INEFFECTIVENESS OF DIFFERENT ADSORBENTS IN ALLEVIATION OF ORAL LESIONS INDUCED BY FEEDING T-2 TOXIN IN BROILER CHICKENS

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ABSTRACT

One hundred and forty one-day-old-male broiler chicks (Ross), were divided into 7 groups (20 chicks/group) and fed T-2 toxin alone or with five different types of adsorbents; Montmorillonite 0.5%, Vermiculite 0.5%, Pigacine 0.5%, Gezilgure 0.5%; and the resin Perlite 0.5%. Gross and microscopic examinations reveal that all adsorbents used were unable to alleviate the oral lesions induced by T-2 toxin in broiler chickens.

INTRODUCTION

Trichotheccene mycotoxins are produced by Fusarium and its perithecial stages (1). T-2 toxin, one member of group A trichotheccenes, was first isolated from fusarium tricictum strain T-2 by Bamburg (2) and Bamburg et al., (3). Feeding T-2 toxin to broilers cause feed refusal, impaired growth and whole body pathology including caustic injury to skin and alimentary mucosa; radiomimetic injury to bone marrow, lymphoid tissues, gastrointestinal tract, and hepatitis; and thyroid alteration(4). T-2 toxin causes erosive and exudative injury to the oral mucosa of poultry fed toxin-appended diets (5, 6, and 7)). These oral lesions were characterized by the presence of focal, yellow oral plaques that progress to yellow gray, raised accumulations of exudates with underlying ulcers located near major
salivary duct openings on the palate, tongue, and buccal floor. Thick crusts accumulate along the interior margin of the beak. Oral histology confirms mucosal necrosis and ulceration, sub mucosal granulation tissue and inflammatory cells, crusts of exudates, bacterial colonies, and feed components. (5; 8; 9; 10; 11; 12; 13; 14). The most applied method for protecting against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastrointestinal tract, (15). Most studies related to the alleviation of mycotoxicosis by the use of adsorbents are focused on aluminosilicates, mainly zeolites and Hydrated sodium calcium aluminosilicates(HSCAS), and aluminumonsilicate-containing clays. All consisting of aluminates, silicates and some interchangeable ions, mainly alkali metal and alkaline earth metal ions (16). Clay minerals are primarily layered silicates with the common chemical formula\[\text{SiO}_2\text{O}_5\]xy, e.g. Bentonit, vermiculite, Kaolin, Pigacinc, Gezilgure. HSCAS contain calcium ions and protons which are exchanged against the naturally occurring sodium ions. They are a type of montmorillonite belonging to phyllosilicates which are composed of layers of aluminum and silicon connected in a 1:1 or 2:1 arrangements. Polymers, an ion exchange mycotoxin adsorbent resins were also tried to be used in vitro studies for adsorption of certain mycotoxins (15,17). As a general aluminosilicates are the preferred adsorbents, followed by activated charcoal and special polymers(16). The aim of the present study was to evaluate different adsorbents; Montmorillonite (HSCAS); new aluminosilicite-containing clays (Vermiculite, Pigacinc, Gezilgure); and new polymer resin (Perlite), in an attempt to alleviate necrotic oral lesions induced by feeding T-2 toxin to broiler chickens.

**MATERIALS AND METHODS**

A total of 140, 1-d-old male meat-type chickens of the Ross line were used. The experimental design consisted of a complete randomized trial with one negative control group, one positive control group, and four feed additives: montmorillonite(HSCAS); Aluminumsilicate-containing clays Vermiculite, pigacinc, gezilgure; and polymer resin, perlite. The experimental treatments consisted of the same commercial mash, based on corn and soybean meal, according to the recommendations of the NRC (1994) (18), with T-2 toxin alone or with different additives as follows:

- **Group 1**: no T-2 toxin or feed additive (negative control).
- **Group 2**: 8 ppm T-2 toxin (positive control).
- **Group 3**: 8 ppm T-2 toxin + 0.5% montmorillonite.
- **Group 4**: 8 ppm T-2 toxin + 0.5% Vermiculite.
- **Group 5**: 8 ppm T-2 toxin + 0.5% pigacinc.
- **Group 6**: 8 ppm T-2 toxin + 0.5% gezilgure.
- **Group 7**: 8 ppm T-2 toxin + 0.5% perlite.

