DETOXIFICATION OF AFLATOXINS-CONTAMINATED DIET BY SOME PHYSICAL AND CHEMICAL MEANS.
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ABSTRACT

In vitro comparative efficacy studies on the addition of fix-a-tox, antitox plus, tafla, autoclaving, microwave, hydrogen peroxide, ammonia solution and extraction with aqueous methanol (50%) as detoxifying agents to a diet containing 3600 ppb or 1800 ppb total aflatoxins. The 0.5% level of antitox plus, fix-a-tox and tafla gave the most constant pattern with aflatoxin reduction of 33.4, 16.7 and 44.4%, respectively at the high level of toxin but at the low level the reduction was 31.6, 15.8 and 42.1%, respectively. The autoclaving for 30 min and using microwave oven at a medium energy for 3 min gave reduction of 33.4 and 52.8% at the high level of toxin but at the low level of toxin the reduction was 31.6 and 51.6%, respectively. The effect of high temperature on the appearance, consistency and composition of a food (consequently its biological value, digestibility and utilization) raises special attention to the choice of protection as the corner stone of the aflatoxin control which is more beneficial than detoxification. Many chemicals have been tested for their ability to structurally degrade or inactivate aflatoxin, including H\textsubscript{2}O\textsubscript{2} and ammoniation. These chemicals resulted in a reduction of 38.9 and 88.9% at the high level of toxin but in the low level contaminated diet, the reduction was 36.8 and 87.9%, respectively. Extraction of aflatoxin from the diet with methanol caused a reduction of 55.6% at the high level of toxin but in the low level contaminated diet, the reduction was 53.2%. However, this method for the removal of aflatoxins via solvent extraction appears to be impractical and expensive when compared with other methods.

There was no difference in the chemical composition for all different treatments, yet the treatment with ammonia increased crude protein (CP)% but the extraction caused a decrease in CP% and hence in the nutritional quality.

Aflatoxin productivity from *Aspergillus flavus* in liquid yeast media in the absence and presence of 0.5% of each of antitox plus, fix-a-tox, tafla, 1% of ammonia and 3% level of H\textsubscript{2}O\textsubscript{2} was determined in the samples. Addition of any of these agents resulted in a decrease in aflatoxin productivity with the maximum effect for ammonia followed by H\textsubscript{2}O\textsubscript{2}, tafla, antitox plus and fix-a-toxin. Ammonia prevented the growth of *Aspergillus flavus* and this resulted in prevention of aflatoxin contamination.

Keywords: Aflatoxin - detoxication - removal - chemical & physical means - production.

