Effects of Vanadium-Containing Egg Yolk Powder on Growth, Oxidative Stress Status, and Related Gene Expression in Rats: Postprint

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Abstract

Vanadium is a heavy metal, and excessive intake can cause oxidative stress in laying hens, reduce egg quality, accumulate in eggs, and compromise egg safety. This study was conducted to investigate the effects of feeding vanadium-containing egg yolk powder to rats on their growth performance, oxidative stress status, and related gene expression, thereby providing a basis for evaluating the biological safety of vanadium (organic vanadium). Twenty-seven 4-week-old female Wistar rats were selected and housed individually, with 3 treatments and 9 rats per treatment. Egg yolk powder prepared from eggs laid by laying hens fed diets containing 3 vanadium levels (0, 5, and 10 mg/kg) was added to the diets at 600 g/kg. The measured vanadium concentrations in the three diets were 0.107, 0.137, and 0.164 mg/kg, respectively. The experimental period was 35 days. Results showed that there were no significant differences among the three treatments in rat growth performance, organ indices, plasma triglyceride, malondialdehyde (MDA), urea nitrogen content, and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), liver and kidney superoxide dismutase activity, MDA content, total antioxidant capacity, liver ALT and AST activities, glutathione content and glutathione S-transferase activity, as well as liver and kidney tissue structure and kidney vanadium residue ($P>0.05$). Compared with treatment 1, hepatic quinone oxidoreductase 1 (NQO1) activity in treatments 2 and 3 decreased significantly ($P<0.05$), and the relative mRNA expression levels of NQO1 and nuclear factor E2-related factor 2 also decreased significantly ($P<0.05$). Results suggest that adding 600 mg/kg egg yolk powder containing vanadium at 0.107, 0.137, and 0.164 mg/kg to rat diets had no significant effect on rat growth performance and body redox status, but vanadium at 0.137 and 0.164 mg/kg could reduce NQO1 enzyme activity and downregulate the expression of antioxidant response-related genes.

Full Text

Effects of Vanadium-Containing Egg Yolk Powder on Growth, Oxidative Stress Status, and Related Gene Expression in Wistar Rats

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Abstract

Vanadium is a heavy metal, and excessive intake can cause oxidative stress in laying hens, reduce egg quality, and accumulate in eggs, thereby affecting egg safety. This study investigated the effects of feeding vanadium-containing egg yolk powder to rats on their growth performance, oxidative stress status, and related gene expression to provide a basis for evaluating the biosafety of organic vanadium. Twenty-seven female Wistar rats (4 weeks old) were individually housed and assigned to three treatments (n = 9 each). The rats were fed diets supplemented with 600 g/kg egg yolk powder prepared from eggs laid by hens fed diets containing three vanadium levels (0, 5, and 10 mg/kg). The measured vanadium concentrations in the three experimental diets were 0.107, 0.137, and 0.164 mg/kg, respectively. The experimental period lasted 35 days. The results showed no significant differences among the three treatments in growth performance, organ indices, plasma triglyceride, malondialdehyde (MDA), and urea nitrogen contents, plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, liver and kidney superoxide dismutase (SOD) activity, MDA content, total antioxidant capacity (T-AOC), liver ALT and AST activities, glutathione content, glutathione S-transferase activity, liver and kidney histomorphology, or kidney vanadium residues ($P > 0.05$). However, compared with treatment 1, treatments 2 and 3 significantly decreased liver quinone oxidoreductase 1 (NQO1) activity ($P < 0.05$) and also significantly reduced the relative mRNA expression levels of NQO1 and nuclear factor erythroid 2-related factor 2 (Nrf2) ($P < 0.05$). These results indicate that dietary supplementation with 600 mg/kg egg yolk powder containing 0.107, 0.137, and 0.164 mg/kg vanadium had no significant differential effects on growth performance or redox status in rats, but vanadium at 0.137 and 0.164 mg/kg decreased NQO1 enzyme activity and downregulated the expression of antioxidant response-related genes.

