Vanadium in high-fat diets sourced from egg yolk decreases growth and antioxidative status of Wistar rats

Jian-Ping Wang 1, Ren-Yong Cui 1, Xue-Mei Ding, Shi-Ping Bai, Qiu-Feng Zeng, Huan-Wei Peng, Ke-Ying Zhang*

Animal Nutrition Institute, Key Laboratory of Animal Disease-Resistance Nutrition, Ministry of Education, Ministry of Agriculture and Sichuan Province, Sichuan Agricultural University, Chengdu, 611130, China

A B S T R A C T

The objective of this paper was to evaluate the effect of vanadium (V) in high-fat diets sourced from egg yolk on body weight gain, feed intake, blood characteristics and antioxidative status of Wistar rats. A total of 72 female Wistar rats were allocated according to a $2^2\times 4$ factorial design throughout a 5-wk trial, including 2 levels of dietary fat (normal and high; ether extract 40.3 and 301.2 g/kg; fat sourced from egg yolk) and 4 levels of dietary V (0, 3, 15 and 30 mg/kg). Vanadium decreased ($P < 0.05$) body weight gain (V at 30 mg/kg during wk 1 and 2; V at 15 and 30 mg/kg during the overall phase), feed intake (V at 30 mg/kg during wk 3 and the overall phase; V at 15 and 30 mg/kg during wk 4), but increased the relative weight of liver (V at 30 mg/kg, $P < 0.05$). Moreover, increasing dietary V significantly increased ($P < 0.05$) plasma aspartate aminotransferase, alanine aminotransferase and malondialdehyde levels and decreased triglyceride level, and V at 30 mg/kg in high-fat treatment had the highest or lowest values (interaction, $P < 0.05$). Under the same dietary V dose, V residual content in liver (dietary V at 15 and 30 mg/kg) and kidney (dietary V at 15 mg/kg) was higher in high-fat diet treatment compared with normal-fat diet treatment ($P < 0.05$). In conclusion, it is suggested that V could decrease the body weight together with the feed intake, and the high fat could enhance oxidative stress induced by V of Wistar rats.

© 2019, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Vanadium (V), as a transition element that exists widely on earth (Nriagu, 1998; Habib and Ibrahim, 2011; Sullivan and Leavely, 2011), is essential in life processes (Schwarz and Milne, 1971). Vanadium compounds have been proposed as new antidiabetic drugs because of their insulin-mimetic and insulin-enhancing effects both in vitro and in vivo (Blondel et al., 1989; Sekar et al., 1996; Marzban and McNeill, 2003). However, excess V was shown to be toxic for animals, especially for layers (Wang et al., 2016; Yuan et al., 2016). Vanadate (V$^{5+}$) and vanadyl (V$^{4+}$) may cause lots of adverse toxic effects in mammals depending on its exposed levels. Hematological and biochemical changes (Uche et al., 2008; Kamal et al., 2012), loss of body weight (Imura et al., 2013), reproductive toxicity (Valko et al., 2005), necrosis of hepatocytes with fatty cell infiltration and vacuolation (Cortizo et al., 2000), necrosis of renal tubules (Wei et al., 1982), gastrointestinal problems, e.g. diarrhea, dehydration (Heyliger et al., 1985), and even death were reported to occur in mammals following excessive V exposure (Strasia, 1971). Rats fed high-fat diets exhibited significantly increases in body weight, basal plasma glucose, insulin, triglycerides and total cholesterol levels as compared to normal-fat diet-fed control rats (Lissner et al., 1987). High-fat diets reduced glucose disappearance rate and impaired the antioxidant status (Storlien et al., 1987), which suggested high-fat diets may increase insulin-resistance (Ng et al., 2010). A previous study has shown that the toxic effects of V
in mice may increase as high dietary fat level increases (Sekar et al., 1996; Imura et al., 2013). Mice fed high-fat diets with V (ammonium metavanadate, NH₄VO₃) at 1 to 5 mg V/kg for 10 d showed severe clinical and pathological changes with decreased survival rate (Imura et al., 2013). Layers are sensitive to the toxicity of V. In our previous study, it was found that layer diet supplemented with V could increase the V deposition in eggs, especially in egg yolk (Wang et al., 2017). Egg yolk, which is enriched in lipids and fatty acid, may increase the safety risk of humans for the person who has V-contaminated egg yolk included high-fat food. However, the exact effect of V in high-fat diets on animal health is still not known. Therefore, the purpose of this research was to study the effect of V toxicity in high-fat diets sourced from egg yolk on growth, blood characteristics and antioxidative status in rats.