INTRODUCTION

Mycotoxins are the silent enemy. Mycotoxins affect as much as 25% of the worlds' feed crops each year (Yegany et al., 2002). Acute aflatoxicosis causes hepatitis, hemorrhage, immune suppression, genetic damage (carcinogenicity, teratogenicity and mutagenicity) and death (Moorthy et al., 1985; Nowar et al., 1987 and 1992 a&b and Pier, 1992). Growth impairment and lowering of reproductive performance are the most sensitive clinical signs of chronic aflatoxicosis (Clark et al., 1980; CAST, 1989 and Nowar et al., 1996). Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Müller, 1993; Abdelhamid et al., 1985, 1986 and 1992 a&b; Abdelhamid, 1993 and Abdelhamid and Mahmoud, 1996). Aflatoxins are heat-stable and not totally
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destroyed by boiling water, autoclaving and a variety of food and feed processing procedures (Pluyer et al., 1987). The degradation of aflatoxins is a direct function of temperature, heating time and moisture content (Mann et al., 1967). A variety of solvents were capable of extracting aflatoxins from different commodities with minimal effect on protein content or nutritional quality (Rayner et al., 1977 and Goldblatt and Dollear 1979). However, these methods appear to be impractical and expensive when compared with other methods. A variety of adsorbent materials were used too including activated carbon (Decker, 1980) and clays (Masimanco et al. 1973 and Nowar et al., 1996 & 2000). Abdelhamid and Mahmoud (1996) studied the efficiency of toxin-adsorbent-agents (florisil, aluminum oxide and fix-a-tox) on detoxification of aflatoxin. Adsorbents used herein did not lower aflatoxin level in rice grains during storage but increased the toxin concentration by storage advance, particularly in the presence of florisil, fix-a-tox and urea. Mahmoud et al. (1994) reported also surprising results by using such detoxifying agents (Al₂O₃, antitox plus, charcoal, fix-a-tox, and florisil). Many chemicals have been tested for their ability to structurally degrade or inactivate aflatoxins, including numerous acids, bases, aldehydes, bisulfite and oxidizing agents (Goldblatt and Dollear 1979 and Anderson 1983). Ammoniation resulted in a significant reduction in the level of aflatoxins in contaminated peanut and cottonseed meal (Dollear et al., 1968; Gardner et al., 1971 and Park et al., 1984). Natural aflatoxin inhibitors, e.g. carotenoids and benzoazolinone compound from corn (Norton, 1998) and glucosannan from yeast cell wall can reduce also aflatoxin level and its effects (Bintvihok, 2001). Also, peptide (D4E1) may help defeat aflatoxin in cotton (STAT, 2001). Additionally, yeast (Stanley et al., 1993), bacteria (El-Nezami et al., 1998) and fungi (El-Sayed 1996), even atoxigenic Aspergillus flavus (Cotty and Bhatnagar, 1994) prevent aflatoxin contamination. However, the aim of the present study was to evaluate the effectiveness of some treatments in reducing level of aflatoxin contamination and/or production.

MATERIALS AND METHODS

Production of aflatoxins: For producing aflatoxin, the strain of Aspergillus flavus NRRL 3357 (from Laboratory of Mycotoxins, National Research Center, Dokki – Cairo) was grown in synthetic media, yeast extract – sucrose broth (YES) containing 2% yeast extract and 20% sucrose. The substrate was dispensed in conical flasks. The flasks were then autoclaved for 15 minutes at 121°C, then coold and inoculated with spores suspension and incubated for 9 days at 25 – 29°C. Aflatoxin concentration was determined using immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization according to Truckes et al. (1991).

The media contained a mixture of aflatoxins B₁, B₂, G₁ and G₂, at a total level of 18 ppm. Half liter of the culture was added to 2.5 or 5.0 Kg feed to be contained 3600 or 1800 ppb of aflatoxins. The ration used to be artificially contaminated was formulated according to Ahmed (1976). The ingredients of this ration are as following: crushed wheat 46%, shredded barley 40%, fish meal powder 9%, dried milk 3%, yeast 1% and minerals and vitamins 1%.

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Detoxification methods: Different methods of aflatoxin inhibition were applied in duplication by weighing 500 g samples from the concentrate mixture mentioned before. The samples were mixed thoroughly together with aflatoxins solution and the mixture refilled into sacks (bags). Ammonia solution at a concentration of 1% was added to some treated sacks and then kept for 3 wks. Another sacks of samples were sprayed by hydrogen peroxide solution at a level of 3% to bring the final moisture content to about 20%. The mixture was incubated in oven at 80°C for 0.5 hr using stainless steel trays. Another samples of the aflatoxicated feed mixture were extracted by methanol (50%) then filterated and air-dried. Sacks of the toxificated feed were autoclaved at 121°C for 30 minutes and 1.5 prs. Approximately 500 g of the aflatoxicated feed were thoroughly mixed with 0.5% of either antitox-plus, talla, or fix-a-tox. The microwave oven used for heating the toxicated feed was Goldstar Model No. ER 535 MD. The frequency of the radiation emitted in this oven was 2450 MHZ. The selected setting of heating corresponding to full power (3) which provided 1300 W (1.3 KW). 250 ml beckers contained the contaminated meal (200 g) were heated using this microwave oven at a medium power for 3 mins. Proximate chemical analysis as well as calcium and phosphorus contents were determined in the treated samples according to A.O.A.C. (1990).