Keywords: egg yolk powder; vanadium; rat; oxidative stress; NQO1; Nrf2

Vanadium is an essential trace element for organisms, participating in the metabolism of proteins, nucleic acids, carbohydrates, and lipids, and main-

taining normal growth and development under trace conditions, including involvement in glucose transport [1-2]. However, as a heavy metal, excessive vanadium can cause severe toxic effects, including reduced body weight and feed intake, decreased heart rate with cardiac damage, pulmonary fibrosis, hepatic and renal oxidative damage [3-5], embryonic malformations, and even death. Studies in rats have shown that the toxic concentration of vanadium is 0.25 mg/L, with a lethal concentration of 6.00 mg/L [5]. Research indicates that vanadium is ingested by laying hens through feed and ultimately transported into eggs, with vanadium residues in eggs increasing with dietary vanadium levels, and the yolk being the primary deposition site [6]. As eggs are an inexpensive, high-quality protein source in the human diet, whether vanadium residues in eggs pose potential safety hazards to human health remains unreported. This study evaluates whether vanadium-containing egg yolk powder (organic vanadium) affects rat growth and health by feeding rats diets formulated with egg yolk powder obtained from hens fed vanadium-supplemented diets.

1.1 Materials, Reagents, and Instruments

Eggs were collected from laying hens fed diets containing 0, 5, and 10 mg/kg vanadium for 12 weeks. Shells and egg whites were carefully removed, and the yolks were freeze-dried to prepare egg yolk powder. Twenty-seven specific-pathogen-free Wistar rats (3 weeks old) were purchased from Chengdu Dossy Biological Co., Ltd. and housed individually at (23 ± 2)°C and (55 ± 2)% relative humidity with free access to water and feed. After a 7-day acclimation period, the formal experiment began. Assay kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen (UN), malondialdehyde (MDA), superoxide dismutase (SOD), triglyceride (TG), total antioxidant capacity (T-AOC), glutathione (GSH), and glutathione S-transferase (GST) were purchased from Nanjing Jiancheng Bioengineering Institute. The quinone oxidoreductase 1 (NQO1) assay kit was purchased from Wuhan GeneMay Co., Ltd. Primers for nuclear factor erythroid 2-related factor 2 (Nrf2) and NQO1, as well as RNA extraction, reverse transcription, and quantitative reagents, were purchased from Tiangen Biotech Co., Ltd. All other reagents were analytically pure.

Instruments included an ultrapure water system (Millipore, USA), electronic balance, microwave digestion apparatus, and inductively coupled plasma mass spectrometer (ICP-MS, 7500a, Agilent, USA). For metal element determination, the instrument sensitivity (Li, Y, Tl), oxide level (CeO/Ce), and double charge (Ce^2 /Ce) were optimized using a 2% HNO tuning solution containing 10 ^{12} g/L of Li, Y, Ce, Tl, and Co as external standards. Additional equipment included a microplate reader (Thermo, USA) and real-time fluorescence quantitative PCR system (C1000, Bio-Rad, Chengdu).

1.2 Basal Diet

The basal diet was formulated primarily with corn starch and egg yolk powder. Crude protein, gross energy, and crude fat levels were designed according to Imura et al. [7], while crude fiber, vitamin, and mineral additions were based on Reeves et al. [8]. The diet was prepared in block form for easy consumption by rats. The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (as-fed basis) g/kg

Item	Content
Ingredients	
Corn starch	
Casein	
Egg yolk powder	
Dextrin	
Saccharose	
Wheat bran	
CMC-Na	
CaCO	
Mineral premix[1]	
Vitamin premix[1]	
L-Cys	
DL-Met	
Choline chloride	
Total	
Nutrient levels[2]	
GE/(MJ/kg)	
CF	
Ca	
AP	
Met	
Cys	
CP	
EE	

[1]The premix provided the following per kg of diet: Cu 6 mg, Fe 35 mg, Mn 11 mg, Zn 35 mg, Se 0.17 mg, I 0.21 mg, Na 1.3 g, VA 10,000 IU, VD 3,000 IU, VE 22.5 IU, VK 3 mg, VB 3 mg, VB 7.5 mg, VB 4.5 mg, VB 30 g, niacin 300 mg, calcium pantothenate 15 mg, folic acid 1.5 mg, biotin 120 g, antioxidant 60 g.
[2]CP and EE were measured values, while the others were calculated values.