2. Materials and methods

2.1. Animals and diets

The experiment protocol was approved by committee of Animal Nutrition Institute of Sichuan Agricultural University. A total of 72 female Wistar rats, weighing 55 to 60 g (3-wk-old), were purchased from DaSuo Biological Science and Technology (Chen Du, China). The rats were randomly allotted into 8 treatments involving a 2×4 factorial arrangement, which included 2 dietary fat levels (normal and high; ether extract 40.3 vs. 301.2 g/kg; fat sourced from egg yolk) and 4 V levels at 0, 3, 15, 30 mg/kg. The high fat was maintained by adding egg yolk powder. Ammonium metavanadate (99.9%) was purchased from Shanghai Hengdelao Trading Co., Ltd. (Shanghai, China). The chilled plasma samples were slowly unfrozen until complete at 4°C. Then, the samples were taken into reaction system in accordance with the manufacture of the reagent kit. After the samples reacted with reagents, the reaction solution were used to colorimetric in Multiskan spectrum (1500, Thermo scientific) and absorbency data were used to calculate the levels.

Table 1 Composition of normal- and high-fat diet (g/kg, as fed basis).

| Item                      | Normal-fat diet | High-fat diet |
|---------------------------|----------------|--------------|
| Ingredients               |                |              |
| Corn starch               | 317.2          | 215.0        |
| Casein                    | 286.0          | 50.0         |
| Egg yolk powder           | 600.0          |              |
| Dextrinized cornstarch    | 100.0          | 10.0         |
| Sucrose                   | 130.0          | 3.2          |
| Soybean oil               | 60.0           | 30.0         |
| Wheat bran                | 24.0           | 49.0         |
| Fiber                     | 28.0           | 1.5          |
| Dicalcium phosphate       | 24.0           |              |
| Limestone                 | 0.3            |              |
| DL-methionine             | 3.0            |              |
| Choline chloride          | 2.5            |              |
| Nutrient levels           |                |              |
| Crude protein             | 213.2          | 210.7        |
| ME, MJ/kg                 | 14.4           | 21.1         |
| Crude fiber               | 51.0           | 51.0         |
| Calcium                   | 7.9            | 7.9          |
| Available phosphorus      | 5.7            | 5.7          |
| Methionine                | 7.4            |              |
| Cysteine                  | 3.9            |              |
| Ether extract             | 40.3           | 301.2        |

1 Mineral premix provided the following per kilogram of diet: Cu 6 mg, Fe 35 mg, Mn 11 mg, Zn 35 mg, Se 0.17 mg, 10.21 mg, Na 1.3 g.

2 Vitamin premix provided the following per kilogram of diet: vitamin A 10,000 IU, vitamin D₃ 3,000 IU, vitamin E 22.5 IU, vitamin K 3 mg, thiamin 3 mg, riboflavin 7.5 mg, pyridoxine 4.5 mg, vitamin B₁₂ 30 μg, nicotinic acid 300 mg, calcium pantothenate 15 mg, folic acid 1.5 mg, D-biotin 120 μg.

2.2. Animal management and sampling

The rats were in a cage with free access to feed and water during the whole experiment. There was a 1-wk adjusting period prior to experiment. Body weight and feed intake were recorded each week. At 35 d of the experiment, all the rats were sampled for blood from eyeballs and sacrificed by cervical dislocation after ether anesthesia. Tissues (kidney, liver, lung, heart and spleen) were taken immediately, weighed and stored at −20°C for further assay.