The experimental ration was checked to contain no detectable levels of aflatoxins, ochratoxins, zearalenone, and T-2 toxin by the method reported by Coker et al (1984) (19). Purified crystalline T-2 toxin was produced by culturing *F. tricinctum* NRRL 3299 according to the method reported by Burmeister et al. (20). The crystalline toxin was dissolved in acetone, added to experimental diets, and mixed to homogeneity by means of a twin- shell blender. Homogeneity was tested by analyzing the presence of T-2 toxin in each diet by thin-layer chromatography. The chemical composition of Aluminum silicate feed additives
Chickens were kept in batteries in a thermo regulated room with continuous light; feed and water were provided ad libitum. On 21 day of age heads were removed from all chicks and visually scored for oral lesions using a four-point scoring system ranging from 1 to 4 (5) by the same individual without knowledge the treatment groups. A lesion score of 1 indicated no visible lesions; a score of 2 was seen as one or two mouth lesions clearly visible on either the lower or upper mandible; a score of 3 was seen as more than one or two mouth lesions clearly visible on either the lower or upper mandible and back of the tongue; a lesion score of 4 was seen as large lesions occurring at several sites within the mouth, principally on the upper and lower mandibles, the corners of the mouth, and the back of the tongue. Histopathological study of oral lesions was performed by fixing affected portions in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin stain. Tissue samples from treatments show oral lesions were examined microscopically. Data (pen means) for all response variables in each treatment were subjected on ANOVA using the SAS software (SAS 2000) general Linear Models procedure for factorial ANOVA. Treatment means were ranked by Duncan’s multiple range test (SAS 2000). All statements of significance are based on the 0.05 level of probability (21).

RESULTS

Oral lesion scores: The oral lesion scores in all treatment groups fed purified T-2 toxin with five different aluminum silicate feed additives show no significant difference in developing oral lesions when compared with group of chicks fed T-2 toxin alone, but all were significantly (p<0.05) differ from control group fed no toxin or additives(Table 1).

Table 1: Effect of T-2 toxin alone and T-2 toxin with feed additives on broiler oral lesions at 21days of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed additive (%)</th>
<th>T-2 toxin (ppm)</th>
<th>Oral lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00</td>
<td>0</td>
<td>0.0 b*</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.00</td>
<td>8</td>
<td>2.30 ±0.14a</td>
</tr>
<tr>
<td>T-2 toxin + montmorillonite</td>
<td>0.5</td>
<td>8</td>
<td>2.20±0.14 a</td>
</tr>
<tr>
<td>T-2 toxin + Vermicullite</td>
<td>0.5</td>
<td>8</td>
<td>2.10±0.11 a</td>
</tr>
<tr>
<td>T-2 toxin + pigacine</td>
<td>0.5</td>
<td>8</td>
<td>2.00±0.14 a</td>
</tr>
<tr>
<td>T-2 toxin + gezilgure</td>
<td>0.5</td>
<td>8</td>
<td>2.30±0.16 a</td>
</tr>
<tr>
<td>T-2 toxin + perlite</td>
<td>0.5</td>
<td>8</td>
<td>2.00±0.12 a</td>
</tr>
</tbody>
</table>

*Values within column with no common subscripts differ significantly (p<0.05)

1 Values = X±SEM of two groups of 10 broilers.

2 Lesions were scored on a scale from 0-4, with a value of 4, representing the more severe lesions.

Gross lesions: Oral lesions were reported in all groups fed T-2 toxin alone or with different feed additives and were characteristic of T-2 toxicosis which first appeared when the chicks were about 1 week old, as raised eaceous yellow-white plaques. By 2-weeks, the lesions increased in size and invaded the lingual papillae at the root of the tongue, margin of the beak, mucosa of the hard palate.
and angle of the mouth. By three weeks, the size of the lesions increased to more extent that of the 2nd week. These lesions were characterized by raised accumulation of exudates with underlying ulcers located near major duct opening of the palate, tongue, and buccal floor. (Figure 1, 2, 3, 4, 5, 6)

Figure 1: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption T-2 toxin (8 ppm), characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.