1.3 Experimental Design

The rat experiment consisted of three treatments, with 600 g/kg of three different egg yolk powder sources added to the basal diet, designated as treatments 1, 2, and 3. The measured vanadium contents in the three egg yolk powders were 0.006, 0.053, and 0.080 mg/kg, respectively, resulting in experimental diets containing 0.107, 0.137, and 0.164 mg/kg vanadium.

1.4 Animal Husbandry and Management

Twenty-seven 21-day-old weaned Wistar rats were individually housed in iron cages (20 cm × 15 cm × 20 cm) with free access to feed and water. After a 7-day pre-feeding period, rats were divided into three treatments according to the principle of no significant difference in body weight and fed three experimental diets. Room temperature and relative humidity were recorded twice daily (morning and afternoon), and rat activity and mental status were observed. The experimental period lasted 35 days.

1.5 Sample Collection and Determination

At the end of the experiment, all rats were weighed and blood was collected via eyeball enucleation. Two milliliters of blood were collected into anticoagulant tubes containing 0.2% heparin sodium, mixed well, and centrifuged at 3,500 r/min for 10 min. The supernatant was collected, aliquoted into 200 L EP tubes, and stored at -20°C for later analysis. After blood collection, rats were euthanized by cervical dislocation. Approximately 0.2 g of liver tissue was placed in a sterile 1.5 mL EP tube and stored at -80°C for gene expression analysis. The liver, kidneys, lungs, spleen, and heart were weighed to calculate organ indices using the formula:

Organ index (%) = 100 × (organ weight / live body weight).

Half of the liver and kidney tissues were fixed in 4% paraformaldehyde solution for histological observation. The remaining liver and kidney tissues were bagged and stored at -20°C for determination of antioxidant biochemical indicators and vanadium residues.

1.5.1 Plasma Biochemical Indicators Frozen plasma samples were thawed at room temperature, and indicators were measured according to the instructions of the assay kits from Nanjing Jiancheng Bioengineering Institute. Plasma ALT activity was determined by the modified Reitman-Frankel method, AST activity by the kinetic enzyme method, UN content by the urease method, MDA content by the thiobarbituric acid (TBA) method, and TG content by the lipase colorimetric method.

1.5.2 Liver and Kidney Antioxidant Indicators Liver and kidney tissues (1.0 g each) were homogenized in ice-cold physiological saline to prepare 10%

tissue homogenates and centrifuged at 3,500 r/min for 10 min. The supernatant was used for the following determinations: liver and kidney SOD activity by the xanthine oxidase method, MDA content by the TBA method, and T-AOC by the reduction method. Liver TG content and ALT and AST activities were determined using the same methods as for plasma. Liver GSH content was measured by the microplate enzyme method, GST activity by the chemical colorimetric method, and NQO1 activity by enzyme-linked immunosorbent assay (ELISA).

1.5.3 Liver and Kidney Histological Observation Liver and kidney tissues fixed in 4% paraformaldehyde were dehydrated, paraffin-embedded, sectioned, and stained with hematoxylin-eosin for routine histological examination. Pathological changes were observed and recorded under a microscope.

1.5.4 Vanadium Residue Determination in Liver and Kidney Retained liver and kidney samples were weighed and subjected to microwave digestion [9]. Vanadium content in tissues was quantitatively determined by inductively coupled plasma mass spectrometry (ICP-MS) using the standard curve method [10].

1.5.5 Liver Gene Expression Analysis Frozen liver samples stored at -80°C were ground in liquid nitrogen using an RNase-free mortar. Cell lysis solution was added to extract RNA, which was then reverse-transcribed. After adding primers, fluorescence quantification was performed to determine the relative expression levels of Nrf2 and NQO1 mRNA in the liver. Nrf2 primers were based on Habeos et al. [11], while NQO1 primers were designed using Primer Premier 6.0 [12] (Table 2). RNA extraction, reverse transcription, and real-time quantitative PCR were performed according to Tiangen Biotech kit protocols. Relative gene expression was calculated using the 2ˆ(-ΔΔCT) method [13].