2.3. Plasma parameters

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), blood urea nitrogen (BUN), triglyceride (TG) of plasma were determined by reagent kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The chilled plasma samples were slowly unfrozen until complete at 4°C. Then, the samples were taken into reaction system in accordance with the manufacture of the reagent kit. After the samples reacted with reagents, the reaction solution were used to colorimetric in Multiskan spectrum (1500, Thermo scientific) and absorbency data were used to calculate the levels.

2.4. Hepatic and renal residual determining

At 35 d of the experiment, livers and kidneys in each group were taken and 0.2 g samples of each treatment were weighed out. These samples were treated with 2 mL chromatographically pure HNO₃ and 1 mL H₂O₂, and dissolved with the automatic microwave-heated digestion system (Multiwave 3000). The digestive produce was diluted to 25 mL with deionized water. Also, the blank of the reagents was carried out following the same procedure without samples. Then the V content in dilutions was determined by inductively coupled plasma mass spectrometry (ICP-MS) (7500a, Agilent Technologies Inc.).

2.5. Statistical analyses

Two levels of dietary fat (normal and high) and 4 levels of dietary V (0, 3, 15 and 30 mg/kg) were analyzed as a 2×4 factorial design by General Linear Model (GLM) using SAS9.0 (SAS Institute Inc., Cary, NC, USA). The main effects included V level and fat level, and their interaction were also determined. Polynomial regression analysis between V residual and dietary V supplementation level was carried out. A level of P ≤ 0.05 was used to indicate statistical significance.

3. Results

3.1. Growth performance

Vanadium (more than 15 mg/kg) decreased (P ≤ 0.05) body weight at wk 2, 4 and 5, whereas high-fat diet alone increased (P ≤ 0.05) body weight of rats from 2 to 5 wk (Table 2). An interaction between V and high fat were also observed on body weight (interaction, P ≤ 0.05), and the supplementation of V at 30 mg/kg in high-fat diet had the lowest body weight from 2 to 4 wk. The linear regression equation between body weight and experiment period is shown in Fig. 1, and the R² of all equation is more than 0.85 (P ≤ 0.05).

As shown in Table 3, the result of body weight gain of rats fed V followed the same trend as that of body weight as shown above. It
was observed that body weight gain was decreased \((P < 0.05)\) as the V supplementation levels increased at all weeks except for wk 4 and during the overall phase, and high-fat diet alone increased body weight gain \((P < 0.05)\) from 2 to 5 wk and the overall phase. An interaction \((P < 0.05)\) between V and high fat also observed on feed intake during wk 2, 4, 5 and the overall phase, and feeding rats with V in no fat addition diet had lower feed intake.

The effect of high-fat diet alone improved \((P < 0.05)\) the feed conversion rate (FCR) since wk 2, whereas inclusion of V decreased (more than 15 mg/kg; \(P < 0.05\)) the FCR in spite of the fat addition during wk 1, 5 and the overall phase, and V at 30 mg/kg decreased \((P < 0.05)\) the FCR (Table 5). Also, adding V in normal-fat diet was found to have lower (interaction, \(P < 0.05\)) feed efficiency in wk 1, 2 and the overall phase.

### 3.2. Relative organ weight

The result of relative organ weight is shown in Table 6. Vanadium (30 mg/kg) alone increased \((P < 0.05)\) the relative weight of

---

**Table 2**

Effect of vanadium (V) and fat level on body weight of Wistar rat.\(^1\)

