IN VITRO EVALUATION OF BINDING ABILITY OF MODIFIED MANNANOLIGOSACCHARIDE, INACTIVATED YEAST AND UTPP ON AFLATOXIN B1 IN LIQUID MEDIA

N. AFZALI¹ and G. DEVEGOWDA²

Summary

The in vitro binding efficiency of modified-mannanoligosaccharide (M-MOS) (0.1%) inactivated yeast (0.1%) and UTPP (aluminosilicate with organic acids) (0.5%) on aflatoxin B₁ in liquid media was evaluated at two pH levels (4.5 and 6.5) under simulated in situ conditions of GI tract of chicken. The aflatoxin B₁ levels were 100 and 200 ppb which were tested with binders at two pH levels on triplicate samples. Based on the aflatoxin B₁ recorded after the incubation and centrifugation, the supernatant of treated and control flasks were taken and the adsorption percentage was calculated. Modified-MOS showed significantly P≤0.01 higher binding ability (93.82%) compared to inactivated yeast and UTPP, (62.26 and 72.02%, respectively). Higher significant binding ability was recorded at higher level of aflatoxin and pH 6.5 for all the three binders tested.

I. INTRODUCTION

Several approaches have been tried to detoxify aflatoxins in feeds and feed ingredients. One promising approach is the use of aluminosilicates in feed (Phillips et al., 1988; Doerr, 1989 Kubena, 1990; Sheila, 1993). Aluminosilicates have high adsorption properties with cationic exchange constituent without altering the structure, they possess active sites which can interact and immobilize certain molecules via electrostatic forces or by formation of covalent bonds.

Live yeast (Saccharomyces cerevisiae), Lactobacillus spp. and other bacterial and fungal spp. have been tried to reverse the adverse effects of aflatoxicosis (Ciegler et al., 1966; Stanley et al., 1993; Trenholm et al., 1994; Morton, 1996). Among these, live yeast has shown promising results. The aflatoxin counteracting ability of live yeast is attributed to the mannanoligosaccharide, present in its cell wall. The specific objective of this study was to compare the ability of modified mannanoligosaccharide (M-MOS), inactivated yeast and aluminosilicate with organic acids (UTPP) to bind aflatoxin B₁ in vitro.

II. METHODS

The concentrations of the binding agents used in the study were; M-MOS (0.1%), inactivated yeast (0.1%) and UTPP (0.5%) in liquid media (buffer solution). The aflatoxin B₁ standards (100 and 200 ppb) were dried in conical flasks and each binder was added separately to all the flasks containing both levels of aflatoxin, along with 50 ml of buffer solution of desired pH (4.5 or 6.5). The test was run on triplicate samples and controls were maintained for each toxin level. The samples (Toxin + Binder + Buffer) were mixed on a horizontal shaker for 30 minutes to facilitate contact of binder with toxin. Samples were then incubated for three hours at 37°C. After incubation, the samples were centrifuged at 1500 rpm for 10 minutes to remove sediments which contained binder and bound toxin, the

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supernatant was extracted for its aflatoxin content using CB method (A.O.A.C., 1990). Aflatoxin concentration was estimated by TLC (Romer, 1975). The percentage values were changed to acine values and the data subjected to ANOVA of the $3 \times 2 \times 2$ factorial design using the General Linear Models procedures of the statistical analysis system (SAS Institute, 1987). The New Duncan Multiple Range test was employed for comparison of means (Duncan, 1955).

III. RESULTS

The results of aflatoxin binding by M-MOS, inactivated yeast and UTPP at two of aflatoxin and two pH levels are presented in Tables 1, 2, 3 and 4. Modified-MOS showed significantly ($P<0.01$) higher aflatoxin binding (93.82%) in comparison to inactivated yeast (62.67%) and UTPP (73.02%) (Table 1). UTPP showed significantly, ($P<0.01$) higher binding than inactivated yeast.

Table 1. Per cent aflatoxin binding by M-MOS, Inactivated yeast and UTPP in liquid media

<table>
<thead>
<tr>
<th>Binder</th>
<th>PH</th>
<th>Aflatoxin ppb</th>
<th>Binding %</th>
<th>Binders $\times$ pH Mean ± SE</th>
<th>Binder Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified-MOS</td>
<td>4.5</td>
<td>100</td>
<td>90.28</td>
<td>91.97±0.81</td>
<td>93.82±0.69</td>
</tr>
<tr>
<td>(0.1%)</td>
<td>6.5</td>
<td>200</td>
<td>93.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated</td>
<td>4.5</td>
<td>100</td>
<td>61.33</td>
<td>62.33±0.80</td>
<td>62.67±0.94</td>
</tr>
<tr>
<td>yeast (0.1%)</td>
<td>6.5</td>
<td>200</td>
<td>63.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTPP (0.5%)</td>
<td>4.5</td>
<td>100</td>
<td>70.10</td>
<td>71.55±0.64</td>
<td>73.02±0.82</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>200</td>
<td>72.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

abc: Means having same superscript do not differ significantly ($P<0.01$)