Table 2 Primers for real-time quantitative PCR

Target genes	Primer sequences (5' –3')	Size/bp
Nrf2		
NQO1		
-actin		

1.6 Statistical Analysis All data were analyzed using SAS 9.0 statistical software, with results expressed as means and pooled standard errors. One-way ANOVA was performed using the General Linear Model (GLM) procedure, and Duncan' s multiple comparison test was used. Significance was set at $P < 0.05$, and $P < 0.10$ was considered a trend toward significance.

2.1 Growth Performance

As shown in Table 3 , there were no significant differences among the three treatments in final body weight, body weight gain, total feed intake, feed intake per unit body weight, or feed conversion ratio (P > 0.05).

Table 3 Effects of vanadium-containing egg yolk powder on growth performance of rats

Items	Treatment 1	Treatment 2	Treatment 3	Pooled SEM	P-value
Initial weight/g					
Final weight/g					
BWG/g					
Total feed intake/g					
Feed intake for unit weight/g					

In the same column, values with different small letter superscripts mean significant difference (P < 0.05), while with the same or no letter superscripts mean no significant difference (P > 0.05). The same as below.

2.2 Organ Indices

As shown in Table 4 , there were no significant differences among the three treatments in liver, kidney, lung, spleen, or heart indices at the end of the experiment (P > 0.05).

Table 4 Effects of vanadium-containing egg yolk powder on organ indices of rats (%)

Items	Liver	Kidney	Lung	Spleen	Heart
Treatment 1					
Treatment 2					
Treatment 3					
Pooled SEM					
P-value					

2.3 Plasma Biochemical Indicators

As shown in Table 5 , there were no significant differences among the three treatments in plasma AST and ALT activities or MDA, UN, and TG contents ($P > 0.05$).

Table 5 Effects of vanadium-containing egg yolk powder on plasma biochemical indices of rats

Items	AST/(U/L)	ALT/(U/L)	MDA/(nmol/mL)	UN/(mmol/L)	TG/(mmol/L)
Treatment 1					
Treatment 2					
Treatment 3					
Pooled SEM					
P-value					

2.4 Vanadium Residues in Liver and Kidney

As shown in Table 6 , compared with treatment 1, there were no significant differences in vanadium residues (fresh weight basis) in liver and kidney of treatments 2 and 3 ($P > 0.05$), but liver vanadium residues differed significantly between treatments 2 and 3 ($P < 0.05$).

Table 6 Effects of vanadium-containing egg yolk powder on liver and kidney vanadium residues of rats (fresh weight basis)

Items	Liver	Kidney
Treatment 1	24.40ab	
Treatment 2	18.54b	
Treatment 3	37.22a	
Pooled SEM		
P-value		

2.5 Liver and Kidney Histology

As shown in Figure 1 [Figure 1: see original paper], panel A exhibited intact and clear hepatic cords with complete nuclei and no vacuolization. Panel B showed relatively intact hepatic cords with unchanged nuclei and only minimal vacuole-like structures indicating mild fatty degeneration. Panel C revealed prominent sinusoids with numerous Kupffer cells and some blood cells visible

in the sinusoidal lumen. Panels D, E, and F show renal cortical structure, demonstrating no significant changes in glomeruli among the three treatments. Panels G, H, and I show no obvious differences in renal tubules among the three treatments. Overall, there were no significant changes or pathological lesions in the kidneys across all three treatments.