| Item                        | Week 0 (Initial) | 1     | 2     | 3     | 4     | 5     |
|-----------------------------|------------------|-------|-------|-------|-------|-------|
| Fat level V, mg/kg          |                  |       |       |       |       |       |
| High                        | 94.4             | 104.3\(^a\) | 124.5\(^a\) | 114.8\(^ab\) | 166.7\(^a\) | 188.4\(^a\) |
| 3                           | 93.5             | 98.1\(^ab\)  | 122.5\(^a\)  | 148.1\(^a\)  | 169.4\(^a\) | 190.7\(^a\) |
| 15                          | 95.7             | 94.7\(^abc\) | 110.9\(^ab\) | 129.2\(^bc\) | 147.7\(^b\) | 165.6\(^b\) |
| 30                          | 95.6             | 83.8\(^ab\)  | 103.4\(^c\)  | 113.2\(^cd\) | 122.2\(^c\) | 129.6\(^c\) |
| Normal                      | 93.6             | 95.4\(^abc\) | 117.2\(^ab\) | 132.2\(^sd\) | 138.1\(^cd\) |       |
| 3                           | 98.9             | 101.7\(^ab\) | 111.7\(^ab\) | 125.2\(^a\)  | 132.6\(^bd\) | 138.7\(^c\) |
| 15                          | 94.1             | 94.5\(^abc\) | 96.6\(^d\)   | 104.2\(^d\)  | 108.9\(^d\) | 110.8\(^d\) |
| 30                          | 92.0             | 99.1\(^ab\)  | 105.3\(^bc\) | 117.0\(^c\)  | 126.9\(^cd\) | 130.7\(^d\) |
| Pooled SEM                  | 4.39             | 3.94   | 4.38   | 5.62   | 7.38   | 7.83   |
| \(P\)-value                 | 0.994            | 0.020  | <0.001 | <0.001 | <0.001 | <0.001 |
| Main effect                 |                  |       |       |       |       |       |
| Fat level                   |                  |       |       |       |       |       |
| High                        | 94.7             | 95.3   | 112.1\(^a\) | 131.4\(^a\) | 150.8\(^a\) | 169.5\(^a\) |
| Normal                      | 94.7             | 97.5   | 104.1\(^b\) | 115.1\(^b\) | 122.6\(^b\) | 128.2\(^b\) |
| Pooled SEM                  | 2.20             | 2.04   | 2.30   | 3.00   | 3.43   | 3.86   |
| V, mg/kg                    |                  |       |       |       |       |       |
| 0                           | 94.0             | 99.6   | 113.2\(^a\) | 128.4\(^a\) | 143.2\(^a\) | 157.3\(^a\) |
| 3                           | 95.9             | 99.7   | 117.3\(^a\) | 138.1\(^a\) | 153.4\(^a\) | 167.9\(^a\) |
| 15                          | 94.9             | 94.6   | 103.8\(^b\) | 116.7\(^bc\) | 128.3\(^b\) | 140.3\(^b\) |
| 30                          | 94.1             | 90.9   | 96.7\(^ab\) | 108.8\(^b\) | 121.2\(^b\) | 130.4\(^b\) |
| Pooled SEM                  | 3.12             | 2.88   | 3.26   | 4.23   | 4.84   | 5.45   |
| \(P\)-value\(^2\)           |                  |       |       |       |       |       |
| Fat                         | 0.984            | 0.456  | 0.018  | <0.001 | <0.001 | <0.001 |
| V                           | 0.970            | 0.098  | <0.001 | <0.001 | <0.001 | <0.001 |
| \(Fat \times V\)            | 0.782            | 0.032  | <0.001 | <0.001 | <0.001 | <0.001 |

\(^a, b\) Means in the same column without common superscripts differ significantly \((P < 0.05)\).

\(^1\) Each mean represents 9 replicates, with 1 rat per replicate.

\(^2\) Main effect was analyzed by 2 fat levels (high and normal) and 4 V levels (0, 3, 15 and 30 mg/kg).

---

**Fig. 1.** The polynomial regression equation between body weight and experiment time. (A) The linear regression equation between body weight in rats fed high-fat diet and experiment time; (B) The linear relationship of body weight in rats fed normal-fat diet and experiment time. Treatment 1: high-fat diet; treatment 2: high-fat diet supplemented with 3 mg/kg vanadium (V); treatment 3: high-fat diet supplemented with V at 15 mg/kg; treatment 4: high-fat diet supplemented with V at 30 mg/kg; treatment 5: normal-fat diet; treatment 6: normal-fat diet supplemented with V at 3 mg/kg; treatment 7: normal fat diet supplemented with V at 15 mg/kg; treatment 8: normal-fat diet supplemented with V at 30 mg/kg.
liver, whereas the high-fat diet led to lower ($P \leq 0.05$) relative weight of kidney in spite of V level. Moreover, fed rats with V at 30 mg/kg in high-fat diet induced much higher (interaction, $P \leq 0.05$) liver weight compared with the groups fed V alone.

