Effects of Feedborne Fusarium Mycotoxins on Brain Regional Neurochemistry of Turkeys

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ABSTRACT An experiment was conducted to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on brain regional neurochemistry of turkeys. The possible preventative effect of a polymeric glucomannan mycotoxin adsorbent (GMA) was also determined. Forty-five 1-d-old male turkey poults were fed wheat-, corn-, and soybean meal-based diets up to wk 6, formulated with control grains, contaminated grains, or contaminated grains + 0.2% GMA. Deoxynivalenol was the major contaminant, and the concentrations were 2.2 and 3.3 mg/kg of feed during starter and grower phases, respectively. Concentrations of brain monoamine neurotransmitters and metabolites were measured in discrete regions of the brain including the pons, hypothalamus, and cortex by HPLC with electrochemical detection. Neurotransmitters and metabolites analyzed included norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). The concentration of 5-HIAA and the 5-HIAA:5-HT-ratio were significantly decreased in pons after feeding contaminated grains. Dietary supplementation with GMA prevented these effects. In the pons, a significant positive correlation (r = 0.52, P < 0.05) was observed between the concentration of 5-HT and BW gain after feeding contaminated diets. The feeding of contaminated diet had no significant effects on the concentrations of neurotransmitters and metabolites in hypothalamus and cortex. It was concluded that consumption of grains naturally contaminated with Fusarium mycotoxins adversely altered the pons serotoninergic system of turkeys. Supplementation with GMA partially inhibited these effects.

Key words: Fusarium mycotoxin, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, turkey

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INTRODUCTION

Trichothecenes, zearalenone (ZEN), and fumonisins are the major Fusarium mycotoxins present in cereal grains, animal feeds, and forages. Moniliformin and fusaric acid (FA) are other important Fusarium mycotoxins (Smith et al., 2005). The toxic effects of Fusarium mycotoxins in animals and poultry include reduced growth, feed refusal and vomiting, immunosuppression, gastrointestinal lesions, and neurological and reproductive disorders (Rocha et al., 2005).

It has been suggested that differences in alterations of brain neurotransmitters and metabolites in poultry and swine might be one of the possible mechanisms for species differences in the severity of Fusarium mycotoxin-induced feed refusal (Swamy et al., 2004b). Differences between species of poultry have also been observed in alterations of brain monoamines after feeding Fusarium mycotoxins (Yegani et al., 2006a). Deoxynivalenol (DON), a trichothecene mycotoxin, causes suppression of feed intake in susceptible species. The neurochemical changes seen in acute DON exposure might cause peripheral signs of toxicity, such as vomiting, rather than feed refusal (Prelusky et al., 1992). Wang et al. (1998) reported that the brain is the main site of trichothecene action, evidenced by alterations in brain monoamine profiles. Increased brain concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT), a transient increase in norepinephrine (NE) in the nucleus raphe magnus, and a transient decrease in NE concentrations in the substantia nigra were observed in rats orally dosed with 0.1 to 2.5 mg of T-2 toxin/kg of BW (Wang et al., 1998). Dihydroxyphenylacetic acid (DOPAC) concentrations were also elevated in different regions of the hypothalamus and olfactory tubercles, but no regional changes were observed in concentrations of epinephrine or dopamine (DA; Wang et al., 1998). Smith and MacDonald (1991) observed vomiting and alterations in brain neurotransmitters in swine after an oral dose of 200 mg of FA/kg of BW. Brain concentrations of crypto-
phian, 5-HT, and 5-HIAA were elevated, but the brain catecholamine concentrations were refractory to FA treatment. These alterations in 5-HT metabolism have been linked to feed refusal (Leathwood, 1987). Inhibition of protein synthesis by trichothecenes, resulting in hyperaminoacidemia, has been related to elevated levels of brain tryptophan and hence elevation of other neurotransmitters (Meloche and Smith, 1995). Serotonin is synthesized from tryptophan, and increased concentrations of tryptophan in the blood can result in tryptophan crossing the blood-brain barrier and increasing concentrations of tryptophan in the brain. Increased concentrations of 5-HIAA and a decrease in regional NE and DA concentrations were observed after 24 h of dosing broilers with 2.5 mg of T-2 toxin/kg of BW (Boyd et al., 1988). Yegani et al. (2006a) reported that the feeding of naturally contaminated diets to laying hens, turkeys, and broiler breeder hens resulted in significantly different patterns of alterations in brain neurotransmitter concentrations. These changes, moreover, predicted the inraspecies differences in the severity of *Fusarium* mycotoxin-induced reductions in feed intake. The feeding of naturally contaminated diets to laying hens increased the concentrations of 5-HT and decreased the 5-HIAA:5-HT ratio in the pons region of the brain. A trend to similar disturbances in the broiler breeder hens was observed. There was no significant effect of diet, however, on brain neurochemistry of turkeys bred for meat. A trend to similar disturbances in the broiler breeder hens was observed after 24 h of dosing broilers with 2.5 mg of T-2 toxin/kg of BW. 