RESULTS

1- Efficacy of the detoxification:
Table (1) presents to what extent the used tetoxification methods were effective in lowering the contamination levels. It is clear that 1% ammonia treatment was the best one, since it reduced the initial levels of aflatoxins by 87.9 – 88.9%, depending on the initial concentration. Then followed by the extraction with 50% methanol (53.2 – 55.6%), microwave (51.6 – 52.8%), 0.5% talla (42.1 – 44.4%), 3% hydrogen peroxide (36.8 – 38.9%), autoclaving (31.6 – 33.4%), 0.5% anti-tox-plus (31.6 – 33.4%) and at the end 0.5% fix-a-tox (15.8 – 16.7%). However, the higher aflatoxins level was associated with slightly elevated degradation rate of aflatoxins. This means that ammoniation of aflatoxins – contaminated diet was the best method among the different means used herein for decomposition of aflatoxins in the faulty feed.

2- Effect of the detoxification on chemical composition:
Data shown in Table (2) are the chemical composition percentages, which were not influenced by the contamination level or by the used means of decontamination.

Table (1): Percentages of losses from two aflatoxins concentrations by various treatments for contaminated diet.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Aflatoxins level (ppb)</th>
<th>% losses</th>
<th>Aflatoxins level (ppb)</th>
<th>% losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated diet</td>
<td>3600</td>
<td></td>
<td>1800</td>
<td></td>
</tr>
<tr>
<td>Autoclaving</td>
<td>2400</td>
<td>33.4</td>
<td>1300</td>
<td>31.6</td>
</tr>
<tr>
<td>Fix-a-tox</td>
<td>3000</td>
<td>18.7</td>
<td>1600</td>
<td>12.8</td>
</tr>
<tr>
<td>Talla</td>
<td>2000</td>
<td>44.4</td>
<td>1100</td>
<td>42.11</td>
</tr>
<tr>
<td>Antitox plus</td>
<td>2400</td>
<td>33.4</td>
<td>1300</td>
<td>31.6</td>
</tr>
<tr>
<td>H2O2</td>
<td>2200</td>
<td>38.9</td>
<td>1200</td>
<td>36.8</td>
</tr>
<tr>
<td>NH3</td>
<td>4000</td>
<td>88.9</td>
<td>2300</td>
<td>87.9</td>
</tr>
<tr>
<td>Methanol</td>
<td>1600</td>
<td>55.6</td>
<td>890</td>
<td>53.2</td>
</tr>
<tr>
<td>Microwave</td>
<td>1700</td>
<td>52.8</td>
<td>920</td>
<td>51.6</td>
</tr>
</tbody>
</table>
Yet, there was some elevation in crude protein content by the ammoniation of the contaminated diets (18.7 – 19.2%) comparing with the control (without treatments), being (16.2 – 17.2%). However, the extraction with methanol appeared to reduce crude protein contents (14.3 – 14.4%) of the treated – aflatoxin contaminated diets. Hence, the last treatment may reduce the feeding value by leaching some protein from the treated diet (besides its toxicity for the presence of aflatoxins). Whereas the best detoxification method (ammoniation) improved the feeding value via N-enrichment of the treated diet.

3- Effect of the detoxicants on the aflatoxin production:
Table (3) shows the aflatoxin productivity and its inhibition by the effect of using some detoxification materials in YES – media for Aspergillus flavus. It was obviously that all used materials led to various inhibition rates in aflatoxin production. But the best treatment was 1% ammonia, which caused 100% inhibition of aflatoxin production. Since ammonia prevent fungal growth for its alkalinity. Hydrogen peroxide also reduced the productivity of aflatoxin by 91.8% comparing with the control. It is a strong oxidative agent for aflatoxin. However, the adsorbents (antitox plus and fix-a-tox) were less effective in reducing aflatoxin productivity, being 62.5 and 47.5%, respectively.