### 3.3. Blood characteristics

As shown in Table 7, plasma AST, ALT, MDA and BUN levels were higher ($P \leq 0.05$) and TG level was lower ($P \leq 0.05$) in

---

**Table 3**

Effect of vanadium (V) and fat level on body weight gain of Wistar rat.

| Item | Week | Overall phase |
|------|------|--------------|
| Fat level | V, mg/kg | 1 | 2 | 3 | 4 | 5 |  |
| High 0 | 9.9<sup>a</sup> | 20.1<sup>ab</sup> | 20.4<sup>b</sup> | 21.9<sup>a</sup> | 21.7<sup>a</sup> | 94.0<sup>a</sup> |  |
| High 3 | 4.5<sup>b</sup> | 24.5<sup>a</sup> | 25.6<sup>a</sup> | 21.8<sup>a</sup> | 20.7<sup>ab</sup> | 97.1<sup>a</sup> |  |
| High 15 | –1.0<sup>b</sup> | 16.1<sup>c</sup> | 18.4<sup>bc</sup> | 18.4<sup>a</sup> | 17.9<sup>ab</sup> | 69.9<sup>b</sup> |  |
| High 30 | –11.8<sup>d</sup> | 5.4<sup>b</sup> | 11.8<sup>d</sup> | 15.2<sup>b</sup> | 14.0<sup>d</sup> | 34.5<sup>d</sup> |  |
| Normal 0 | 1.8<sup>b</sup> | 7.8<sup>d</sup> | 10.6<sup>d</sup> | 8.4<sup>b</sup> | 7.4<sup>c</sup> | 36.0<sup>d</sup> |  |
| Normal 3 | 2.8<sup>b</sup> | 10.0<sup>cd</sup> | 13.5<sup>bc</sup> | 7.1<sup>b</sup> | 6.5<sup>c</sup> | 39.8<sup>c</sup> |  |
| Normal 15 | 0.5<sup>b</sup> | 2.2<sup>d</sup> | 7.5<sup>d</sup> | 4.1<sup>c</sup> | 1.1<sup>c</sup> | 15.6<sup>d</sup> |  |
| Normal 30 | 6.6<sup>b</sup> | 6.2<sup>d</sup> | 12.4<sup>d</sup> | 9.1<sup>b</sup> | 3.8<sup>c</sup> | 38.2<sup>c</sup> |  |
| P–value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |  |
| Main effect | Fat level | 0.6 | 16.8<sup>a</sup> | 19.3<sup>a</sup> | 19.4<sup>a</sup> | 18.7<sup>a</sup> | 74.8<sup>a</sup> |  |
| | Normal | 2.8 | 6.6<sup>b</sup> | 11.0<sup>b</sup> | 7.4<sup>b</sup> | 5.0<sup>b</sup> | 33.2<sup>b</sup> |  |
| | Pooled SEM | 1.3 | 1.28 | 1.34 | 1.34 | 1.05 | 3.45 |  |
| | V, mg/kg | 0 | 13.6<sup>ab</sup> | 15.2<sup>ab</sup> | 14.7 | 14.1<sup>b</sup> | 63.3<sup>a</sup> |  |
| | 3 | 3.8<sup>a</sup> | 18.1<sup>a</sup> | 20.3<sup>a</sup> | 15.3 | 14.5<sup>s</sup> | 72.1<sup>a</sup> |  |
| | 15 | –0.3<sup>b</sup> | 9.2<sup>bc</sup> | 13.0<sup>b</sup> | 11.6 | 10.1<sup>b</sup> | 44.8<sup>b</sup> |  |
| | 30 | –3.2<sup>b</sup> | 5.8<sup>c</sup> | 12.1<sup>b</sup> | 12.4 | 9.2<sup>b</sup> | 36.3<sup>b</sup> |  |
| | P–value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |  |
| a, b, c | Means in the same column without common superscripts differ significantly ($P < 0.05$).

