

Dietary Vanadium Induces Decrease in Antioxidant Enzyme Activities and Oxidative Stress in the Spleens of Broilers

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Abstract

The purpose of this 42-day study was to evaluate the risk of oxidative stress in the spleens induced by dietary vanadium through determining changes in antioxidant enzymes and oxidation products. A total of 420 one-day-old avian broilers were divided into six groups. There were 70 broilers in each group. The broilers were fed on a corn-soybean basal diet as a control diet (vanadium 0.073 mg/kg) or the same diet amended to contain 5 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg vanadium supplied as ammonium metavanadate (NH_4VO_3). When compared with those of the control group, the splenic and serum vanadium contents were increased in the 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg groups. Also, the splenic and serum superoxide dismutase (SOD) activities were greatly depressed in the 45 mg/kg and 60 mg/kg groups; the glutathione peroxidase (GSH-Px) activities and the ability to inhibit hydroxyl radical were markedly depressed in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups; the malondialdehyde (MDA) content was significantly increased in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups. At the same time, the splenic glutathione (GSH) content was significantly decreased in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups, and the serum GSH content was significantly decreased in the 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg groups. Also, the splenic and serum glutathione (oxidized form, GSSG) content was significantly higher in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups than that in the control group. These results indicated that dietary vanadium in the range of 30–60 mg/kg caused substantial oxidative stress in the spleen, which then affected the antioxidant function; this may be possible pathway leading to spleen injury. At the same time, it was found that dietary vanadium in the range of 5–15 mg/kg was relatively safe for the spleens of young broilers.

Keywords: Dietary vanadium; Antioxidant enzymes; Oxidative stress; Spleen; Broilers

Introduction

Vanadium (V) is an essential trace nutrient for animals and humans [1]. Vanadium is necessary for normal structure and the biological importance of vanadium has been suggested by a number of studies [2]. Vanadium deficiencies result in slow growth in rats [3] and poultry, and increased abortion rates in goats [4]. An important physiological role for vanadium was suggested by the finding that it mimics the action of insulin [5,6] as an antidiabetic agent [7]. Vanadium has also been showed to be a potent inhibitor of several phosphohydrolases, such as Na^+K^+ -ATPase and Ca^{2+} -ATPase, an activator of adenyl cyclase [8–10] and a potent anti-carcinogenic agent [11].

Vanadium is an important industrial mineral that is widely distributed in nature [12]. Due to concern for the biological effects of vanadium, the toxicology of this element has been established in human and animals [13,14]. As a chemically active ionized element, vanadium can impel the free radical formation and transformation [15]. Previous research has showed that a high level of dietary vanadium can induce the depression of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and an increase in malondialdehyde (MDA) content in the muscles of rats [16].

According to the aforementioned references, there are very few reports on the effect of oxidative damage in the spleen in human and animals at present. In our previous research, dietary vanadium more than 30 mg/kg was showed to inhibit splenic growth and cause lesions in the spleen [17]. In the present study, the experiment was conducted with the objective of examining the oxidative damage of the spleen induced by different levels of dietary vanadium, which is a part of a larger study to examine the effects of dietary vanadium on lymphoid organs (including the bursa of Fabricius, spleen and thymus), and to provide helpful information for the similar studies in both human and animals in the future.

Materials and Methods

Chickens and Diets

A total of 420 one-day-old healthy avian broilers were divided into six groups. There were 70 broilers in each group. The broilers were housed in cages with electrically heated units and were provided with water as well as experimental diets *ad libitum* for 42 days.

A corn-soybean basal diet formulated by the National Research Council (NRC, 1994) was the control diet (vanadium 0.073 mg/kg). Ammonium metavanadate (NH_4VO_3) was mixed into the corn-soybean basal diet to produce 5 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg vanadium diets.

Determination of vanadium contents in the spleen and serum

The vanadium contents in the spleen and serum were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). At the end of experiment, five spleens from each group were collected and torrefied, and five chickens in each group were phlebotomized from the jugular vein to obtain serum. These samples were treated with 2 mL HNO_3 and 1 mL H_2O_2 , and then dissolved with an automatic microwave-heated digestion system (Multiwave 3000). Digestive production was made up to 25 mL with deionized water

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resulting in a clear colorless solution. Also, the reagent blanks were carried out following the same procedure but without sample. Then, samples were analyzed by ICP-OES (5300V, PE Ltd., USA).