Table (3): Effect of various treatments on aflatoxin productivity by Aspergillus flavus on YES media.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxins (ppb)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>160,000</td>
<td>-</td>
</tr>
<tr>
<td>0.5% Fix-a-tox</td>
<td>84,000</td>
<td>47.5</td>
</tr>
<tr>
<td>0.5% Antitox plus</td>
<td>60,000</td>
<td>62.5</td>
</tr>
<tr>
<td>0.5% Tafía</td>
<td>13,600</td>
<td>91.5</td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td>13,200</td>
<td>91.8</td>
</tr>
<tr>
<td>1% NH₃</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

DISCUSSION
Aflatoxins are very toxic and carcinogenic mycotoxins. Their occurrence is outspreading in various food and feeds all over the world, particularly the developing countries, e.g. Egypt (Abdelhamid, 1981, 1983 a&b, 1985 and 1990; Kiermeir, 1985; Aziz and Youssef, 1991 and Wood, 1992). Even it was found in herb tea, medicinal plants (Halt, 1998) and tobacco, therefore aflatoxin is identified as silent killer (ASH, 1999). Thus, aflatoxin residues may be often found in animal products and human tissues (Vries et al., 1990; Jonsyn et al., 1995; Abdelhamid and Saleh, 1996 and Changbumrung et al., 1999). Therefore, aflatoxins threaten animal and human health (Sun et al., 1985; Hsieh et al., 1988; Abdelhamid et al., 1998 and 1999; Costantini et al., 1999 and Med Nets, 2001). Hence, the prophylaxis against fungal invasion is a must to avoid the harmful effects of mycotoxicoses. But when the aflatoxin is existed, then it is necessarily to
alleviate its toxic severity. The present research is an attempt in this direction, i.e. to overcome (or inhibit) aflatoxin production and to reduce level (or detoxify) of the present aflatoxin.

Among the physical and chemical means of detoxification used herein, ammonia treatment was beneficial in reducing most of the present aflatoxin levels (by up to 88.9%). Moreover, this treatment elevated crude protein content of the contaminated diet by about 13.8% comparing with the untreated (control) diet. Additionally, ammonia treatment inhibits totally aflatoxin production in the *A. flavus* media. In accordance, Mann *et al.* (1971), Jensen *et al.* (1977) and Bagley (1979) inactivated aflatoxin in cottonseed meal and corn. Yet, Mann *et al.* (1971) found that ammonia treatments lowered nitrogen solubility and lysine availability. But Jensen *et al.* (1977) reported that ammoniation lowered feed intake. In this context, Abdelhamid *et al.* (1994) reported higher crude protein content of the treated materials with ammonia or hydrogen peroxide. Also, urea (as a source of ammonia) destroyed the aflatoxin (Abdelhamid and Mahmoud, 1996). Similar results were recorded by Abdelhamid *et al.* (1985) concerning inhibition of aflatoxin production via using the preserving power of some additives, mainly medical herbs, spices, organic acids and fungicides. Yet, in another study (Abdelhamid *et al.*, 2002b), it was concluded that no one of the tested medicinal herbs completely overcome the effects of foodborne aflatoxicosis.

The moderate or low effects of extraction (methanol), microwave, autoclaving, oxidation (H₂O₂), and adsorbents (tafla, antitox plus, and fix-a-tox) used on lowering level of contamination with aflatoxin were reviewed. Since Decker (1980) and Abdelhamid *et al.* (1986) reported slight positive effect of charcoal in overcoming aflatoxicosis. Yet, autoclaving was effective in decontamination of aflatoxins by 92 – 100%, depending on the initial contamination level, time course of the treatment or its temperature in addition to the aflatoxin type (Abdelhamid, 1993). On the other hand, the last author added that autoclaving led to hard-sticky food with dark coloration. Therefore, the choice of protection (prevention) is preferable as the cornerstone of the aflatoxin control, which is more beneficial than detoxification. However, the effect of the used adsorbents may be due to their characteristics including adsorbing water (leading to lowering water activity against the required level for fungal growth and aflatoxin production as cited by Adebaje *et al.*, 1994), which has the same effect of drying (Ozay *et al.*, 1995), fungicides or fumigation on mould inhibition (Nilipour, 1996). They also bind aflatoxin leading to reduction of its contamination level (Horvath, 1998).