1 Each mean represents 9 replicates, with 1 rat per replicate.

2 Main effect was analyzed by 2 fat levels (high and normal) and 4 V levels (0, 3, 15 and 30 mg/kg).

---

**Table 4**

Effect of vanadium (V) and fat level on daily and accumulated feed intake of Wistar rat.

| Item | Week | Overall phase |
|------|------|--------------|
| Fat level | V, mg/kg | 1 | 2 | 3 | 4 | 5 |  |
| High 0 | 7.5<sup>abc</sup> | 8.0<sup>d</sup> | 9.4<sup>de</sup> | 10.6<sup>e</sup> | 10.8<sup>e</sup> | 323.6<sup>e</sup> |  |
| High 3 | 7.8<sup>ab</sup> | 8.4<sup>d</sup> | 9.6<sup>b</sup> | 10.7<sup>c</sup> | 11.0<sup>c</sup> | 312.6<sup>c</sup> |  |
| High 15 | 6.9<sup>bc</sup> | 7.8<sup>d</sup> | 8.6<sup>bc</sup> | 9.6<sup>bc</sup> | 10.6<sup>bc</sup> | 304.1<sup>bc</sup> |  |
| High 30 | 6.0<sup>d</sup> | 6.6<sup>b</sup> | 6.6<sup>d</sup> | 7.6<sup>bc</sup> | 7.6<sup>bc</sup> | 244.8<sup>de</sup> |  |
| Normal 0 | 6.9<sup>bc</sup> | 7.5<sup>ab</sup> | 8.1<sup>cde</sup> | 8.1<sup>cde</sup> | 7.2<sup>ce</sup> | 264.2<sup>cde</sup> |  |
| Normal 3 | 8.1<sup>b</sup> | 7.9<sup>c</sup> | 8.8<sup>bcd</sup> | 8.9<sup>bd</sup> | 8.5<sup>c</sup> | 295.2<sup>de</sup> |  |
| Normal 15 | 6.4<sup>ab</sup> | 7.0<sup>b</sup> | 7.1<sup>d</sup> | 6.5<sup>d</sup> | 6.2<sup>c</sup> | 230.3<sup>de</sup> |  |
| Normal 30 | 7.2<sup>bc</sup> | 7.9<sup>b</sup> | 8.1<sup>bc</sup> | 8.2<sup>bc</sup> | 7.8<sup>bc</sup> | 274.3<sup>de</sup> |  |
| P–value | 0.062 | 0.017 | <0.001 | <0.001 | <0.001 | <0.001 |  |
| Main effect | Fat level | 7.1 | 7.7 | 8.7 | 9.6<sup>a</sup> | 10.1<sup>a</sup> | 302.2<sup>a</sup> |  |
| | Normal | 7.1 | 7.5 | 8.0 | 7.9<sup>b</sup> | 7.5<sup>b</sup> | 267.1<sup>b</sup> |  |
| | Pooled SEM | 0.23 | 0.2 | 0.23 | 0.29 | 0.28 | 6.77 |  |
| | V, mg/kg | 0 | 7.7<sup>bc</sup> | 7.7<sup>bc</sup> | 8.7<sup>bc</sup> | 9.3<sup>a</sup> | 8.6<sup>b</sup> | 292.2<sup>de</sup> |  |
| | 3 | 8.0<sup>b</sup> | 8.2<sup>d</sup> | 9.3<sup>b</sup> | 9.9<sup>a</sup> | 9.9<sup>a</sup> | 316.2<sup>a</sup> |  |
| | 15 | 6.6<sup>b</sup> | 7.2<sup>d</sup> | 7.9<sup>bc</sup> | 8.1<sup>b</sup> | 8.6<sup>b</sup> | 270.0<sup>d</sup> |  |
| | 30 | 6.6<sup>b</sup> | 7.2<sup>d</sup> | 7.4<sup>bc</sup> | 7.9<sup>b</sup> | 8.0<sup>a</sup> | 258.6<sup>a</sup> |  |
| | P–value | 0.34 | 0.28 | 0.32 | 0.42 | 0.40 | 9.56 |  |
| a, b, c | Means in the same column without common superscripts differ significantly ($P < 0.05$).