A, B, and C were liver slices of treatments 1, 2, and 3, respectively. D, E, and F were glomerular slices, and G, H, and I were kidney tubular slices of treatments 1, 2, and 3, respectively. (400×)

Figure 1 Effects of vanadium-containing egg yolk powder on histopathological changes in liver and kidney tissues of rats

2.6 Liver and Kidney Biochemical Indicators

As shown in Tables 7 and 8 , there were no significant differences among the three treatments in liver and kidney SOD activity, MDA content, T-AOC, or liver ALT, AST, GST activities, and GSH content ($P > 0.05$). However, compared with treatment 1, treatments 2 and 3 significantly decreased liver NQO1 activity ($P < 0.05$).

Table 7 Effects of vanadium-containing egg yolk powder on oxidative stress status in liver and kidney of rats

Items	Liver	Kidney
	SOD/(U/mg prot)	MDA/(nmol/mg prot)
Treatment 1		
Treatment 2		
Treatment 3		
Pooled SEM		
P-value		

Table 8 Effects of vanadium-containing egg yolk powder on liver biochemical indices of rats

Items	ALT/(U/g prot)	AST/(U/g prot)	TG/(mmol/g prot)	GSH/(mol/g prot)	GST/(U/g prot)	NQO1/(g/mL)
Treatment 1	9.45a					
Treatment 2	8.36b					
Treatment 3	8.69b					
Pooled SEM						

Items	ALT/(U/g prot)	AST/(U/g prot)	TG/(mmol/g prot)	GSH/(mol/g prot)	GST/(U/g prot)	NQO1/(g/mL)
P-value						

2.7 Liver NQO1 and Nrf2 mRNA Expression

As shown in Table 9 , compared with treatment 1, treatments 2 and 3 significantly downregulated the expression of liver NQO1 and Nrf2 mRNA ($P < 0.05$).

Table 9 Effects of vanadium-containing egg yolk powder on relative mRNA expression levels of NQO1 and Nrf2 in liver of rats

Items	NQO1	Nrf2
Treatment 1	1.00a	1.00a
Treatment 2	0.79b	0.75b
Treatment 3	0.94b	0.73b
Pooled SEM		
P-value		

3.1 Effects of Vanadium-Containing Egg Yolk Powder on Rat Growth Performance

Vanadium is an essential trace element for the body [14], and low doses can promote animal growth and development [1]. Daniel et al. [15] reported that dietary vanadium levels below 23 mg/kg did not affect rat growth. Previous studies have shown that the dose of vanadate causing poisoning in mammals is 17 mg/kg body weight [16], the toxic dose of ammonium metavanadate in rats is 11.2 mg/kg body weight, and the critical value for vanadium toxicity in humans is 3 mg/day [17]. In this experiment, the dietary vanadium contents in the three treatments were 0.107, 0.137, and 0.164 mg/kg, all relatively low levels. The study found no changes in mental status or feed intake among treatments, and no significant differences in final body weight or body weight gain. The highest vanadium intake per unit body weight among the three treatments was 0.38 mg/kg, with a maximum daily vanadium intake of 1.49×10^{3} mg/day, far below the toxic dose, indicating that vanadium-containing egg yolk powder had no negative effects on rat growth. Assuming humans consume one egg (60 g) daily from hens fed a diet containing 10 mg/kg vanadium, the estimated vanadium intake would be approximately 0.001 mg, far below the critical toxicity threshold.

3.2 Effects of Vanadium-Containing Egg Yolk Powder on Rat Health

Organ indices can intuitively reflect changes in animal organs and indicate health status. Faulkner et al. [18] reported that the minimum toxic dose of vanadium (ammonium metavanadate) causing poisoning in rats is 11.2–16.8 mg/kg. Domingo et al. [19] found that dietary vanadium supplementation below 50 mg/kg had no effect on organ indices of liver, kidney, spleen, or heart in rats. In this experiment, feeding rats diets with vanadium contents of 0.107, 0.137, and 0.164 mg/kg had no significant effects on liver, kidney, spleen, heart, or lung indices, consistent with previous reports. These results indicate that feeding laying hens diets containing 5 and 10 mg/kg vanadium resulted in low vanadium residues in the resulting egg yolk powder, insufficient to cause changes in organ indices in rats.