Determination of the oxidative stress parameters in spleen and serum

At 14, 28 and 42 days of age during the experiment, five chickens in each group were phlebotomized from the jugular vein to obtain serum. Then, euthanasia carried out and spleens were taken immediately. 9 mL ice-cold isotonic saline (0.9% wt/vol NaCl) was added to a 1.00 g spleen. Then, 10% tissue homogenate was prepared using a glass homogenizer and was centrifuged at $3000 \times g$ for 10 min at 4°C . After determining the amount of total protein in the supernatant of the spleen homogenate and serum by the method of Bradford [18], the SOD and GSH-Px activities, the ability to inhibit hydroxyl radical, and GSH, GSSG and MDA contents in the splenic supernatant and serum were measured by a biochemical method following the instructions of reagent kits (SOD, A001-1; GSH-Px, A005; the ability to inhibit hydroxyl radical, A018; MDA, A003-1; GSH, A006; GSSG, A061; total protein, A045-2). All reagent kits were provided by the Nanjing Institute of Jiancheng Biological Engineering.

Statistical analysis

The significance of difference among the six groups was analyzed by variance analysis, and results were presented as means \pm standard deviation ($\bar{x} \pm S$). The analysis was done using SPSS 12.0 for Windows.

Results

Clinical Observation

The broilers grew much faster in the 5 mg/kg group and much slower in the 30 mg/kg, 45 mg/kg, and 60 mg/kg groups than in the control group. Broilers in the 45 mg/kg and 60 mg/kg groups showed depression and 35.31%, 44.03%, and 68.05% loss of body weight were in 30 mg/kg, 45 mg/kg, and 60 mg/kg groups, respectively, at the end of the experiment when compared with that of the control group.

Changes of vanadium contents in the spleen and serum

Our data, as shown in Figure 1, suggested that the vanadium

contents in the spleen and serum were increased when the dietary vanadium levels were increased. The splenic vanadium content was significantly increased ($P < 0.01$) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups and was increased in the 15 mg/kg group ($P < 0.05$). Also, the changes in the serum vanadium content were the same as the changes in the spleen.

Changes of the SOD and GSH-Px activities

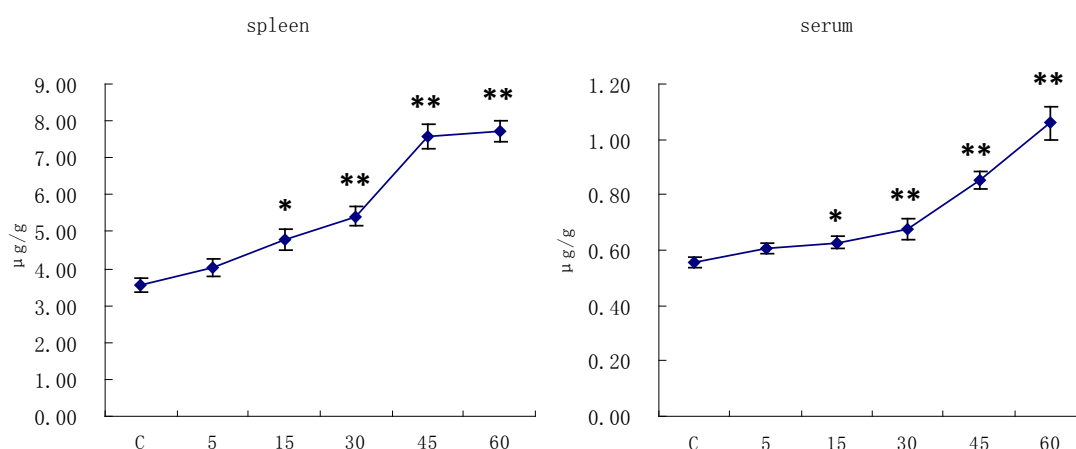
When compared with those of the control group, the splenic SOD activity was significantly decreased ($P < 0.05$ or $P < 0.01$) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age, but was increased ($P < 0.05$) in the 5 mg/kg group at 14 days of age. Meanwhile, the serum SOD activity was significantly lower ($P < 0.05$ or $P < 0.01$) in the 60 mg/kg group from 14 to 42 days of age and in the 45 mg/kg group at 14 days of age, and was higher ($P < 0.05$) in the 5 mg/kg group at 28 days of age than that in the control group. The results were shown in Figure 2.