### Experimental Design

A total of 45 one-day-old male Hybrid turkey poult (Hybrid Turkeys, Kitchener, Ontario, Canada) were individually weighed, wing-banded, and distributed randomly into groups of 3 poult per floor pen at the Arkell Poultry Research Station of the University of Guelph. Five pens were randomly assigned to each of the 3 diets with each diet fed to 15 poult. Poult were initially maintained at 32°C, and the temperature was gradually reduced by 3°C per week to reach a temperature of 21°C by the end of wk 4. This temperature was maintained for the duration of the experiment. Turkey poult were fed wheat, corn and soybean meal-based starter (0 to 3 wk) and grower (4 to 6 wk) diets formulated with control grains, contaminated grains, or contaminated grains + 0.2% GMA (Mycosorb, Alltech Inc., Nicholasville, KY). The contaminated grains were obtained from farmers in southern Ontario, Canada, and were naturally contaminated with mycotoxins preharvest. The control diet was formulated to meet or exceed the minimum nutrient requirements of turkeys according to the NRC (1994). Mycotoxin-contaminated and GMA-supplemented diets were prepared, and the experimental diets were analyzed for chemical composition (AOAC, 1980) and mycotoxin concentrations. Feed and water were provided ad libitum. The diet formulations and nutrient contents are presented in Table 1. The experimental procedures were approved by the University of Guelph Animal Care Committee following the guidelines of the Canadian Council on Animal Care. This study on effects of feedborne *Fusarium* mycotoxins on brain serotonergic neurochemistry was a part of a larger study of *Fusarium* mycotoxicoses in turkeys (Girish et al., 2008).

### Analysis of Dietary Mycotoxin Concentrations

Dietary mycotoxin concentrations were analyzed at the Veterinary Diagnost Laboratory, North Dakota State University, Fargo, using HPLC and a combination of gas chromatography and mass spectrometry (Leung et al., 2007). Fusaric acid was estimated by the HPLC method of Matsui and Watanabe (1988) as modified by Smith and Sousadias (1993) and confirmed by Porter et al. (1995).

### Experimental Parameters Measured

#### Analysis of Brain Regional Monoamine Concentrations

Turkey poult were killed after 6 wk of feeding, and brain sections including hypothalamus, pons, and cortex were dissected (Glowinski and Iversen, 1966) and immediately frozen in liquid N and stored at −80°C until further analysis for neurotransmitters. Brain sections were analyzed for DA, DOPAC, 5-HT, 5-HIAA, and NE using HPLC with electrochemical detection. The method was adapted from Food and Drug Administration (2001)
Table 1. Composition of experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter diet</th>
<th>Contaminated + GMA</th>
<th>Contaminated</th>
<th>Starter diet</th>
<th>Contaminated + GMA</th>
<th>Contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Contaminated</td>
<td></td>
<td>Control</td>
<td>Contaminated</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>10.20</td>
<td>0.20</td>
<td></td>
<td>17.22</td>
<td>7.22</td>
<td>7.02</td>
</tr>
<tr>
<td>Wheat</td>
<td>36.50</td>
<td>1.50</td>
<td>1.50</td>
<td>35.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Contaminated corn</td>
<td>10.00</td>
<td>10.00</td>
<td></td>
<td>34.80</td>
<td>34.80</td>
<td></td>
</tr>
<tr>
<td>Contaminated wheat</td>
<td>35.00</td>
<td>35.00</td>
<td></td>
<td>6.00</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>40.20</td>
<td>40.20</td>
<td>40.20</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>5.80</td>
<td>5.80</td>
<td>5.80</td>
<td>1.40</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>Animal and vegetable fat</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.40</td>
<td>2.40</td>
<td>2.40</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.60</td>
<td>1.60</td>
<td>1.60</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin and mineral mixture¹</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Enzymes</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>GMA²</td>
<td></td>
<td></td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculated values