1 Each mean represents 9 replicates, with 1 rat per replicate.

2 Main effect was analyzed by 2 fat levels (high and normal) and 4 V levels (0, 3, 15 and 30 mg/kg).
rats fed diets containing V in spite of fat addition, whereas high-fat diet alone increased \((P \leq 0.05)\) TG and decreased \((P \leq 0.05)\) BUN. Aspartate aminotransferase and BUN were increased (interaction, \(P \leq 0.05\)) in V and high-fat containing diet, and high-fat diet with V (30 mg/kg) had the highest level.

### 3.4. Hepatic and renal V residual content

The linear equation between hepatic and renal V residual in both high- and normal-fat and experiment time were shown in Fig. 2. Under the same V dose, V residual content in liver and kidney were higher in high-fat diet compared with normal-fat diet \((P \leq 0.05)\).

### 4. Discussion

In the current study, we found that V given in feed at 15 and 30 mg/kg decreased body weight, body weight gain and feed intake, and V at 30 mg/kg in high-fat diet had the lowest body weight throughout the whole experimental period. It has been demonstrated that oral or injection of high dosage of V (more than 50 mg/kg) induce growth retardation and feed intake reduction in rats (Parker and Sharma, 1977; Kurt et al., 2011). It was also reported that injections of V at 10 mg/kg per day for 8 consecutive days led to a diarrhea, decreased feed intake and weight gain in rats (Varga et al., 2005). Moreover, the growth reduction was more obvious in high-fat diets at the present study. This is in accordance with the results of Imura et al. (2013), who reported that the body weight in groups given V at 20 mg/kg in high-fat diet per day was significantly lower compared to normal-fat diet.

Upon supplementation, V can be incorporated in various organs and tissues including the liver, kidney, brain, muscle and bone (Borges et al., 2003; Srinivasan et al., 2005; Wang et al., 2017). As shown in our study, V addition at levels ranging from 15 to 30 mg/kg increased the V deposition in the liver and kidney. Previous studies also revealed that the V content in kidney and liver were increased by V (30, 45, and 60 mg/kg) in a dose dependent manner in broilers and layers (Liu et al., 2012; Wang et al., 2016;
Yuan et al., 2016). Our results also suggested high fat level increased V deposition in the kidney, not in the liver; however, the reason is still not known. It was reported that V is poorly (only about 10%) absorbed in the gastrointestinal tract (Nriagu, 1998), and the high fat level may increase the V absorption by increasing passing time in the intestine and to increase its deposition in the target tissues, such as the bone, kidney, and liver.

Oxidative stress induction effect of V has been reported in many previous studies, which may be because V could alter antioxidant enzymes and lipid peroxidation (MDA). Plasma parameter changes reflect partly inner organ capacity. When hepatic damage suffered from poison or heavy metal, plasma ALT and AST activities increased. Increase of plasma MDA content reflected raise of hepatic and renal lipid peroxidation extent. Blood TG content showed hepatic lipid metabolism status and increasing plasma BUN content suggested renal damage. This paper showed that given V at 15 and 30 mg/kg significantly increased blood ALT, AST, BUN, and MDA levels of groups received high-fat diet. Similarly, previous studies showed that the injecting or orally ingestion of more than 10 mg V/kg resulted in the increased serum TG, AST and ALT levels of rats (Aarati and Ani, 2004; Liu et al., 2012; Hosseini et al., 2013). But the result on BUN is not in consistent with the studies of Cam et al. (1993) and Clark et al. (2004), who found that the V (0.75 mg/mL vanadyl sulfate; 30 mg/mL sodium) supplied in drinking water did not affect the plasma BUN content. The difference may be due to the different inclusion levels and administration method. In other studies, high-fat diet was shown to reduce the antioxidant enzyme activities and increase AST, ALT and MDA production (Sekar et al., 1990; Nanji et al., 1995; Shyamala et al., 2003). Therefore, the result of this research suggested that high-fat diet with can decrease the hepatic detoxifying capacity.

Liver is the main detoxification organ whereas kidney is the primary route for drug excretion, so the residual content of V in liver and kidney are much higher than in other organs, and there is a significant difference in the V content between liver and kidney.

