Activity of Pomegranate Peels and Clove Powders in Detoxification of Aflatoxin B1 and Ochratoxin A from Contaminated Poultry Diet

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Abstract

The study was conducted to evaluate the effect of contaminated poultry diet with AfB1, OTA on chicks feds on this diet and the efficiency of addition pomegranate peels and clove powders in detoxifying the mycotoxins. Results showed that two mycotoxins caused significant reduction in chick's weight with high mortality percentage compared with control (non-contaminated diet). The chick weights were found to be 518.85, 532.90, 418.97 g/chick associated with mortality of 20, 25, 35% for chick fed on diet contaminated with AfB1, OTA, and a combination of each respectively compared with 801.63 g/chick in control. The two mycotoxins caused significant reduction in packed cell volume, hemoglobin concentration, red blood cell and protein concentration, 27.62%, 7.24 g/100 ml, 2.07 × 10^6/ml, 1.88 × 10^6/ml in chicks blood fed on diet contaminated with OTA, AfB1, and combination of each respectively compared with 38.55%, 11.56 g/100 ml, 2.98 × 10^6/ml, 4.50 g/100 ml in control respectively. The amendment of the contaminated diet with 5% pomegranate peels powder and 2% clove powder induced significant increases in chicks weight that attained to 740.30, 730.25, 680.50 g/chick, 730.25, 725.00, 675.25 g/chick of chicks fed on diet contaminated with AfB1, OTA, and combination of each respectively compared with 535.90, 518.85, 418.79 g/chick in control respectively associated with high reduction in mortality, 5% compared with 20, 25, 35% in control respectively.

Keywords: AfB1; Detoxification; Medicinal plant powders; OTA; Poultry diet

Introduction

The contamination of poultry diet with fungi producing mycotoxins is considered as one of the most important problems confronting poultry breeders worldwide [1]. Corn seeds, the main constituent of poultry diet, has been reported to be infected with many fungi producing mycotoxins in the field and in storage and considered the main source of diet contamination with mycotoxins [2,3]. Of the fungi found associated with poultry diet and producing mycotoxin, Aspergillus flavus producing aflatoxin B1, and A. ochraceus producing ochratoxin A were the more prevalent.

Both mycotoxin caused enormous problems to both chicks feds on contaminated diet and to human consumed meat of chicken previously feds on such diet [4-6]. The two mycotoxins reported to cause renal failure, hydropnephrosis and chlrosis of kidney, necrosis in liver cells, anemia and reduction in blood components in birds feds on contaminated diet [7,8]. The problem became more complicated in case of diet contaminated with more than one mycotoxin that exert synergistic effect [9-12]. A preliminary study at our department showed that poultry diet is highly contaminated with AfB1 producing by Aspergillus flavus and ochratoxin A producing by A. ochraceus that may be introduced with corn seeds, main part in the diet, previously infected in the field or contaminated and developed under storage condition [13].

The study was conducted to evaluate the effect of double contamination of poultry diet with aflatoxin B1 and ochratoxin A on chicks and the efficiency of pomegranate pericarps and clove powders in detoxification the mycotoxins from the diet.

Materials and Methods

Fungal isolates

The fungal isolates were isolated from poultry diet collected from different locations in Iraq on potato dextrose agar (PDA) in petriplates of 9 cm diameter. The growing fungi were purified and identified as describe by Klich and Pitt [14].

Test of isolates ability to produce mycotoxin

The isolates of A. flavus and ochraceus were cultivated on rice seeds for producing mycotoxin. Hundred ml distilled water were added to 150 g rice seeds in 250 ml flasks, the flasks were autoclaved at 121°C and 1.5 kg/cm² for 20 min in two successive days. Two discs, 0.5 cm diameter, from fungal colony 7 days old were added into each flask. The flasks were agitated for homogenization and incubated at 25 ± 2°C for 21 days [13]. The contaminated seeds were oven dried at 50°C in paper sacks and ground to fine powder.

Mycotoxin extraction and detection

A sample of contaminated rice seeds powder was used for mycotoxin extraction as described by Hussein et al. [15]. The extracted mycotoxin was identified by thin layer chromatography on plates of silica gel G60, 20 × 20 × 0.25 cm using standard AfB1 , OTA (sigma chemical co.) as control as described by Asonio et al. [16].

Evaluation of mycotoxin concentration

The mycotoxin concentration was estimated by High Performance Liquid Chromatography (HPCL) using mobile phase is a mixture of methanol, water, acetic acid and phosphoric acid 60:38:2:6 (v/v/v) at 0.5 ml/min flow rate, UV detector at 254 nm, with reference to standard solution of mycotoxin.

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Liquid Chromatography (HPLC) system, model LC 20/OA, shimadzu co. Kyoto, Japan, in reverse phase column C18 DB (50 × 4.6 mm) 3 mm particle size with mobile phase 0.01 N potassium phosphate solution (KH_2PO_4) PH 6.0 at flow rate 1 ml/min. The absorbance values were followed by spectrophotometer at 220 nm, and the concentration of the mycotoxin was evaluated by comparison the absorption curve obtained with mycotoxin standard curve by the following equation:

Mycotoxin concentration=area of sample curve/area of mycotoxin× standard conc. × dilution factor [17].

Plant powders

Note: Pomegranate peel and clove powders were purchased from local commercial market.

Activity of medicinal plant powders in mycotoxin detoxification:

A poultry diet composed of, 39% corn seeds, 30% wheat seeds, 20% soybean, 10% concentrated protein, 0.7 calcium, 0.3% NaCl was used in this experiment. Powders of rice seeds containing aflatoxin B1 and ochratoxin A were mixed separately and in combination with the diet to obtain final concentration 2 ppm of each mycotoxin. The diet was homogenized with water to obtain relative humidity 15%. A part of each diet was amended with 5% pomegranate peel powder and the other with 2% clove powder, chicks of 51 g each (2 days old) were left to feed on the contaminated diet and other chicks on mycotoxin free diet as control. The chicks were distributed in Complete Randomized Design (CRD) with 10 treatments and 3 replications, 6 chicks/replication as control.