- ME, kcal/kg: 2,800 vs. 2,900
- Crude protein: 28.00 vs. 26.00
- Lysine: 1.60 vs. 1.50
- Methionine: 0.56 vs. 0.49
- Calcium: 1.20 vs. 1.00
- Available phosphorus: 0.60 vs. 0.50

Analyzed values

- Crude protein: 27.76 vs. 29.00 vs. 29.11 vs. 26.13 vs. 26.64 vs. 28.02
- DM: 87.55 vs. 87.05 vs. 87.33 vs. 88.35 vs. 88.03 vs. 87.95
- Ash: 6.8 vs. 7.6 vs. 7.08 vs. 6.33 vs. 6.5

¹Vitamin-mineral mixture provided the following per kilogram of diet: vitamin A (all-trans-retinyl palmitate), 8,800 IU; cholecalciferol, 3,300 IU; vitamin E (all-rac-α-tocopheryl acetate), 40 IU; menadione, 3.3 mg; thiamin, 4.0 mg; riboflavin, 8.0 mg; pantothenic acid, 15.0 mg; niacin, 50 mg; pyridoxine, 3.3 mg; choline, 600 mg; folic acid, 1.0 mg; biotin, 220 μg; vitamin B12, 12 μg; ethoxyquin, 120 mg; manganese, 70 mg; zinc, 70 mg; iron, 60 mg; copper, 10 mg; iodine, 1.0 mg; and selenium, 0.3 mg.

²Polymeric glucomannan mycotoxin adsorbent.

Guidelines. Preparation and analyses of samples for brain neurotransmitters were according to Yegani et al. (2006a).

Statistical Analyses

Data were analyzed by ANOVA using the PROC GLM procedure of SAS as a completely randomized block design (Kuehl, 2000; SAS Institute, 2000). Multiple comparisons were made using Dunnett’s test to determine the nature of the response to control and contaminated diets. The correlation between the brain neurotransmitters and metabolite concentrations and BW gains was determined in the birds fed contaminated diets. Statements of statistical significance were based on $P \leq 0.05$.

RESULTS

Dietary Mycotoxin Concentrations

To maintain similar concentrations of mycotoxins in the starter and grower phases of the experiment, 10 and 35% of control corn and wheat were replaced with contaminated corn and wheat, respectively, in both starter and grower diets (Table 1). The levels of replacement of control grains with the contaminated grains were the same in all growth phases to achieve as close as possible a constant mycotoxin challenge. Dietary concentrations of DON, 15-acetyl-DON, ZEN, and FA are given in Table 2. Other mycotoxins were in concentrations below the detection limits, which were 0.02 mg/kg for aflatoxin, 2 mg/kg for fumonisin, 0.77 mg/kg for FA, and 0.2 mg/kg for the remaining mycotoxins analyzed. The mycotoxin screening technique used in the present study, however, will not screen for all possible mycotoxins that could be present in naturally contaminated feeds.

Brain Regional Neurotransmitter Concentrations

The feeding of contaminated grains significantly decreased 5-HIAA concentrations and the 5-HIAA:5-HT concentration ratio in the pons region (Table 3). Dietary supplementation with GMA prevented these effects. There was a significant positive correlation ($r = 0.52, P < 0.05$) between the concentration of 5-HT in the pons and BW gain over wk 6 in the groups fed a contaminated diet (Figure 1). There were no significant correlations, however, between the concentrations of 5-HT and BW gains in the hypothalamus and cortex (data not shown). Dietary supplementation with GMA in contaminated diets reduced the concentration of DOPAC in hypothalamus compared with controls.