In accordance with the present results; antitox plus, charcoal, fix-a-tox and florisil-as aflatoxin detoxifying agents were of low effect. Meanwhile, addition of any of these agents resulted in an increase in aflatoxin productivity of *A. parasiticus* in sorghum (Mahmoud *et al.*, 1994). Moreover, Abdelhamid and Mahmoud (1996) concluded that the usage of such adsorbents was of no benefits during feed-storage, since their toxin-adsorbent effect is only immediately during their addition. Since many absorbents are sold based on their activity in laboratory trials (*in-vitro* tests) without actual testing in live animals (*in-vivo* tests) in the field (Anon., 1999). Anyhow, aflatoxin was found to be less adsorbed than other mycotoxins by different adsorbents and the
adsorbance of mycotoxins from aqueous media is more than from corn (Shehata, 2001). Additionally, Biogen® did not detoxify aflatoxic diets (Abdelhamid et al., 2001). However, another studies (Hertrampf, 1994; Smith et al., 1994 and Edrington et al., 1996) referred to the benefits of such materials (alumino silicates). In addition, detoxification of aflatoxin in peanut meal was completely by hydrogen peroxide at a pH of 9.5 involved heat treatment of the meal at 80°C (Sreenivasamarthy et al., 1967). Also, methanol extraction was used for removing aflatoxin from oilseeds meal (Sargent et al., 1961; Feuell, 1966 and Müller, 1983). Moreover, microwave heating may reduce also other mycotoxins (DON) level to a certain extent (Horvath, 1998). In general, to choose an aflatoxin detoxification method (physical, chemical, and/or biological), it must be depended on its availability, costs, effectiveness, applicability, suitability for the contaminated material and on its side effects.

CONCLUSION

From the foregoing results, it could be concluded that ammonia treatment of aflatoxin contaminated diets strongly reduces the level of contamination and enhances crude protein level of the treated materials. The presence of ammonia can prevent fungal growth and consequently aflatoxin production. Thus, any suspicious material could be safely treated with ammonia to avoid aflatoxin occurrence.

REFERENCES


Abdelhamid, A.M. et al.


البحث

العنوان: إزالة سمية العلاق الملوثة بالأفلاتوكسين ببعض الطرق الطبيعية والكيميائية

الناشر: Abdelhamid, A.M. et al.

النص:


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** المعمل المركزى للأغذية والأعلاف – مركز البحوث الزراعية