Figure 2: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption 8 ppm T-2 toxin and 0.5% montmorillonite, characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.

Figure 3: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption 8 ppm T-2 toxin and 0.5% vermicullkite, characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.
Figure 4: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption 8 ppm T-2 toxin and 0.5% pegacine, characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.

Figure 5: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption 8 ppm T-2 toxin and 0.5% gezilgure, characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.

Figure 6: Beak and palate ulceration and crusting in broiler chickens following 21 days consumption 8 ppm T-2 toxin and 0.5% perlite, characterized by raised accumulations of exudates with underlying ulcers located near major salivary duct openings on the palate.
Microscopical findings: Histopathological results of 21-day-old experimentally fed broiler chickens 8 ppm T-2 toxin showed intense inflammation and localized necrosis in the oral cavity. The outer layer of the lesion consisted of sloughing fibrous material with bacterial colonies, while the underlying tissue was heavily infiltrated with granular leukocytes. Hyperplasia of the main salivary glands in the oral cavity was also evident (Figure 6).

Montmorillonite, hydrated sodium calcium aluminosilicate (HSCAS), when added at a rate of 0.5% to T-2 toxin contaminated diet was ineffective in counteracting adverse effect of T-2 toxin inducing oral lesion. Lesions were characterized by sloughing upper fibrous material which was heavily contaminated with bacterial aggregations, while underlying tissue was infiltrated with granular leukocytes (Figure 7).
Addition of 0.5% vermiculite, one of the clay mineral feed additive when added to T-2 toxin contaminated diet, didn’t restore the normal oral mucous membrane structure. Erosive and exudative injury still present in the broilers buccal cavity. Multiple focal necroses, interrupting the continuity of mucous membrane were noticed. fibrinosuppurative inflammation and bacterial colonies were also found in birds of this group. The underlying tissue show inflammatory response mainly of heterophils. Hyperplasia of the epithelial layer, were noted. Hypertrophy and hyperplasia of oral salivary glands were recorded (Figure 8).

Pigacin, like other mineral clays used, when added to T-2 toxin contaminated diet, did not alleviate oral lesions induced by T-2 toxin. Microscopically, lesions show hypertrophy and hyperplasia of the epithelial cells.
Increase in the thickening of the mucous membrane was noticed as a result of an increase in the amount of exudates, granulocytes and subcutaneous edema (Figure 9).

Figure 9: Photomicrograph of the mucous membrane of the buccal cavity 21-day-old broiler fed purified 8 ppm T-2 toxin with 0.5% Pigacine,(A) showing fibrinous exudates, clumps of bacteria, granulocytosis and subcutaneous edema. HY&E. X36. (B). Higher magnification of (A) H&E.X 110. (C) Higher magnification of (B), showing clumps of bacteria. H&E.X 700

The addition of 0.5% Gezilgure, the third mineral clay used, to T-2 toxin contaminated diet, was also failed to relief the degenerative effect in broiler buccal mucosa induced by the toxin. Oral histopathology confirms mucosal necrosis which varied in shape and size and had random distribution. The lesions were consisted predominantly of lower hyperplastic epithelium, layer of inflammatory cells, crusts of exudates, bacterial colonies and feed component (Figure 10).
Perlite, the polymer resin used in this experiment addition at a rate of 0.5% to T-2 toxin contaminated diet, was also ineffective in ameliorating oral lesions induced by feeding 8 ppm T-2 toxin. Oral lesions in birds buccal cavity of this group was characterized by focal fibrinoid necrosis, with degeneration of the epithelial cells. Microscopically there was a focal fibrinoid necrosis, with degeneration and sloughing off of the epithelial cells, edema and granulocytosis with bacterial colonies aggregations (Figure 11).