DISCUSSION

Dietary Mycotoxin Concentrations

Experimental diets were formulated to achieve similar concentrations of mycotoxins in starter and grower
Table 2. Mycotoxin concentrations (μg/g) in experimental diets

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>DON1</th>
<th>15-acetyl-DON</th>
<th>ZEN2</th>
<th>FA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter (0 to 3 wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>ND5</td>
<td>ND</td>
<td>13.1</td>
</tr>
<tr>
<td>Contaminated diet</td>
<td>2.2</td>
<td>0.2</td>
<td>0.2</td>
<td>12.4</td>
</tr>
<tr>
<td>Contaminated diet + GMA6</td>
<td>2.8</td>
<td>0.2</td>
<td>0.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Grower (4 to 6 wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
<td>7.9</td>
</tr>
<tr>
<td>Contaminated diet</td>
<td>3.3</td>
<td>0.2</td>
<td>ND</td>
<td>18.8</td>
</tr>
<tr>
<td>Contaminated diet + GMA</td>
<td>2.7</td>
<td>0.2</td>
<td>ND</td>
<td>8.7</td>
</tr>
</tbody>
</table>

1Other mycotoxins, including T-2 tetraol, fusarenone-X, diacetoxyscirpenol, T-2 triol, T-2 toxin, scirpentriol, nivalenol, 15-acetoxyscirpenol, neosolaniol, HT-2 toxin, acetyl T-2 toxin, zearalenol, aflatoxin, 3-acetyl-DON, and fumonisin, were also measured in the experimental diets but were not detected.

2Deoxynivalenol.

3Zearalenone.

4Fusaric acid.

5Not detected.

6Polymeric glucomannan mycotoxin adsorbent.

phases by incorporating the same percentages and sources of contaminated corn and wheat. The major contaminant in all contaminated diets was DON, and its concentration was similar in both phases (Table 2). It was found that 15-acetyl-DON and ZEN were present as minor contaminants. The concentration of ZEN was below the detection limit in grower diets. Toxicological synergism between DON and ZEN has not been observed in swine (Cote et al., 1985) or mice (Forsell et al., 1986). The cytotoxicity of 15-acetyl-DON is similar to that of DON (Sundstol Eriksen et al., 2004), and the toxicity of 15-acetyl-DON in the present study may be additive to that of DON. This co-contamination of mycotoxins is more indicative of commercial situations than feeding of purified and semipurified diets. It has been reported that the same level of inclusion of contaminated grains resulted in 1.9 mg of DON/kg of feed in one experiment and 4.4 mg of DON/kg of feed in another experiment (Smith et al., 1997). Failure to achieve the same concentrations of DON in the present experimental diets may be attributable to a lack of uniformity in distribution of mycotoxins in contaminated corn and wheat (Hamilton, 1978) and the limitations of sampling (Davis et al., 1980). It has also recently been shown that some of the Fusarium mycotoxins including DON and ZEN form conjugates with glucose, thereby escaping routine analytical detection procedures (Schneweis et al., 2002; Berthiller et al., 2005). This situation might have contributed to an underestimation of the total amount of Fusarium mycotoxins in the present study.

Deoxynivalenol concentrations of 0.5 to 1.0 mg/kg of feed (starter and grower) were detected in the control diets, thereby indicating that control corn and wheat contained, nevertheless, detectable amounts of mycotoxins.

There is no evidence for DON toxicity in turkeys at the concentrations detected in the control diets used in the present study.

It is possible that FA may act synergistically with trichothecene mycotoxins to increase the toxicity of contaminated feedstuffs. Recent studies with purified T-2 toxin and FA, however, failed to demonstrate such synergism.

Table 3. Effects of Fusarium mycotoxins on brain neurotransmitter concentrations of turkeys

