Cytokines are key regulators of the immune system that shapes both the innate and adaptive immune responses. Present knowledge allows a deeper understanding of the cytokine network and their complex roles in the development of immune responses. The purpose of this study was to examine the effect of dietary vanadium on the contents of cytokines such as interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in the mucosa of different parts of intestines (duodenum, jejunum and ileum) by Enzyme-Linked Immunosorbent Assay (ELISA). A total of 420 one-day-old avian broilers were divided into six groups and fed on a corn-soybean basal diet (control diet) or the same basal diet supplemented with 5 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg vanadium in the form of ammonium metavanadate, respectively. During the experimental period of 42 days, the results showed that the IL-2, IL-10, IFN-γ and TNF-α contents were significantly lower (p<0.05 or p<0.01) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups than those in the control group, and the IL-6 contents were significantly decreased (p<0.05 or p<0.01) mainly in the 45 mg/kg and 60 mg/kg groups in comparison with those of the control group. It was concluded that dietary vanadium in excess of 30 mg/kg significantly reduced the contents of above cytokines in the intestinal mucosa of broilers, which could impact the function of intestinal mucosal immunity by affecting the pathways that reduced the lymphocyte population and / or lymphocyte activation.
A corn-soybean basal diet formulated by the National Research Council (NRC, 1994) [17] was used as the control diet. Ammonium metavanadate was mixed into the corn-soybean basal diet to produce experimental diets with 5, 15, 30, 45, and 60 mg/kg of vanadium, respectively.

**Sample preparation**

At 14, 28, and 42 days of age during the experiment, five broilers in each group were humanely killed and the intestinal tract were removed and separated into duodenum, jejunum and ileum. A 4 cm length of tissues was taken from the middle section of each of these intestinal regions and thoroughly cleaned free of digesta by gentle squeezing. The mucosa was carefully scraped from the luminal face of the gut samples and stored at -80°C prior to antioxidant analyses. A certain amount of mucosa was weighed and rinsed with ice-cold isotonic saline (0.9% wt/vol NaCl). Ten percent tissue homogenate was prepared using glass homogenizer and centrifuged at 3,000 × g for 10 min at 4°C. The pellet was discarded and a portion of supernatant was preserved [18].

**Determination of the cytokines contents in intestinal mucosa**

Contents of the IL-2, IL-6, IL-10, IFN-γ, and TNF-α in the intestines were determined using ELISA as described by Gaca et al [19]. The cytokines of the intestinal mucosa were quantified using the IL-2 (1039-09), IL-6 (1046-09), IL-10 (1057-09), IFN-γ (1015-09), and TNF-α (1041-09) ELISA kits (GBD Ltd, USA) specific for chicken. The contents of detected cytokine were determined according to standard curve and were expressed as nanogram per milliliter or nanogram per liter (ng/mL or ng/L).

**Statistical analysis**

The significance of difference among groups was analyzed by variance analysis, and results presented as means ± standard deviation (\(\bar{X} \pm S\)). The statistical analysis was done under SPSS 12.0 for windows.

**Results**

**Changes of the IL-2 contents**

The results were shown in Figures 1 and 2. The duodenal IL-2 contents were significantly lower (\(P<0.01\)) in the 45 mg/kg at 42 days of age and in the 60 mg/kg group from 14 to 42 days of age than those in the control group. The jejunal IL-2 contents were significantly decreased (\(P<0.05\) or \(P<0.01\)) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age and in the 15 mg/kg group at 28 days of age in comparison with those of the control group. The changes of the ileac IL-2 contents have been shown in our previous report [20].

**Changes of the IL-6 contents**

The IL-6 contents in duodenum were significantly reduced (\(P<0.05\) or \(P<0.01\)) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age when compared with those of the control group. The IL-6 contents in jejunum were significantly lower (\(P<0.05\) or \(P<0.01\)) in 45 mg/kg group from 14 to 28 days of age and in 60 mg/kg group from 14 to 42 days of age than those in the control group. The results were shown in Figures 3 and 4. The changes of the ileac IL-6 contents have been shown in our previous report [20].

**Changes of the IL-10 contents**

As showed in Figures 5, 6 and 7, the duodenal IL-10 contents were significantly lower (\(P<0.01\)) in the 60 mg/kg group at 14 days of age and significantly lower (\(P<0.01\)) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups than those in the control group from 28 to 42 days of age. The jejunal IL-10 contents were decreased (\(P<0.05\) or \(P<0.01\)) in the 45 mg/kg and 60 mg/kg groups at 14 and 42 days of age when compared with the control group from 14 to 28 days of age and in 60 mg/kg group at 14 days of age when compared with the control group.

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**Figure 1:** Changes of the duodenal IL-2 contents (ng/L) in broilers.

**Figure 2:** Changes of the jejunal IL-2 contents (ng/L) in broilers.

**Figure 3:** Changes of the duodenal IL-6 contents (ng/L) in broilers.
with those of the control group. Also, the ileac IL-10 contents were significantly reduced (*p<0.05 or **p<0.01) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age.

Changes of the IFN-γ contents

The IFN-γ contents in duodenum and jejunum were significantly reduced (*p<0.05 or **p<0.01) in the 60 mg/kg group from 14 to 42 days of age when compared with those of the control group. Also, the duodenal IFN-γ contents were lower in the 45 mg/kg group than those in the control group at 42 days of age. The results were shown in Figures 8 and 9. The changes of the ileac IFN-γ contents have been shown in our previous report [20].

