CHANGES INDUCED BY HIGH DIETARY FLUORINE IN THE CECAL TONSIL CYTOKINE CONTENT OF BROILERS

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SUMMARY: As part of our recent studies on the effects of high fluorine (F) on the cecal tonsil of newly hatched chickens, the same broilers with 400, 800, and 1200 mg F/kg in their diet were used to investigate the changes in the cecal tonsil content of the cytokine proteins interleukin-4 (IL-4), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) by enzyme-linked immunosorbent assay (ELISA). The results showed that the content of these cytokines in the cecal tonsil was significantly lower (p<0.05 or p<0.01) in the high F groups II and III than in the control group. Lower cytokine levels in the cecal tonsil can impact the local immune function of intestines by affecting pathways that decrease the lymphocyte numbers and/or lymphocyte activation.

Keywords: Broilers; Cecal tonsil; Cytokines; ELISA; High fluorine diet; IL-4; IL-6; IFN-α; TNF-γ.

INTRODUCTION

The relationship between fluoride (F) and immunity in animals is an ongoing topic of discussion and debate. F can be an adjuvant for mucosal and systemic immunity and is reported to affect oral immunity in chickens. Studies with sodium fluoride (NaF), however, indicate that F can damage human lymphocyte chromosomes, induce adverse effects in the spleen, inhibit growth and general health in rabbits and increase nonspecific immune-related acid phosphatase and lysozyme activities. In agreement with these reports, our studies have shown that high dietary F (800 to 1200 mg/kg) can impair the function of the immune system in chickens. Recently we have reported that high dietary F can induce oxidative damage and decrease the percentages of the T-cell subsets in the cecal tonsil. Cytokines are immunoregulatory proteins that are important host mediators for response to stress, infection, and other forms of antigen invasion. Consequently, the cytokine environment is critical to sustain protective immunity and to avoid immunopathology. However, no study appears to have been reported about the changes of the cytokine contents induced by dietary high F in the cecal tonsil of broilers so far. As a part of our recent studies, the same broilers fed diets high in F were used in the present research to investigate changes of the interleukin-4 (IL-4), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) contents in the cecal tonsil by the method of enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Broilers and diets: The same 280 one-day-old healthy avian broilers involved in our recent studies were used in this research: a control group, and three high F groups I, II, and III with 400, 800, and 1200 mg F/kg diet, respectively. There were

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70 broilers in each group, housed in cages with electrically heated units. They were provided with water and nutritionally adequate diets\textsuperscript{13} as reported below \textit{ad libitum} for 42 days.

\textit{Determination of cytokines}: 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\(^\circ\) in 0.85\% NaCl solution. The dissected tonsils were weighed and homogenized in nine volumes of ice-cold 0.85\% NaCl solution in a chilled homogenizer, and immediately centrifuged at 3500 \(\times\) g at 4\(^\circ\)C. The supernatant fluids were immediately assayed for the concentrations of IL-4, IL-6, IFN-\(\gamma\), and TNF-\(\alpha\) by enzyme-linked immunosorbent assay (ELISA) as described by Gaça.\textsuperscript{14} The final contents were determined by the standard curve and were expressed as nanograms per milliliter (ng/mL).

\textit{Statistical analysis}: The significance of the difference between means was determined by analysis (ANOVA). All results were expressed as means \(\pm\) standard deviation (\(\pm\)S), representing five broilers in each group. A value of \(p<0.05\) was considered significant.

**RESULTS**

\textit{Clinical symptoms}: These were reported earlier.\textsuperscript{9-10}

\textit{Changes in the IL-4 content}: As shown in Table 1, the IL-4 content of the cecal tonsil was significantly decreased \((p<0.01)\) in the high F groups II and III compared with the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-4 content (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 days</td>
</tr>
<tr>
<td>Control group</td>
<td>1.25(\pm)0.06</td>
</tr>
<tr>
<td>High F group I</td>
<td>1.30(\pm)0.04</td>
</tr>
<tr>
<td>High F group II</td>
<td>1.13(\pm)0.06(^*)</td>
</tr>
<tr>
<td>High F group III</td>
<td>1.01(\pm)0.04(^*)</td>
</tr>
</tbody>
</table>

Compared with the control \(^*p<0.01\).