The splenic GSH-Px activity was significantly decreased ($P < 0.01$) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age, and decreased ($P < 0.05$) in the 30 mg/kg groups at 28 days of age. Meanwhile, the serum GSH-Px activity was significantly lower ($P < 0.05$ or $P < 0.01$) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age than that in the control group. The results were shown in Figure 3.

Changes of the GSH and GSSG contents

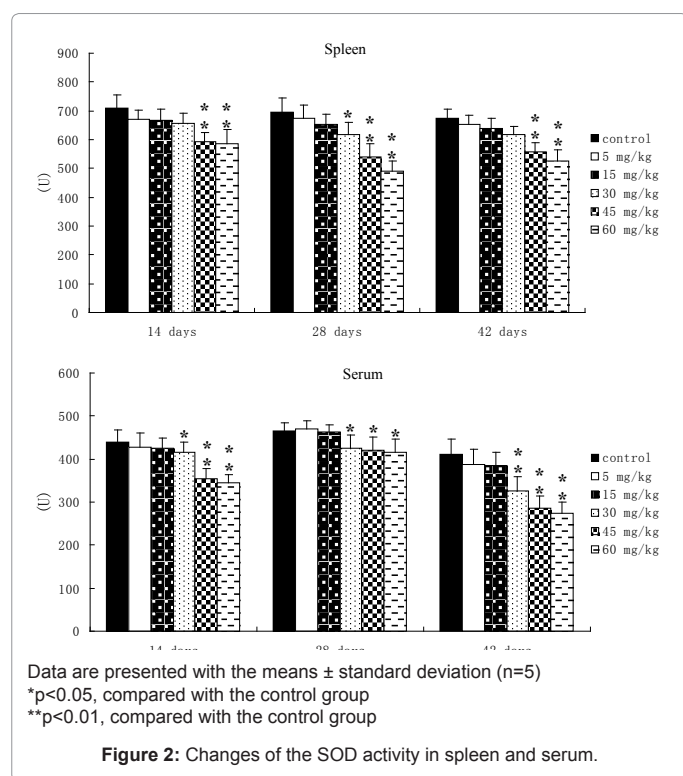
As shown in Figure 4, the splenic GSH content was significantly decreased ($P < 0.01$) in the 45 mg/kg and 60 mg/kg groups and was decreased ($P < 0.05$) in the 30 mg/kg group from 14 to 42 days of age in comparison with that of the control group. The serum GSH content was significantly lower ($P < 0.01$ or $P < 0.05$) in the 15 mg/kg, 30 mg/kg, 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age than that in the control group.

However, the changes in GSSG content were contrary to GSH content. The splenic GSSG content was significantly increased ($P < 0.05$ or $P < 0.01$) in the 60 mg/kg group and was increased ($P < 0.05$) in the 30 mg/kg and 45 mg/kg groups from 14 to 42 days of age. The serum GSSG content was significantly higher ($P < 0.05$ or $P < 0.01$) in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age than that in the control group. Also, the serum GSSG content was increased