<table>
<thead>
<tr>
<th>Neurotransmitter concentrations2</th>
<th>NE</th>
<th>DA</th>
<th>DOPAC</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>DOPAC:DA</th>
<th>5-HIAA:5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.78</td>
<td>0.50</td>
<td>0.14</td>
<td>3.31</td>
<td>1.17</td>
<td>0.27</td>
<td>0.36</td>
</tr>
<tr>
<td>Contaminated</td>
<td>3.81</td>
<td>0.45</td>
<td>0.11</td>
<td>3.64</td>
<td>0.87</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Contaminated + GMA3</td>
<td>4.13</td>
<td>0.49</td>
<td>0.11</td>
<td>3.87</td>
<td>1.14</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>SEM</td>
<td>0.17</td>
<td>0.03</td>
<td>0.01</td>
<td>0.17</td>
<td>0.07</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Control vs. contaminated NS4 NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.009</td>
<td>NS</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.05</td>
<td>1.22</td>
<td>0.20</td>
<td>4.95</td>
<td>0.94</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Contaminated</td>
<td>5.39</td>
<td>1.11</td>
<td>0.18</td>
<td>5.13</td>
<td>0.82</td>
<td>0.17</td>
<td>0.16</td>
</tr>
<tr>
<td>Contaminated + GMA</td>
<td>5.00</td>
<td>1.05</td>
<td>0.13</td>
<td>4.92</td>
<td>0.82</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>SEM</td>
<td>0.26</td>
<td>0.07</td>
<td>0.02</td>
<td>0.15</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Control vs. contaminated NS NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.28</td>
<td>0.29</td>
<td>ND5</td>
<td>1.71</td>
<td>0.35</td>
<td>ND</td>
<td>0.22</td>
</tr>
<tr>
<td>Contaminated</td>
<td>1.52</td>
<td>0.33</td>
<td>ND</td>
<td>1.80</td>
<td>0.28</td>
<td>ND</td>
<td>0.16</td>
</tr>
<tr>
<td>Contaminated + GMA</td>
<td>1.29</td>
<td>0.29</td>
<td>ND</td>
<td>1.80</td>
<td>0.32</td>
<td>ND</td>
<td>0.18</td>
</tr>
<tr>
<td>SEM</td>
<td>0.08</td>
<td>0.04</td>
<td>0.15</td>
<td>0.02</td>
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<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Control vs. contaminated NS NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Values are the least squares means; for each diet, n = 5 pens and 3 birds per pen.

2nmol/g of wet tissue. NE = norepinephrine; DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; 5-HT = 5-hydroxytryptamine, 5-HIAA = 5-hydroxyindoleacetic acid.

3Polymeric glucomannan mycotoxin adsorbent.

4P > 0.05.

5Not detected.
in broilers and turkeys (Ogunbo et al., 2007). Analyses of North American feeds and feedstuffs for Fusarium mycotoxins have shown DON and FA as frequent contaminants, whereas ZEN is a less common problem (Smith and Sousadias, 1993). It has been shown that acute doses of FA caused vomiting and lethargy in swine (Smith and MacDonald, 1991). Fusaric acid was a common contaminant in the experimental diets (Table 2), and the concentrations were slightly higher or similar in the control diet (13.1 mg/kg) compared with contaminated diets (10.2 to 12.4 mg/kg) during the starter phase. The concentration of FA in the control diet, however, was lower compared with contaminated diets in the grower phase. The concentration of FA in the contaminated diet was similar to that of the control diet during the starter phase, and it is possible, therefore, that contaminated corn and wheat might not have contributed to FA concentrations. Higher or equivalent concentrations of FA in broiler diets including the control diet, the low level of contaminated grains, and the high level of contaminated grains have been previously reported (Swamy et al., 2002a). Swamy et al. (2002a) found FA concentrations of 18 mg/kg of feed in the control diet, 20.6 mg/kg of feed in the low level of contaminated grains, and 20.3 mg/kg of feed in the high level of contaminated grains. The concentration of FA was slightly lower in the high level of contaminated grains (20.3 mg/kg) compared with the low level of contaminated grains (20.6 mg/kg). This indicates that control grains including corn and wheat grown in Ontario contained fairly high levels of FA. The concentration of FA in swine feeds has been reported to range from 11.61 to 35.76 mg/kg of feed (Smith and Sousadias, 1993). It could be hypothesized that occurrence of FA in naturally contaminated diets is not unusual and may result in synergistic toxic effects when present with other Fusarium mycotoxins.

**Brain Regional Neurotransmitter Concentrations**

It has been established that the serotonergic raphe nuclei are involved in appetite and feeding. These pontine nuclei innervate most regions of the brain. Thus, any disturbances in 5-HT neurotransmission in this region could reduce feed intake (Cooper et al., 2003).

The location of the pons region (associated with vomiting) outside of the blood-brain barrier (Miller and Leslie, 1994) could account for the higher sensitivity of pontine serotonergic neurons to toxin-induced changes. This might contribute to disturbances in serotonergic neurotransmitter and metabolite concentrations. Larger alterations in neurotransmitter and metabolite concentrations, however, were seen in rats in other regions of the brain including cortex, hippocampus, and hypothalamus in response to drugs (Yamane et al., 1999).