The levels of aflatoxin had a significant influence ($P<0.01$) on the amount of the toxin bound (Table 2). All three binders showed higher binding values at the higher level of aflatoxin (200ppb) than at the lower level (100 ppb). Aflatoxin binding was significantly ($P<0.01$) influenced by the pH of the medium (Table 2). Higher binding of aflatoxin (77.72%) was seen at pH 6.5 than at pH 4.5 (75.28%).

Table 2. Mean values of binders at levels of aflatoxin bound at different aflatoxin concentrations (aflatoxin 100 and 200 ppb) and pH (6.5 and 4.5)

<table>
<thead>
<tr>
<th>Aflatoxin (ppb)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>74.82±3</td>
<td>78.18±3</td>
</tr>
<tr>
<td>±3.37</td>
<td>±2.98</td>
</tr>
</tbody>
</table>

ab: means having same superscript within each row and factor do not differ significantly ($P<0.01$)
Significant (P<0.01) interaction was noted between the binders and the level of aflatoxin (Table 3). Level of aflatoxin in media had significant influence on the binding abilities of inactivated yeast and UTPP, while no such trend was seen with M-MOS. On the other hand, percent binding of aflatoxin by inactivated yeast and UTPP was significantly (P<0.5) higher at 200 ppb level of aflatoxin than at 100 ppb level.

Table 3. Interaction between the various binders and aflatoxin levels for per cent binding

<table>
<thead>
<tr>
<th>Aflatoxin Ppb</th>
<th>Binders</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Modified-MOS</td>
<td>Inactivated yeast</td>
<td>UTPP</td>
</tr>
<tr>
<td>100</td>
<td>93.3&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>60.2&lt;sup&gt;bC&lt;/sup&gt;</td>
<td>71.1&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>94.4&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>65.2&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>75.0&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ab: means having same superscript within each column do not differ significantly (P<0.01)  
ABC: means having same superscript within each row do not differ significantly (P<0.01)

The liquid media pH and binders also showed significant interaction (P<0.01) for binding percentage (Table 4). Modified-MOS and UTPP showed significantly higher binding percentage (95.7 and 74.5, respectively) at pH 6.5 than at pH 4.5 (92.0 and 71.5%, respectively), while the binding percentage for inactivated yeast at both pH levels was statistically not different.

Table 4. Interaction between the various binders and pH level for percent aflatoxin binding

<table>
<thead>
<tr>
<th>pH</th>
<th>Binders</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Inactivated yeast</td>
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<td>71.6&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.5</td>
<td>95.7&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>63.0&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>74.5&lt;sup&gt;aB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ab: means having same superscript within each column do not differ significantly (P<0.01)  
ABC: means having same superscript within each row do not differ significantly (P<0.01)

IV. DISCUSSION

The results of this study show that more aflatoxin is bound to binder at higher compared to lower concentrations of the toxin. This may be due to an increase in the availability of substrate for binding to binders as supported by the findings of the present studies and also the study conducted by Mahesh and Devegowda (1996a,b).

The results obtained in the trial with UTPP are in close agreement with those reported by Mahesh and Devegowda (1996b). However, the percent binding recorded with M-MOS in this trial (93.82%) is marginally higher than that observed by Mahesh and Devegowda with MOS (85.12%). This increase in binding with M-MOS over that of MOS might have been due to its modified chemical nature (esterified glucomannan).

Trenholm (unpublished as reported by Devegowda et al., 1998b) also recorded higher binding of aflatoxin with M-MOS than with aluminosilicate. However, the total binding percentages reported by him are higher those observed in this study. This may be due to the differences in the methodology applied in the determination of in vitro binding.

Morton (1996) while working with live yeast, recorded 56 percent degradation of aflatoxin at 48 hrs of incubation in Sabbaroud's broth cultures employing 250 ppb aflatoxin.
This value is close to the mean binding percentage (62.7) observed with inactivated yeast in the present trial. Higher binding percentage were observed at pH 6.5 than at 4.5 with modified-MOS. Further, Trenholm et al. (1994) also reported higher binding of zearalenone by modified oligosaccharide from yeast cell wall at pH 9 than at pH 4 in aqueous solutions.

REFERENCES