{النص باللغة العربية}
Table (2): Effect of various treatments on chemical composition (%) of the contaminated – diet with two aflatoxin concentrations.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Ether extract</th>
<th>Ash</th>
<th>NFE</th>
<th>Calcium</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>High contaminated diet (H)</td>
<td>10.2</td>
<td>89.8</td>
<td>17.2</td>
<td>4.7</td>
<td>2.78</td>
<td>8.8</td>
<td>56.32</td>
<td>1.97</td>
<td>0.72</td>
</tr>
<tr>
<td>Low contaminated diet (L)</td>
<td>11.1</td>
<td>88.9</td>
<td>16.2</td>
<td>4.3</td>
<td>3.02</td>
<td>9.2</td>
<td>56.48</td>
<td>2.09</td>
<td>0.73</td>
</tr>
<tr>
<td>H + Autoclaving</td>
<td>11.8</td>
<td>88.2</td>
<td>17.2</td>
<td>4.6</td>
<td>3.05</td>
<td>9.1</td>
<td>54.25</td>
<td>2.01</td>
<td>0.71</td>
</tr>
<tr>
<td>L + Autoclaving</td>
<td>11.9</td>
<td>88.1</td>
<td>16.8</td>
<td>4.7</td>
<td>2.88</td>
<td>9.4</td>
<td>54.32</td>
<td>1.85</td>
<td>0.70</td>
</tr>
<tr>
<td>H + antitox plus</td>
<td>10.9</td>
<td>89.1</td>
<td>16.8</td>
<td>4.6</td>
<td>2.74</td>
<td>8.6</td>
<td>56.36</td>
<td>1.98</td>
<td>0.71</td>
</tr>
<tr>
<td>L + antitox plus</td>
<td>11.1</td>
<td>88.1</td>
<td>16.8</td>
<td>4.7</td>
<td>2.89</td>
<td>8.8</td>
<td>55.71</td>
<td>2.15</td>
<td>0.74</td>
</tr>
<tr>
<td>H + Fix-a-toxin</td>
<td>11.3</td>
<td>88.7</td>
<td>16.8</td>
<td>4.3</td>
<td>3.29</td>
<td>8.7</td>
<td>55.61</td>
<td>2.03</td>
<td>0.69</td>
</tr>
<tr>
<td>L + Fix-a-toxin</td>
<td>10.9</td>
<td>89.1</td>
<td>16.8</td>
<td>4.5</td>
<td>3.10</td>
<td>8.8</td>
<td>55.90</td>
<td>2.02</td>
<td>0.67</td>
</tr>
<tr>
<td>H + tafla</td>
<td>11.4</td>
<td>88.6</td>
<td>16.7</td>
<td>4.6</td>
<td>2.95</td>
<td>8.8</td>
<td>55.55</td>
<td>1.94</td>
<td>0.64</td>
</tr>
<tr>
<td>L + tafla</td>
<td>11.2</td>
<td>88.8</td>
<td>17.1</td>
<td>4.8</td>
<td>2.68</td>
<td>9.2</td>
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<td>0.62</td>
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<tr>
<td>H + H₂O₂</td>
<td>13.9</td>
<td>86.1</td>
<td>16.4</td>
<td>4.4</td>
<td>2.91</td>
<td>8.7</td>
<td>53.69</td>
<td>2.02</td>
<td>0.66</td>
</tr>
<tr>
<td>L + H₂O₂</td>
<td>14.3</td>
<td>85.7</td>
<td>16.7</td>
<td>4.9</td>
<td>3.15</td>
<td>8.2</td>
<td>52.75</td>
<td>1.98</td>
<td>0.63</td>
</tr>
<tr>
<td>H + NH₃</td>
<td>12.2</td>
<td>87.8</td>
<td>18.7</td>
<td>4.8</td>
<td>3.01</td>
<td>8.3</td>
<td>53.99</td>
<td>2.02</td>
<td>0.72</td>
</tr>
<tr>
<td>L + NH₃</td>
<td>12.1</td>
<td>87.9</td>
<td>19.2</td>
<td>4.8</td>
<td>2.89</td>
<td>8.3</td>
<td>53.71</td>
<td>1.98</td>
<td>0.65</td>
</tr>
<tr>
<td>H + Methanol</td>
<td>15.5</td>
<td>83.5</td>
<td>14.3</td>
<td>4.6</td>
<td>2.69</td>
<td>8.5</td>
<td>53.91</td>
<td>1.99</td>
<td>0.66</td>
</tr>
<tr>
<td>L + Methanol</td>
<td>15.6</td>
<td>83.4</td>
<td>14.4</td>
<td>4.6</td>
<td>2.65</td>
<td>8.4</td>
<td>54.35</td>
<td>1.88</td>
<td>0.62</td>
</tr>
<tr>
<td>H + Microwave</td>
<td>11.4</td>
<td>88.6</td>
<td>16.4</td>
<td>4.9</td>
<td>2.66</td>
<td>9.1</td>
<td>55.54</td>
<td>1.90</td>
<td>0.60</td>
</tr>
<tr>
<td>L + Microwave</td>
<td>11.2</td>
<td>88.8</td>
<td>16.8</td>
<td>4.8</td>
<td>3.17</td>
<td>8.7</td>
<td>55.33</td>
<td>1.95</td>
<td>0.69</td>
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