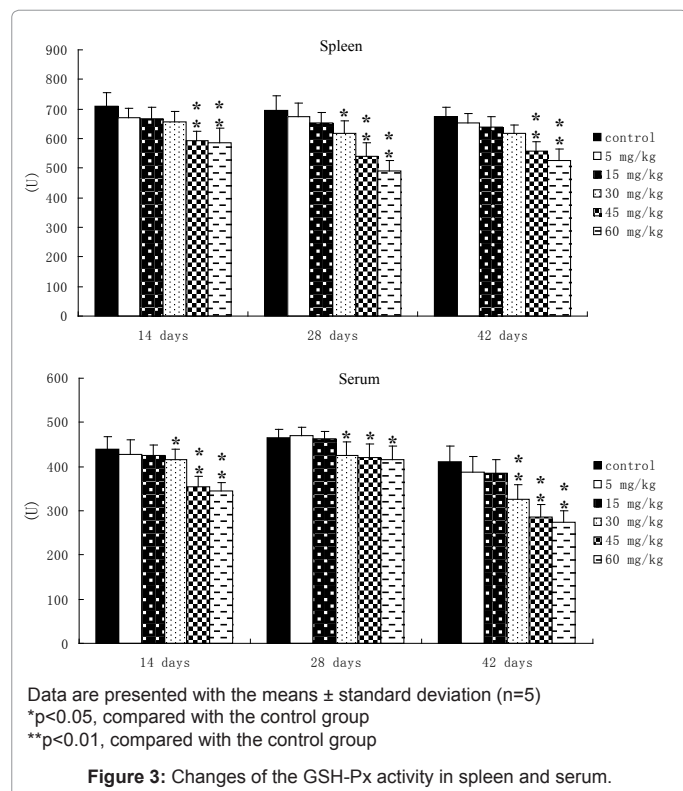
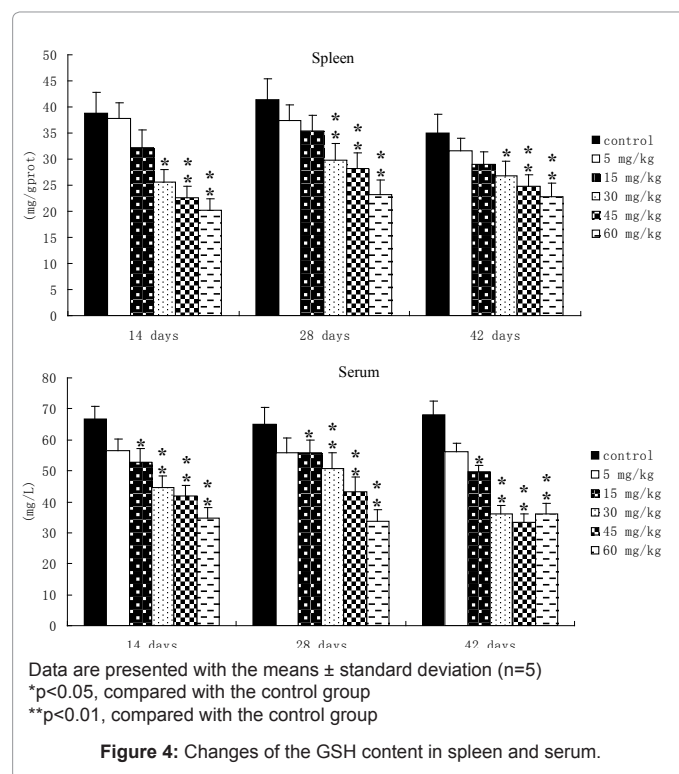


Data are presented with the means \pm standard deviation ($n=5$)
 * $p < 0.05$, compared with the control group
 ** $p < 0.01$, compared with the control group

Figure 1: Changes of vanadium content in spleen and serum.



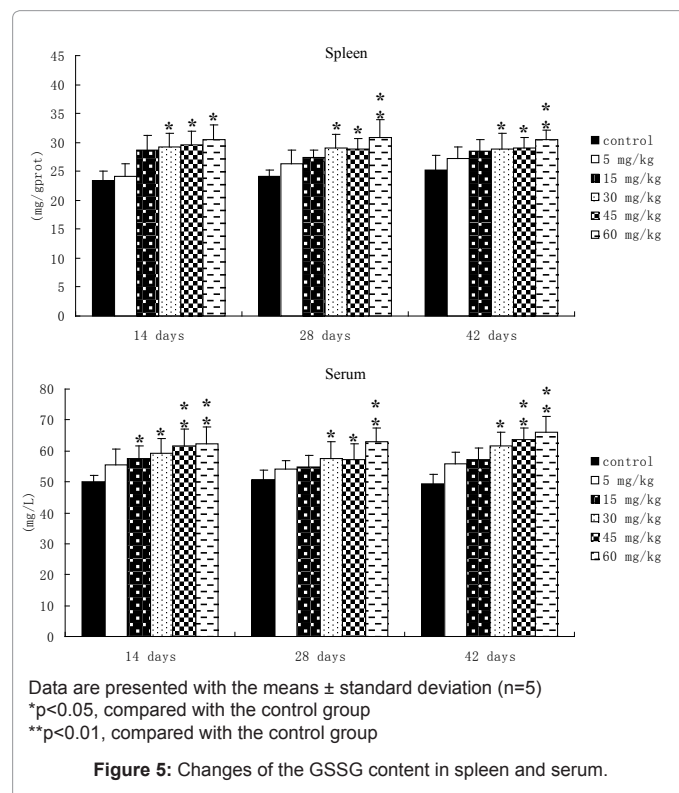
dietary vanadium levels were increased. When compared with that of the control group, the splenic ability to inhibit hydroxyl radical was significantly depressed ($P<0.05$ or $P<0.01$) in the 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age, and was depressed ($P<0.05$) in the 30 mg/kg group from 28 to 42 days of age.



($P<0.05$) in the 15 mg/kg group at 14 days of age. The results were shown in Figure 5.