Changes in plasma AST and ALT activities can reflect liver health status, UN content changes can reflect kidney structural changes, TG content can reflect hepatic lipid metabolism function, and MDA content changes can indicate the degree of lipid peroxidation. Sun et al. [20] administered 5 mg/kg vanadium (ammonium metavanadate) to rats via drinking water and found no differences in serum ALT and AST activities compared with the control group. Liu et al. [4] fed 1-day-old broilers a diet containing 5 mg/kg vanadium until 42 days of age and found no significant changes in plasma SOD activity, T-AOC, or MDA content on days 14, 28, and 42. In this experiment, there were no significant differences in plasma ALT and AST activities or TG, UN, and MDA contents among treatments, consistent with previous studies.

Changes in tissue vanadium residues reflect deposition patterns and sites of vanadium in tissues and can serve as reference indicators of vanadium effects, while histomorphological observation can directly reveal structural changes in organs and reflect functional changes. Daniel et al. [15] showed that low-dose vanadium intake (1 mg/L sodium vanadate in drinking water) had no significant effect on tissue vanadium residues in rats. This experiment similarly found no significant differences in liver and kidney vanadium residues among rats fed the three types of egg yolk powder.

Liu et al. [4] added 5 mg/kg vanadium to broiler diets and observed no effects on organ histomorphology, indicating that low-dose vanadium had no obvious effect on liver and kidney structure. Histological sections from the three treatments in this experiment showed no obvious damage to liver or kidney tissue structure in treatments 2 and 3, possibly because the vanadium content was too low to cause tissue damage [4]. Changes in liver and kidney antioxidant capacity reflect the ability of these organs to resist external oxidants. GSH and GST are important components of the antioxidant system, and their content and activity can reflect changes in antioxidant capacity. SOD activity, T-AOC, and MDA content are fundamental indicators of antioxidant capacity. High-valence vanadium can cause oxidative stress and affect liver and kidney antioxidant capacity [21], resulting in organ damage. Liu et al. [4] fed 1-day-old broilers

a diet containing 5 mg/kg vanadium and confirmed no significant changes in liver SOD activity, T-AOC, GSH, or MDA content on days 14, 28, and 42. In this experiment, there were no significant differences in liver and kidney MDA content, SOD activity, T-AOC, or liver GSH and GST activities among the three treatments, indicating that vanadium in the egg yolk powder did not reach levels sufficient to affect these biochemical indicators in rats.

Numerous in vivo and in vitro studies in humans and rats have confirmed that vanadium can induce oxidative stress and oxidative damage in lung, liver, kidney, and small intestinal epithelial cells [7,21]. Nrf2 is a basic leucine zipper transcription factor that serves as a key nuclear factor regulating reactive oxygen species balance. NQO1 is a phase II detoxification enzyme regulated by Nrf2, and its activity increases under oxidative stress conditions [22]. Studies have found that vanadium (NH VO) can decrease NQO1 mRNA expression and increase oxidative stress in human liver cells (HepG2 and Hepa 1c1c7) [22]. Nrf2 also regulates the antioxidant enzyme system, modulating the activities of SOD, NQO1, and GST. In this experiment, treatments 2 and 3 significantly decreased liver NQO1 activity and reduced the mRNA expression of Nrf2 and NQO1, though the biological significance of these findings requires further investigation.

In conclusion, the three vanadium-containing egg yolk powders had no significant differential effects on rat growth performance, plasma biochemical indicators, or liver and kidney pathology. However, vanadium at 0.137 and 0.164 mg/kg significantly decreased liver NQO1 activity and downregulated the relative expression of liver NQO1 and Nrf2 mRNA.