Changes of the TNF-α contents

The results in Figures 10, 11 and 12 showed that the duodenal TNF-α contents were decreased (*p<0.05) in the 45 mg/kg and 60 mg/kg groups at 28 days of age and markedly decreased (*p<0.01 or **p<0.05) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups at 14 and 42 days of age in comparison with those of the control group. The TNF-α contents in both jejunum and ileum were significantly decreased (*p<0.01 or **p<0.05) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age.

Discussion

The immune system is composed of a network of cells and soluble components that cooperate to enhance the effective responses to microbes and other foreign substances. There are two major systems involved in host defense, i.e., the phylogenetically older innate immune system and the more recent adaptive immune system; while the former includes natural killer (NK) cells and phagocytes together with antimicrobial peptides, complements and cytokines, the latter generates antigen-directed responses involving T and B lymphocytes together with specific antibodies and a range of cytokines and chemokines. It has become clear that the innate and adaptive immune system cooperatively provide the optimal host defense [21]. At the same time, mucosal immunity is also a main composition of the body’s immune system. In most animals, the mucosal immune system effectively controls the expression of active immune responses to pathogen and the tolerance to harmless antigens. The intestinal mucosa plays a central role in the initial immune activation and subsequent maturation of regulation [22]. Current understanding of the function and control of the mucosal immune system has been further advanced as a result from the studies on broilers and pigs [10,23].

The source of cytokines and growth factors produced under

Figure 4: Changes of the jejunal IL-6 contents (ng/L) in broilers.

Figure 5: Changes of the duodenal IL-10 contents (ng/mL) in broilers.

Figure 6: Changes of the jejunal IL-10 contents (ng/mL) in broilers.

Figure 7: Changes of the ileac IL-10 contents (ng/mL) in broilers.

on immune responses is to determine T cell subset differentiation. Upon activation, T cells derived from the lamina propria have been proven to produce mainly the primary Th1 cytokines such as IL-2, TNF-α and IFN-γ or the primary Th2 cytokines such as IL-6 and IL-10 [27]. Th1 cytokines, which enhances the cell-mediated immunity, are produced by Th1 lymphocytes, and a predominant Th1 action results in the activation of T lymphocytes. IL-2 is the essential cytokine that is secreted by activated T-cells, playing a pivotal role in the replication, maturation and differentiation of lymphocytes, and exerting important regulatory role in mucosal immunity [28]. IFN-γ is a Th1 pro-inflammatory cytokine at elevated levels in the intestinal mucosa. In addition to its immunomodulatory role during inflammation, IFN-γ acts to modify epithelial and endothelial barrier function. In model cell culture systems of inflammation, direct treatment with IFN-γ increases the permeability-per-cells of endothelial and epithelial monolayer [29]. TNF-α which is secreted by activated T-cells has the ability to increase intestinal permeability too [30]. Th2 cytokines, which enhances the humoral immunity, are produced by Th2 lymphocytes. A predominant Th2 action results in the activation of B lymphocytes and the up-regulation of antibody production. The Th2 cytokines such as IL-6 and IL-10 produced by a variety of cells including monocytes and T cells exert their effects on both myeloid and lymphocytes [31]. The distinct profile of cytokines produced by the T cells and monocytes under various conditions has been investigated in numerous studies. These mediators may be produced by lymphocytes, monocytes, natural killer cells, neutrophils, endothelial cells, and macrophages. The intestine has been proposed as a possible source of proinflammatory cytokines under various pathologic circumstances [24,25]. Regulatory cytokines and growth factors are present in the normal intestinal mucous membrane, and mucosal immunity is one of the main functions of mucous membrane. In our study, the contents of IL-2, IL-6, IL-10, IFN-γ and TNF-α in different parts of the intestine were respectively lower in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups than those in the control group, and the rank of effects of dietary vanadium on the abovementioned cytokines was as the following: 60 mg/kg > 45 mg/kg > 30mg/kg. The results imply that the decrease in these cytokine contents may impact the immune function of intestinal mucosa. However, many aspects of the immune responses are initiated or regulated by the cytokine network. As our understanding of these relationships expands, it is likely that an increasing number of undefined clinical phenotypes manifesting recurrent infections will be linked to the defects in cytokine production, receptor function, or downstream intracellular signaling cascades [21]. Deregulation of cytokine balance is also observed in the onset and maintenance of several pathological non-infectious conditions [26]. One of the main ways that cytokines exert key effects.
may be the starting point to enhance the differential functions of the systemic and mucosal immune system [32,33]. In this study, the contents of both Th1 and Th2 cytokines were decreased in the groups with high levels of dietary vanadium. Results demonstrate that dietary vanadium in excess of 30 mg/kg can impact not only the humoral immunity but also the cellular immunity in the intestinal mucosa. Our previous data have indicate that dietary vanadium in excess of 30 mg/kg reduces T cells population and IL-2 contents in the cecal tonsil ileum, and cause cecal tonsil lesions [10,20]. Based on all these results and aforementioned discussion, it is speculated that the decline in contents of cytokines such as IL-2, IL-6, IL-10, TNF-α and IFN-γ is closely related to the reduction of T and B cell population induced by high levels of vanadium, for these cytokines are mainly produced by T and B cell. Thus, the abovementioned cytokines impact mucosal immunity by affecting the pathways that reduce the lymphocyte population and activation.

In conclusion, dietary vanadium in excess of 30 mg/kg reduces the contents of cytokines such as IL-2, IL-6, IL-10, TNF-α and IFN-γ in the different part of the intestines, which impacts the function of mucosal immunity by affecting the pathways that reduce the lymphocyte population and activation.

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References


