyme activity pre- and post-challenge was similar. Welker et al. (2007) also found that lysozyme levels were similar in catfish fed Bio-Mos®. Further studies must be conducted to understand the role of Bio-Mos® in modulating the immune system in fish.

While genetic gains toward developing lines of catfish that show improvement in disease resistance are slow, feeding yeast-derived mannans in the form of Bio-Mos® may prove beneficial in increasing resistance to diseases such as ESC. Future research will focus on “ideal” inclusion levels of Bio-Mos® into catfish diets as well as a more in-depth effort into understanding the mechanism(s) through which Bio-Mos® effects immune function.

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