Yegani et al. (2006a) reported a significant increase in 5-HT and a decrease in the 5-HIAA:5-HT concentration ratio in the pons of laying hens that had reduced feed intake and egg production after 4 wk of feeding of grains contaminated with Fusarium mycotoxins (Chowdhury and Smith, 2004). The feeding of contaminated grains to turkey poults, however, did not result in any significant alterations in brain neurotransmitter concentrations (Yegani et al., 2006a). Previously, no significant decline in feed intake was observed after feeding contaminated grains to turkey poults for 4 wk (Chowdhury and Smith, 2007). Swamy et al. (2004b) observed significant increases in the concentration of 5-HT in the pons, with simultaneous reductions in feed intake of broilers fed feedborne Fusarium mycotoxins for 8 wk (Swamy et al., 2004a). Ogunbo et al. (2005) observed no significant alteration in the forebrain concentrations of NE, epinephrine, DA, and 5-HT in poults fed 100 to 400 mg of FA/kg of diet for 21 d. The feeding of FA to broilers at concentrations of 100, 200, and 300 mg/kg of diet, however, elevated brain concentrations of NE (Ogunbo et al., 2005). Though there were some differences in the FA levels between the diets, even the highest level was many times lower than the minimal dose eliciting effects in the study of Ogunbo et al. (2005), and therefore, it is unlikely that the results found here are due to these small differences in the FA levels in the diets. These differences in pattern of elevation of brain neurotransmitter concentrations might explain the intraspecies differences in feed intake of animals fed Fusarium-contaminated diets. These previous reports, however, support the concept that feedborne Fusarium mycotoxins alter brain serotonergic activity and cause subsequent reductions in feed consumption (Smith and MacDonald, 1991; Swamy et al., 2002b, 2004a).

In the present study, turkeys were not resistant to the effects of Fusarium mycotoxins on brain 5-HT neurotransmission. Though there was no significant reduction in feed intake (control: 145 g/bird per day compared with contaminated: 135 g/bird per day, \( P > 0.05 \)) of the contaminated diets to turkey poults for 6 wk, the turkey poults consuming the contaminated diet without GMA did exhibit a statistically significant decline in their growth rates (control weight gain 1.74 ± 0.04 kg compared with contaminated weight gain 1.62 ± 0.03 kg; \( P < 0.05 \)). There was also a significant decrease in the concentrations of 5-HIAA and in the 5-HIAA:5-HT ratio in pons after feeding
contaminated grains to turkeys for 6 wk in the current study. This contradicts previous data (Yegani et al., 2006a), and this may be due to differences in age of birds, duration of treatment, and ingested dose of mycotoxins, which are important factors when turkeys are exposed to Fusarium mycotoxins. In the study of Yegani et al. (2006a), though the dietary concentrations of mycotoxins were somewhat higher, the duration of exposure was shorter (4 wk) compared with the current study. Feeding low levels of contaminated diet for 6 wk might have evoked the decreased turnover of neurotransmitter in the serotonergic neurons of the pons. It could be hypothesized that as birds gain weight, they eat more and in that way ingest more of mycotoxin, even if not on a per-BW basis. Another reason for the discrepancies in the results compared with the study conducted by Yegani et al. (2006a) could be that young birds (below 4 wk) have an immature metabolic capacity. Birds less than 4 wk old may not be able to completely metabolize the mycotoxins to their reactive metabolites. At a later age, turkey poult might be able to more efficiently metabolize the mycotoxins to reactive metabolites, which might result in the disturbed 5-HT neurotransmission.

The pattern of alterations in the 5-HIAA:5-HT concentration ratio in the current study was similar to that seen previously in laying hens (Yegani et al., 2006a) and broilers (Swamy et al., 2004b). Laying hens, however, were found to be sensitive to Fusarium mycotoxins exhibiting a disruption in serotonergic activity after 4 wk of feeding contaminated grains, as were broilers fed for 8 wk.