References

[1] SCHWARZ K, MILNE D B. Growth effects of vanadium in rats [J]. Science, 1971, 174(4007): 426-428.
[2] HAMEL F G, DUCKWORTH W C. The relationship between insulin and vanadium metabolism in insulin target tissues [J]. Molecular and Cellular Biochemistry, 1995, 153(1/2): 95-102.
[3] ADACHI A, ASAI K, KOYAMA Y, et al. Subacute toxicity of vanadium in rats [J]. Japanese Journal of Toxicology and Environmental Health, 1997, 43(1): 27.
[4] LIU J, CUI H M, LIU X D, et al. Dietary high vanadium causes oxidative damage-induced renal and hepatic toxicity in broilers [J]. Biological Trace Element Research, 2012, 145(2): 189-200.
[5] DOMINGO J L, GOMEZ M, SANCHEZ D J, et al. Toxicology of vanadium compounds in diabetic rats: the action of chelating agents on vanadium accumulation [J]. Molecular and Cellular Biochemistry, 1995, 153(1): 233-240.
[6] UEBERSCHÄR K H, VOGT H, MATTHES S. Der einfluss von vanadiumzusätzen zum broiler- und legehennenfutter auf die leistungen der tiere und

auf rückstandsgehalte in den geweben und eiern [J]. Archiv fur Geflugelkunde, 1985, 49: 4-8.
[7] IMURA H, SHIMADA A, NAOTA M, et al. Vanadium toxicity in mice: possible impairment of lipid metabolism and mucosal epithelial cell necrosis in the small intestine [J]. Toxicologic Pathology, 2013, 41(6): 842-856.
[8] REEVES P G, NIELSEN F H, FAHEY G C, Jr., et al. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet [J]. The Journal of Nutrition, 1993, 123(11): 1939-1951.
[9] 梁立娜, 李中阳, 陈登云, 等. ICP-MS 技术测定生物样品中重金属的前处理方法研究 [J]. 环境化学, 2003, 22(1): 99-100.
[10] 梁彦秋, 潘伟, 铁梅, 等. ICP-MS 测定动物肝脏中铅、镉和砷 [J]. 光谱实验室, 2006, 23(2): 234-237.
[11] HABEOS I G, ZIROS P G, CHARTOUMPEKIS D, et al. Simvastatin activates Keap1/Nrf2 signaling in rat liver [J]. Journal of Molecular Medicine, 2008, 86(11): 1279-1285.
[12] YURYEV A. PCR primer design [M]. Totowa, New Jersey: Humana Press, 2007.
[13] LIVAK K J, SCHMITTGEN T D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-ΔΔCT) method [J]. Methods, 2001, 25(4): 402-408.
[14] 魏磊磊. 钒营养研究进展 [J]. 中国饲料, 2004(9): 8-10.
[15] DANIEL E P, LILLIE R D. Experimental vanadium poisoning in the white rat [J]. Public Health Reports, 1938, 53(19): 765-777.
[16] LUCKEY T D, VENUGOPAL B, HUTCHESON D. Heavy metal toxicity, safety, and hormonology [M]. New York: Academic Press, 1975.
[17] ANKE M. Vanadium—An element both essential and toxic to plants, animals and humans [J]. Anales de la Real Academia Farmacia, 2005, 70(4): 961-999.
[18] FAULKNER G. Vanadium: toxicology and biological significance [M]. London: Elsevier, 1964.
[19] DOMINGO J L, LLOBET J M, TOMAS J M, et al. Short-term toxicity studies of vanadium in rats [J]. Journal of Applied Toxicology, 1985, 5(6): 418-421.
[20] 孙素玲, 周源, 刘森, 等. 偏钒酸铵对小鼠血清和肝脏转氨酶活力的影响 [J]. 生态毒理学报, 2011, 6(4): 445-448.
[21] CANO-GUTIÉRREZ G, ACEVEDO-NAVA S, SANTAMARÍA A, et al. Hepatic megalocytosis due to vanadium inhalation: participation of oxidative stress [J]. Toxicology and Industrial Health, 2012, 28(4): 353-360.
[22] KENNEDY G C, MITRA J. Body weight and food intake as initiating factors for puberty in the rat [J]. The Journal of Physiology, 1963, 166(2): 408-418.
[23] ADACHI A, ASAI K, KOYAMA Y, et al. Subacute vanadium toxicity in rats [J]. Journal of Health Science, 2000, 46(6): 503-508.

Note: Figure translations are in progress. See original paper for figures.