Changes of the ability to inhibit hydroxyl radical

The ability to inhibit hydroxyl radical was depressed when the



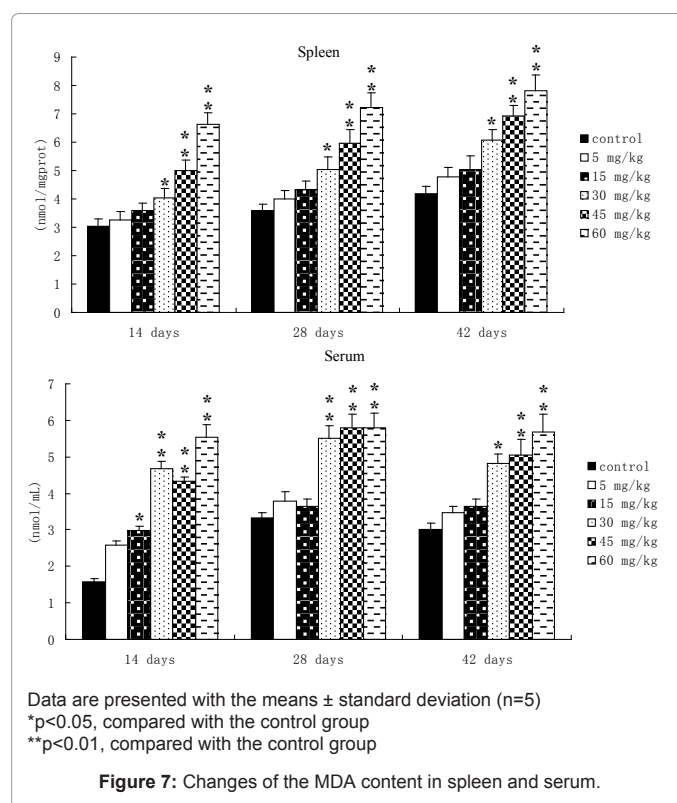
Meanwhile, the serum ability to inhibit hydroxyl radical was significantly lower ($P<0.05$ or $P<0.01$) in the 60 mg/kg group from 14 to 42 days of age and significantly lower ($P<0.05$ or $P<0.01$) in the 45 mg/kg group from 28 to 42 days of age than that in the control group. Also, the serum ability to inhibit hydroxyl radical was depressed ($P<0.05$) 30 mg/kg group at 42 days of age. The results were shown in Figure 6.

Changes of the MDA content

The splenic and serum MDA contents were increased in a dose-dependent manner. As described in Figure 7, the splenic MDA content was significantly increased ($P<0.01$) in the 45 mg/kg and 60 mg/kg groups, and increased ($P<0.05$) in the 30 mg/kg group from 14 to 42 days of age. Meanwhile, the serum MDA content was significantly higher ($P<0.01$ or $P<0.05$) in 30 mg/kg, 45 mg/kg and 60 mg/kg groups from 14 to 42 days of age, and was higher ($P<0.05$) in 15 mg/kg group at 14 days of age than that in the control group.

Discussion

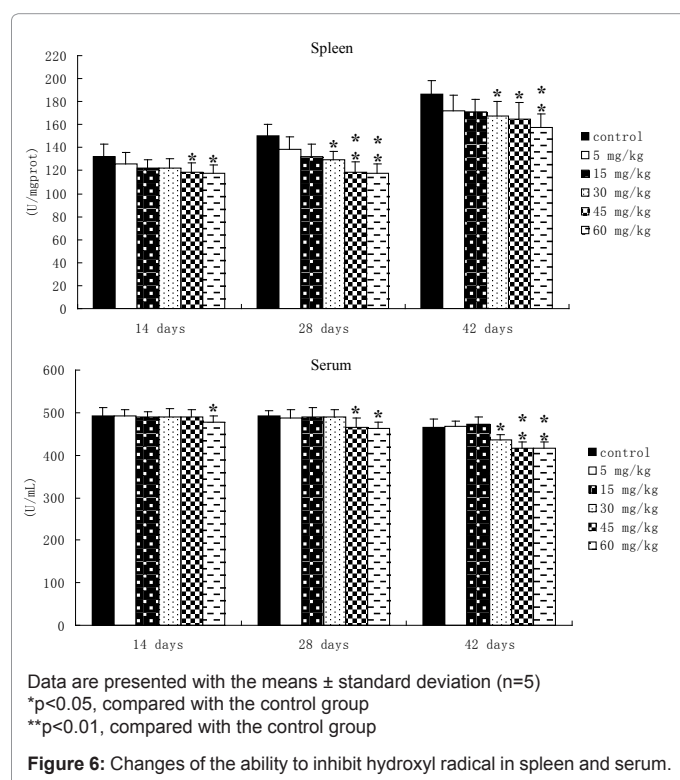
It is widely known that antioxidant defense systems of the body operate with activities of antioxidant enzymes to scavenge reactive oxygen species (ROS) and to prevent the oxidative stress. Both SOD and GSH-Px are antioxidant enzymes, which are considered as important physiological antioxidant defense mechanisms in removing excess oxygen negative ions [19]. Thus, the reduced activities of SOD and GSH-Px could lead to the accumulation of free radicals in the body. In the present research, the splenic and serum SOD activities were greatly depressed in the 45 mg/kg and 60 mg/kg groups, and the splenic and serum GSH-Px activities were greatly depressed in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups. The results indicated that dietary vanadium in excess of 30 mg/kg caused a decrease in antioxidant enzyme activities. These results were similar to the previous reports in rat [16]. However, the mechanism for high level vanadium reducing the activities of SOD and GSH-Px has been not clearly identified. Our data indicated that the



