HIGH DIETARY FLUORINE INDUCTION OF OXIDATIVE DAMAGE IN THE CECAL TONSIL OF BROILERS

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SUMMARY: As part of our study on the effects of high F on lymphoid organs and tissues of broilers, the oxidative damage to their cecal tonsil induced by dietary high F was observed while feeding them a control diet containing 22.6 mg F/kg and three high F diets containing 400, 800, and 1200 mg F/kg for high F groups I, II, and III throughout a 42-day experimental period. The results showed that malondialdehyde (MDA) content was significantly higher (p<0.01) in high F groups II and III than in the control group. In contrast, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), glutathione (GSH) contents, and ability to inhibit hydroxyl radical were significantly decreased (p<0.01) in high F groups II and III in comparison with those in the control group. In conclusion, dietary F, in the range of 800–1200 mg/kg, could induce oxidative damage and impair the antioxidation and immune function of the cecal tonsil in broilers.

Key words: Antioxidation; Broilers; Cecal Tonsil; Fluorine; Lipid peroxidation.

INTRODUCTION

Excess fluoride (F) can cause deleterious effects on the skeleton, teeth, and soft tissues. 1-7 Fluorosis has been a major environmental problem in many regions of the world. 8 Many studies have indicated that excessive fluoride can induce free radical toxicity and oxidative damage in the brain, muscle, thyroid, ovary, liver, and kidney in mice 4,5,9-11 or rabbit. 6 In our recent studies, the results have shown that dietary high F decreases the percentages of the peripheral blood T-cell subsets and the serum interleukin-2 (IL-2) contents, 12 increases the apoptotic splenocytes, 13 inhibits splenocyte proliferation and spleen growth, 14 and induces splenic oxidative stress. 15 However, there have been no studies on the effects of fluoride on oxidative damage in the cecal tonsil of poultry. In our present study, we investigated the oxidative damage in the cecal tonsil by detecting malondialdehyde (MDA) content, activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), ability to inhibit hydroxyl radical and glutathione (GSH) content in the serum and cecal tonsil in broilers.

MATERIALS AND METHODS

Broilers and diets: Two hundred and eighty one-day-old healthy avian broilers were divided into four groups with 70 broilers in each group and fed on diets as follows: control group (22.6 mg F/kg), high F group I (400 mg F/kg), high F group II (800 mg F/kg), and high F group III (1200 mg F/kg). Broilers were housed in cages with electrically heated units and were provided with water (fluoride =1 mg/L) as well as the undermentioned diets ad libitum for 42 days. Nutrition requirements were adequate according to the US National Research Council (NRC 1994). 16

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**Determination of the oxidative damage parameters in the cecal tonsil:** At 14, 28, and 42 days of age, five broilers in each group were humanely sacrificed, and the cecal tonsils were immediately removed and chilled to 0°C in 0.85% NaCl solution. The tonsils were weighed and homogenized in nine volumes of ice-cold 0.85% NaCl solution in a chilled homogenizer, and then immediately centrifuged at 3500×g at 4°C. After the total protein in the supernatant of the cecal tonsil homogenate was determined using Bradford’s method, the MDA content, activities of SOD, CAT and GSH-Px, GSH content, and ability to inhibit hydroxyl radicals were determined using the reagent kits manufactured by Nanjing Jiancheng Bioengineering Institute of China, following the manufacturer’s instructions.

**Statistical analysis:** The significance of the difference between means was determined by analysis (ANOVA). A value of p<0.05 was considered significant.

**RESULTS**

**Clinical symptoms:** The broilers grew much slower in all high F groups than those in control group (Figure 1).

Meanwhile, broilers in high F groups II and III showed decreased feed intake and depression. However, there were no obvious clinical symptoms in high F group I.

**Changes of the MDA content in the cecal tonsil:** As seen in Table 1, the MDA content increased significantly in high F groups II and III compared with that in the control group during the experiment.
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Changes of the activities of SOD, CAT, GSH-Px in the cecal tonsil: The activities of SOD, CAT and GSH-Px were significantly lower (p<0.01) in high F groups II and III than those in the control group from 14 to 42 days of age (Table 2).