\textit{Changes in the IL-6 content}: The IL-6 content was lower \((p<0.01)\) in the high F group II at 28 and 42 days and in the high F group III from 14 to 42 days of age than those in the control group. Moreover, at 28 days the IL-6 content decreased \((p<0.05)\) in high F group I. The results are shown in Table 2.
Table 2. Change in the IL-6 content in the broiler cecal tonsil (Values are mean±SD, n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.06±0.03</td>
<td>1.20±0.01</td>
<td>1.20±0.01</td>
</tr>
<tr>
<td>High F group I</td>
<td>1.05±0.02</td>
<td>1.16±0.03</td>
<td>1.19±0.03</td>
</tr>
<tr>
<td>High F group II</td>
<td>1.07±0.04</td>
<td>1.09±0.01</td>
<td>1.01±0.04</td>
</tr>
<tr>
<td>High F group III</td>
<td>0.95±0.01†</td>
<td>0.91±0.03†</td>
<td>0.93±0.01†</td>
</tr>
</tbody>
</table>

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

Changes in the TNF-α content: The TNF-α content was significantly decreased (p<0.01) in the high F groups II and III in comparison with the control group (Table 3).

Table 3. Change in the TNF-α content in the broiler cecal tonsil (Values are mean±SD, n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>3.79±0.13</td>
<td>4.23±0.17</td>
<td>4.30±0.09</td>
</tr>
<tr>
<td>High F group I</td>
<td>3.75±0.11</td>
<td>4.14±0.12</td>
<td>4.28±0.17</td>
</tr>
<tr>
<td>High F group II</td>
<td>3.28±0.07*</td>
<td>3.71±0.11*</td>
<td>3.53±0.10*</td>
</tr>
<tr>
<td>High F group III</td>
<td>2.75±0.04*</td>
<td>3.05±0.10*</td>
<td>2.89±0.08*</td>
</tr>
</tbody>
</table>

Compared with the control *p<0.01.

Changes in the IFN-γ content: The IFN-γ content was lower (p<0.05 or p<0.01) in the high F group III from 14 to 42 days of age, and in the high F group II at 28 and 42 days of age than in the control group (Table 4).

Table 4. Change in the IFN-γ content in the broiler cecal tonsil (Values are mean±SD, n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.16±0.05</td>
<td>1.31±0.09</td>
<td>1.41±0.04</td>
</tr>
<tr>
<td>High F group I</td>
<td>1.21±0.10</td>
<td>1.24±0.07</td>
<td>1.39±0.06</td>
</tr>
<tr>
<td>High F group II</td>
<td>1.11±0.02</td>
<td>1.10±0.08‡</td>
<td>1.03±0.02†</td>
</tr>
<tr>
<td>High F group III</td>
<td>1.04±0.08*</td>
<td>1.01±0.03†</td>
<td>0.99±0.01†</td>
</tr>
</tbody>
</table>

Compared with the control *p<0.05; ‡p<0.01; †p<0.01

DISCUSSION

The immune system is composed of a network of cells and soluble components that complement immunoglobulin, cytokines, etc. These cells and soluble components cooperate to mount effective responses to microbes and other foreign substances. Two major parts are involved in host defense: the phylogenetically
innate system and the adaptive system. The innate immune system includes natural killer (NK) cells and phagocytes together with complementary antimicrobial peptides and cytokines. The adaptive immune system generates responses involving T and B lymphocytes together with specific antibodies and a range of cytokines and chemokines. The innate and adaptive immune systems function cooperatively to provide optimal host defense, and a defect in either system can have a significant adverse effect on immune function. Therefore the determination of the number of T or B cells, the quantitative or qualitative measure of the cytokines and antibody levels, and the study of cellular function such as phagocytic activity is used to evaluate the state of the immune system. Refsnes et al. have reported that F can induce IL-6 and IL-8 synthesis in human epithelial lung cells, which illustrates that F can affect the synthesis and secretion of cytokines. Here, in our work, we have found that the content of IL-4, IL-6, TNF-α, and IFN-γ in the cecal tonsil of broilers is decreased in the high dietary F groups II and III.