vanadium contents in the spleen and serum were increased when the dietary vanadium levels were increased. Some reports showed that a high concentration of vanadium could directly aggress DNA and RNA [20,21], which may affect the expression of SOD and GSH-Px.

Meanwhile, the GSH and GSSG are other aspects of the antioxidant system. The GSH consists of glutamic acid, cysteine and glycine that combine and remove free radicals with sulfhydryl (-SH). In vivo, the GSH could be oxidated and translated to GSSH by combining free radical or lipid peroxidation. Meanwhile, the GSSG could translate to GSH by catalysis of glutathione transferase [22]. In the present research, the splenic and serum GSH contents were greatly decreased in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups. And, the splenic and serum GSSG contents were greatly increased in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups. These results indicated that dietary vanadium in excess of 30 mg/kg caused the GSH content to decrease and the GSSG content to increase and that the balance between GSH and GSSG was broken. The aforementioned changes of GSH and GSSG contents may be related to the level of the cationic vanadyl ion (VO^{2+}). A report has suggested that most of the ingested vanadium is transformed into VO^{2+} which can be absorbed [23]. VO^{2+} is the most common intracellular form of vanadyl [24], which has the biological activity. The ingested multivalent vanadium, such as VO^{3+} , was transformed into VO^{2+} by GSH *in vivo* [25]. However, the VO^{2+} is a peroxide and a high concentration of VO^{2+} can induce oxidative damage, which interferes with the free radical metabolism and impaires the tissue cells [26,27]. Thus, the increase of ingested vanadium promotes the consumption of GSH to transform the VO^{3+} into VO^{2+} , which causes the GSH content to decrease and GSSG content to increase, and induces the elevation of VO^{2+} concentrations. At the same time, a high concentration of VO^{2+} induces generous free radical accumulation in the body and promotes the consumption of free radical scavengers.

Depressed antioxidant enzyme activities and reduced GSH content



in the body caused the free radical accumulation *in vivo*. Meanwhile, in our study, the ability to inhibit hydroxyl radical of the spleen and serum was significantly depressed in 30 mg/kg, 45 mg/kg and 60 mg/kg groups. Vanadate catalyzed hydroperoxy radicals, hydroxy radicals and secondarily derived radicals in oxidative reactions [28]. Furthermore, hydroxyl radical is one of the major oxygen radicals that produce oxidative stress. Also, as reported previously, vanadium can promote the hydroxyl radical production that induces the lipid peroxidation of the cell membrane [27]. The MDA is the end production of lipid peroxidation. In the present research, the splenic and serum MDA contents were significantly increased in the 30 mg/kg, 45 mg/kg and 60 mg/kg groups. The results shown that dietary vanadium in the range of 30~60 mg/kg induced hydroxyl radical and an increase in lipid peroxides, which impaired splenic antioxidant function in broilers.

In conclusion, dietary vanadium can affect the antioxidant function. Dietary vanadium in the range of 30~60 mg/kg is found to cause inhibition in the activities of antioxidant enzymes, enhancement of lipid peroxidation and finally induction of oxidative damage in the spleen. Thus, oxidative stress induced by vanadium plays an important role in the pathogenesis and toxicity of vanadium. At the same time, dietary vanadium in the range of 5 ~ 10 mg/kg is relatively safe for the spleens of young chickens.

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