**DISCUSSION**

Results of the current study indicate that broiler chicks fed diet contaminated with 8 ppm T-2 toxin develop oral lesions. Levels of this toxin reported to induce oral lesion in broilers are: 0.4 (8); 1 ppm (13,14); 4ppm(22); 5
Gross and microscopic oral lesions induced here were resemble those reported early by Wyatt et al. (12,28) in broilers fed diets containing 1ppm T-2 toxin and more. These oral lesions were also reported in broilers fed purified T-2 toxin (produced and extracted from *F. tricinctum*) at a rate of 4-16 ppm for 3 weeks, and some microorganisms were isolated from these lesions (10). The erosive and exudative injury to the broiler oral mucosa, and the increase in the bacterial clumps in the sloughed outer fibrous layers appear to bear some resemblance to those described for Alimentary Toxic Aleukia in humans (12; 29). In humans, necrotic lesions were reported in gums, buccal mucosa, throat, larynx and vocal cords, contaminated with a variety of avirulent bacteria. The necrotic areas are an excellent medium for bacterial infection due to lowered resistance caused by damage to the haemopoietic and reticuloendothelial systems (30). These bacterial infections induced by T-2 causes an unpleasant odor from the mouth due to the enzymatic activity of the bacteria on proteins. In the results here, it is interesting to note that in all groups fed T-2 toxin amended with all used adsorbents, there was an increasing tendency to bacterial colonies aggregations in the necrotized, sloughed superficial layer of oral lesions. The exacerbating effect of these silica containing adsorbents (montmorillonite, 33.00%; Vermiculite, 35.12%; pigacine, 61.09%; gezilgure, 88.3%; perlite, 73.35%) in predisposing bacterial colonization may be due to the specific silica-macrophage cytotoxic interaction (31). This condition was also confirmed and reported when broiler chickens fed siliceous Ninivite (6). The increase in bacterial infection could be attributed to T-2 toxin itself in leucopenia and anemia induction (6, 7). The dramatic consequences of T-2 toxin feeding to broiler chicks could be attributed to large extent to its caustic property. The mucosal necrosis caused by Y-2 toxin is very painful to the affected birds, thus feed consumption decreases. Subsequently weight gain and feed conversion are reduced (7).

The most important feature of the adsorption is the physical structure of the adsorbent, i.e. the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbate molecules, the mycotoxins, like polarity, solubility, size, shape and – in case of ionized compounds- charge distribution and dissociation constants play a significant role, too. Therefore, the efficacy of every adsorption process has to be investigated in regard to the particular properties of the adsorbate (16). The applicability of aluminosilicates for the adsorption of mycotoxins has been studied for more than 20 years (32). All these studies refer to inability of aluminosilicates to counteract the negative effect of T-2 toxin and other trichotheccenes (16). Of these studies which confirm the present results those used hydrated calcium sodium aluminosilicate (HSCAS) at a rate of 0.25%; 0.37%; 0.5%; and 0.8%, and showed that there was no effect against toxicity induced by 8 ppm T-2 toxin in broiler chickens (25, 26). No references are available in using clay minerals or resin polymers for counteracting T-2 toxicosis in broilers. So, it is the first time that these clay minerals and resin polymers were tested to elucidate their effectiveness in alleviating oral lesions induced by feeding purified T-2 toxin to broiler chickens. T-2 toxin has a toxic specific group on position (12, 13) responsible for the molecules negative impact on performance and health of animals. Enzymes (de-epoxydases and esterase) are at the present the only way to detoxify T-2 toxin and reduce its toxicity by converting the toxin to nontoxic product. These enzymes are contained in Mycofix® plus 3.0, and are
able to biotransformation T-2 toxin, resulting in the non toxic metabolite, de-epoxy –HT-2 toxin. This product gives a promising solution to the problem of unadsorbable trichothecenes mycotoxins in broiler feed. (7).

REFFERENCE


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