It should also be noted that T lymphocytes, which are required for both cell-mediated immune responses and the production of antibody by B lymphocytes, are composed of two distinct subsets—T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 cells produce IL-2, IFN-γ, and TNF and contribute to cytotoxic T cell differentiation, mediate cellular immune responses, etc., whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-13, promote B cell proliferation and differentiation, and mediate humoral immune response. The source of cytokines produced in various conditions has been investigated in numerous studies. The intestine has been proposed as a possible source of proinflammatory cytokines in various pathological conditions. IL-4, also called B lymphocytes growth factor-1 (BCGF-1), can promote mast cell, B and T lymphocyte proliferation, differentiation and the process of forming SIgA, and plays an important role in the immune system. IL-6, which was originally identified as a B lymphocyte differentiation factor, is now known to be a multifunctional cytokine that regulates the immune response, hematopoiesis, and inflammation. Many clinical data and animal models suggest that IL-6 plays critical roles in the pathogenesis of autoimmune diseases. As Th2 cytokines, IL-4 and IL-6 can enhance humoral immunity. A predominant Th2 effect results in activation of B lymphocytes and up-regulation of antibody production. However, in our study, the content of both of these two cytokines in the cecal tonsil was reduced, thereby indicating that IL-4 and IL-6 were involved in countering the toxicity induced by that high dietary F can impair the local humoral intestinal immunity in broilers.

TNF-α, secreted by activated T cells, is a potent pro-inflammatory and immunomodulatory cytokine implicated in inflammatory conditions. Moreover, intoxication, as by F, can also reduce the secretion of the TNF-α. The cytokine IFN-γ was originally identified as an antiviral factor but also has central roles in the activation of macrophages, stimulation of antigen presentation through class I and class II major histocompatibility complex (MHC) molecules, and regulation of T cell differentiation. IFN-γ can also stimulate expression of other cytokines that activate and induce proliferation of CD4+ cells. Thus, IFN-γ makes a major
contribution to the cell-mediated immune response. IL-2, TNF-α and IFN-γ are produced by Th1 lymphocytes, which enhance cell-mediated immunity. A predominant Th1 effect results in activation of T lymphocytes, particularly cytotoxic functions and macrophages. However, both TNF-α and IFN-γ contents were decreased in the present study, and dietary F could reduce IL-2 contents in the cecal tonsil of our previous report. These results all showed that the cell-mediated immune response had been damaged by dietary high F.

The distinct profiles of cytokines may be the starting point for enhancing the differential functions of the systemic and mucosal immune system. However, both the Th1 and Th2 cytokine contents were decreased in the present study, which indicates that the effects of some cytokines in certain situations actually may deviate functionally from previously ascribed Th1 or Th2 characteristics. In another report, we have found that high dietary F can decrease the percentages of the T-lymphocytes subsets and the IL-2 content in the cecal tonsil, a change which is related to the reduced content of cytokines IL-4, IL-6, TNF-α, and IFN-γ observed here.

According to the results of the present study and the aforementioned discussion, it is concluded that dietary F in the range of 800 to 1200 mg/kg can reduce the cytokine IL-4, IL-6, TNF-α and IFN-γ content in the cecal tonsil of broilers, which impairs the cecal tonsil function. Moreover, the local immune function of intestines is adversely impacted by effect of F on pathways that decrease the lymphocyte numbers and/or lymphocyte activation.

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REFERENCES


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