Changes of the GSH content in the cecal tonsil: The GSH content was significantly decreased (p<0.01) in high F groups II and III when compared with that in the control group from 14 to 42 days of age (Table 3).
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Changes of the ability to inhibit hydroxyl radicals in the cecal tonsil: As shown in Table 4, the ability to inhibit hydroxyl radicals was significantly lower (p<0.01) in high F groups II and III than that in the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>14 days (U/mg protein)</th>
<th>28 days (U/mg protein)</th>
<th>42 days (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>12.26±0.56</td>
<td>13.04±0.47</td>
<td>13.49±0.32</td>
</tr>
<tr>
<td>High F group I</td>
<td>11.78±0.15</td>
<td>12.42±0.66</td>
<td>13.26±0.22</td>
</tr>
<tr>
<td>High F group II</td>
<td>9.60±0.24*</td>
<td>8.10±0.24*</td>
<td>7.53±0.22*</td>
</tr>
<tr>
<td>High F group III</td>
<td>3.81±0.13*</td>
<td>6.41±0.03*</td>
<td>6.37±0.45*</td>
</tr>
</tbody>
</table>

Compared with the control *p<0.01, †p<0.05.

DISCUSSION

The cecal tonsil is located in the proximal end of the rectum-ecum-ileum, and is the largest lymphoid organ of the avian gut-associated lymphoid tissue. Moreover, it is an important component of the mucosal immune system and performs important and unique immune functions. However, there have been no studies on the effect of high F on the mucosal immune functions in poultry at present. In the present study, we investigated the oxidative damage and immune injury of the cecal tonsil induced by dietary high F in broilers.

Lipid peroxidation represents one of the most frequent reactions caused by free radical attack on biological structures. In the present study, the MDA content, a marker of lipid peroxidation, increased in the cecal tonsil, which is consistent with a previous report that individual and combined exposure to sodium arsenite (NaAs) and NaF can increase the MDA contents in the liver of male mice. Furthermore, our previous study found that dietary high F can also increase MDA content in the spleen of chickens. The presence of MDA above normal levels indicates disturbance of the oxidant/antioxidant balance in the biological system. Such an imbalance between oxidants and antioxidants is referred to as oxidative stress. Oxidative stress is eliminated through enzymatic and non-enzymatic
mechanisms, which constitute the cellular defense system. SOD, CAT, GSH-Px and GSH play an important role in the enzymatic and non-enzymatic mechanisms. Moreover, our data further demonstrate that F improved free radical production and inhibited the activities of SOD, CAT, and GSH-Px, probably making the tissue more susceptible to biochemical injury. The results are in agreement with an earlier report that the activities of SOD, CAT, GSH-Px can be decreased by either individual exposures to fluoride or co-treatment of fluoride and ethanol in rat intestine. Chen found that dietary F in the range of 800~1200 mg/kg could reduce the activities of SOD and GSH-Px in the spleen of chickens. Likewise, Hassan and Yousef have reported that NaF can decrease the activity of CAT and GSH-Px and induce hepatotoxicity and oxidative stress in rats. The observed decrease in the activity of GSH-Px may also be due to the reduced availability of GSH, as seen in the decreased contents of GSH, in agreement with this conjecture. In our study, the GSH content was significantly decreased in the cecal tonsil in high F groups II and III, which is consistent with Mittal and Flora’s report that individual and combined exposure to NaAs and NaF can decrease the GSH content in male mice.

Lower activities of SOD, CAT, and GSH-Px as well as the GSH contents suggest that F toxicity might induce the accumulation of free radicals and consumption of the enzymes. In our study, the ability of F to inhibit hydroxyl radical in the cecal tonsil was decreased in the high F groups II and III. The results indicate that free radicals were accumulated in vivo. At present, however, there is no clear evidence on the extent F can inhibit hydroxyl radical production in animals.

We conclude that dietary F, in the range of 800–1200 mg/kg, can inhibit antioxidant enzyme activities, enhance lipid peroxidation, and increase the production of free radicals, thereby causing oxidative damage and impairing the antioxidation and immune function of the cecal tonsil in broilers.

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