In the present study, there was no significant increase in the 5-HT concentrations, but there was a significant decline in the ratio of 5-HIAA to 5-HT, which is a widely used indicator of neurotransmitter turnover. Frequently, the measurement of turnover is more sensitive and informative than simply measuring the levels of the transmitter. This may be due to the fact that turnover is a more dynamic index of the situation in the synapse, because it assesses the amount of transmitter metabolite (i.e., released transmitter) in relation to the level of innervations (i.e., level of transmitter in the storage vesicle). By measuring the turnover, differences in the neuroanatomy tend to be lessened. In this case, the decreased turnover can be traced to a decline in the levels of the 5-HT metabolite 5-HIAA. Therefore, it seems as if the conversion of 5-HT to 5-HIAA was limited in groups fed contaminated diet, and in turn, this is likely attributable to a reduction in the release rate of transmitter into the synapse.

In the present study, a significant positive correlation between the concentrations of 5-HT in pons and BW gain was observed in the groups fed the contaminated diet. No other statistically significant associations were noted between any of the other neurochemical parameters in any other parts of the brain or in any of the other groups. Thus, altered 5-HT neurotransmission in pons was the only apparent change in brain monoamine systems in turkeys consuming the contaminated diet. This may point to differences in the strategies used by the birds to cope with the mycotoxin-induced effects. Some turkeys may be able to limit their exposure to the toxin by restricting their feed intake, but those that try to maintain the same growth as controls consume more of the diet with its toxins and suffer greater disruption to their serotonergic neurons in pons. The significance of disturbances in the serotonergic neurons in this part of the brain is poorly characterized in birds, but in addition to their well known role in the regulation of appetite and food intake in rats, these neurons are also thought to be responsible for controlling behavioral characteristics such as arousal and attention (Robbins et al., 1998). It is apparent that these changes cause emotional distress to turkey poult, and it is clearly beneficial that GMA can at least partially reduce the disturbances in serotonergic turnover in the brain. This characteristic disruption of the pons serotonergic system implies that this area of the brain is important for regulating behavioral changes characteristic of Fusarium mycotoxins in poult.

In the present study, central dopaminergic and noradrenergic systems were not affected by feeding contaminated diets, and only the indoleamine system was sensitive to effects of Fusarium mycotoxins. Boyd et al. (1988) observed that the intubation of 2.5 mg of T-2 toxin/kg of BW to rats significantly affected the serotonergic system, whereas dopaminergic and noradrenergic systems were less sensitive. Fitzpatrick et al. (1988) compared the effects of DON and T-2 toxin on brain biogenic monoamines in rats. An oral dose of DON and T-2 toxin at 2.5 mg/kg of BW significantly elevated the concentrations of indoleamines including 5-HT and 5-HIAA in different brain sections, and this suggests that the central nervous system actions of these trichothecenes were similar (Fitzpatrick et al., 1988). There is a possibility that Fusarium mycotoxins might affect the serotonergic system to a greater extent than other neurotransmitter systems. Disruption of this system after feeding contaminated diets, therefore, could result in feed refusal or reduced feed intake. Inter- and intraspecies differences in disruption of the serotonergic system have also been elucidated (Swamy et al., 2004b; Yegani et al., 2006a). Boyd et al. (1988), however, observed elevation of 5-HIAA concentrations and a decrease in brain regional NE and DA concentrations 24 h after dosing broilers with 2.5 mg of T-2 toxin/kg of BW. This contradicts the observed greater sensitivity of the serotonergic system to Fusarium mycotoxins, but the nature of the mycotoxin challenge was different.

**Effect of GMA Supplementation**

Several strategies to prevent mycotoxicoses in animals and poultry, including physical, chemical, and biological, have been investigated (Doll and Danicke, 2004; Diaz and Smith, 2005). Polymeric glucosamann mycotoxin adsorbent has been shown to have beneficial effects in preventing adverse effects of Fusarium mycotoxins in turkeys (Chowdhury et al., 2005c; Chowdhury and Smith, 2007), broiler chickens (Swamy et al., 2002a, 2004a), laying hens (Chowdhury and Smith, 2004), and broiler breeders (Ye-
gani et al., 2006b). In the current study, GMA prevented the adverse effects on pons neurotransmitter and metabolite concentrations caused by feeding of feedborne *Fusarium* mycotoxins. The mechanism by which adsorbents prevent mycotoxicooses is through adsorption of mycotoxins in the intestinal lumen and preventing transfer to target tissues (Ramos et al., 1996).

It was concluded that consumption of grains naturally contaminated with *Fusarium* mycotoxins results in adverse effects on the pons serotonergic system of turkeys, and supplementation with GMA prevented these effects. The feeding of contaminated grains should, therefore, be minimized.

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