Effect of feeding chicks on diet contamination with AfB1, OTA on chicks weight and mortality.

Table 1:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet contaminated with OTA</td>
<td>102.51</td>
<td>198.80</td>
<td>375.80</td>
<td>518.82</td>
<td>25</td>
</tr>
<tr>
<td>Diet contaminated with AfB1</td>
<td>104.67</td>
<td>208.57</td>
<td>389.57</td>
<td>532.90</td>
<td>20</td>
</tr>
<tr>
<td>Diet contaminated with OTA + AfB1</td>
<td>93.87</td>
<td>150.00</td>
<td>204.22</td>
<td>418.79</td>
<td>35</td>
</tr>
<tr>
<td>Non-contaminated Diet (control)</td>
<td>129.82</td>
<td>312.27</td>
<td>524.43</td>
<td>801.63</td>
<td>0</td>
</tr>
<tr>
<td>LSD=0.05</td>
<td>5.11</td>
<td>16.71</td>
<td>60.21</td>
<td>72.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Effect of feeding chicks on diet contamination with AfB1, OTA on chicks weight and mortality.
a combination of the two mycotoxin, and amended with pomegranate peels and clove powders were found to be 740.30, 730.25, 680.5 g/chick, 730.25, 725.00, 675.25 g/chick respectively, compared with 532.90, 518.83, and 418.97 g/chick in control respectively, associated with high reduction in mortality percentage, 5% for all the treatment compared with 20, 25, 35% respectively in control.

Discussion

Result of this study demonstrated that the mycotoxin AfB1, OTA exerted high reduction in chicks weight and in blood components with high mortality percentage. The reduction of chicks weight may be due to the toxic effects of the mycotoxins on different organelles in the cell leading to refuse the diet by the chicks.

The toxic effect of the mycotoxin could be attributed to the ability of the mycotoxin to form a complex with DNA that leading to inhibit DNA replication and transcription and reduce protein synthesis. Several previous studies reported that mycotoxin interact with nucleic acid in liver cells causing modification in DNA structure, disturbance in liver function and liver toxicosis [18-20]. Mcmasters and Vindani [21], reported that mycotoxin interact with nucleic acid in liver cells causing modification in DNA structure, disturbance in DNA replication and transcription and reduce protein synthesis. Other studies reported that medicinal plant extracts contain herbal constituents such as steroids, triterpens and volatile oil [24-26]. Powders and extracts of many medicinal plants were found inhibit the growth of A. flavus and aflatoxin production [27,28]. Other studies reported that some herbal extract have the potential to degrade AfB1 [8,28,29]. Velzhazhan et al. [30,31] reported the detoxification of AfB1 by seed extract of Trachyspermum ammi.

Natural medicinal plant products may provide alternative way, safer and more effective than synthetic chemical to prevent fungal growth and degrading mycotoxins produced in poultry diet.

References


### Table 2: Effect of feeding chicks on diet contaminated with AfB1, OTA on blood components.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% PVC</th>
<th>Hb concentration</th>
<th>Red blood cells number (RBC) 10^6/ml</th>
<th>white blood cells number (WBC) 10^6/ml</th>
<th>Total protein g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet contaminated with OTA</td>
<td>27.62</td>
<td>8.24</td>
<td>2.07</td>
<td>30.11</td>
<td>3.25</td>
</tr>
<tr>
<td>Diet contaminated with AfB1</td>
<td>27.25</td>
<td>8.77</td>
<td>2.19</td>
<td>28.98</td>
<td>3.74</td>
</tr>
<tr>
<td>Diet contaminated with OTA+AfB1</td>
<td>24.07</td>
<td>7.22</td>
<td>1.88</td>
<td>35.97</td>
<td>3.10</td>
</tr>
<tr>
<td>Non-contaminated Diet (control)</td>
<td>38.55</td>
<td>11.56</td>
<td>2.98</td>
<td>23.13</td>
<td>4.50</td>
</tr>
<tr>
<td>LSD=0.05</td>
<td>2.43</td>
<td>0.62</td>
<td>0.57</td>
<td>3.11</td>
<td>0.89</td>
</tr>
</tbody>
</table>

### Table 3: Effect of amendment of poultry diet with clove and pomegranate powder on chicks weight and mortality.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chick/weight/g</th>
<th>Mortality percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>532.90</td>
<td>20</td>
</tr>
<tr>
<td>T2</td>
<td>740.30</td>
<td>5</td>
</tr>
<tr>
<td>T3</td>
<td>730.25</td>
<td>5</td>
</tr>
<tr>
<td>T4</td>
<td>518.83</td>
<td>25</td>
</tr>
<tr>
<td>T5</td>
<td>730.25</td>
<td>5</td>
</tr>
<tr>
<td>T6</td>
<td>725.00</td>
<td>5</td>
</tr>
<tr>
<td>T7</td>
<td>418.97</td>
<td>35</td>
</tr>
<tr>
<td>T8</td>
<td>680.50</td>
<td>6</td>
</tr>
<tr>
<td>T9</td>
<td>675.25</td>
<td>5</td>
</tr>
<tr>
<td>T10</td>
<td>822.40</td>
<td>0</td>
</tr>
<tr>
<td>LSD=0.05</td>
<td>45.61</td>
<td></td>
</tr>
</tbody>
</table>
in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Br Poult Sci 41: 640-650.