### Table 7

The effect of dietary vanadium (V) in high-fat on blood characteristics.

| Item          | AST, U/L | ALT, U/L | MDA, nmol/mL | TG, mmol/L | BUN, mmol/L |
|---------------|----------|----------|--------------|------------|-------------|
| **Fat level** |          |          |              |            |             |
| High 0        | 16.98d   | 11.84c   | 15.07c       | 2.78a      | 14.78c      |
| High 3        | 18.20d   | 13.27c   | 15.76bc      | 1.81b      | 18.11c      |
| High 15       | 19.66bc  | 14.26c   | 16.78ab      | 1.60a      | 18.39bc     |
| High 30       | 21.48c   | 15.70a   | 17.87a       | 1.51b      | 25.20b      |
| Normal 0      | 18.66c   | 12.71bc  | 15.21bc      | 1.54c      | 22.11c      |
| Normal 3      | 18.96bc  | 12.94bc  | 15.77bc      | 1.56c      | 23.74c      |
| Normal 15     | 19.21bc  | 13.17bc  | 15.94bc      | 1.54c      | 24.70c      |
| Normal 30     | 20.70ab  | 13.81b   | 16.17bc      | 1.60c      | 24.50c      |
| **Pooled SEM** | 0.55     | 0.59     | 0.50         | 0.10       | 1.33        |
| **P-value**   |           |          |              |            |             |
| Fat level      |          |          |              |            |             |
| High          | 19.08    | 13.76    | 16.37        | 1.92a      | 19.31b      |
| Normal        | 19.32    | 13.16    | 15.77        | 1.56b      | 23.76c      |
| **Pooled SEM** | 0.27     | 0.29     | 0.25         | 0.05       | 0.66        |
| V, mg/kg      |          |          |              |            |             |
| 0             | 17.97c   | 12.27c   | 15.14c       | 2.16a      | 18.77c      |
| 3             | 18.62bc  | 13.11bc  | 15.76bc      | 1.68b      | 20.92b      |
| 15            | 19.43bc  | 13.72ab  | 16.36ab      | 1.57b      | 21.55b      |
| 30            | 20.78a   | 14.75a   | 17.02a       | 1.55b      | 24.85a      |
| **P-value**   |           |          |              |            |             |
| Fat           | 0.541    | 0.153    | 0.098        | <0.001     | <0.001      |
| V             | <0.001   | 0.004    | 0.027        | <0.001     | <0.001      |
| Fat × V       | 0.020    | 0.130    | 0.237        | <0.001     | 0.018       |

AST = aspartate aminotransferase; ALT = alanine aminotransferase; MDA = malondialdehyde; TG = triglyceride; BUN = blood urea nitrogen.

+ a, b, c Means in the same column without common superscripts differ significantly (P < 0.05).

1 Each mean represents 9 replicates, with 1 rat per replicate.

2 Main effect was analyzed by 2 fat levels (high and normal) and 4 V levels (0, 3, 15 and 30 mg/kg).
a linear relationship between V residual and dietary V contents (Sharma et al., 1980). Many studies obtained the same results in different animals. Liu (Liu et al., 2012) proved that there is a linear relationship between hepatic and renal residual V content and dietary V in boilers, and Bogden et al. (1982) achieved similar results in rats. When dietary V over 30 mg/kg was added, the hepatic V residual content in high-fat group was more than that in normal-fat group, and the renal V residual content was also higher. These results suggested that V was deposited easier in the kidney of rats fed high-fat diets compared to the normal-fat diet.

5. Conclusion

In conclusion, dietary V over 15 mg/kg can cause body weight loss, feed intake and liver relative weight reduction, and can increase plasma ALT, AST, MDA and BUN levels, and hepatic and renal V residuals. Moreover, the adverse effect of V in its deposition, and renal and hepatic oxidative stress are more obvious in high-fat diets of Wistar rats.

Conflict of interest

We declare that we have no financial and personal relationships with or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This project was fanatically supported by Ministry of Science and Technology Support Program (2014BAD13B04), National Natural Science Foundation of China (31402031), Department of Education Project of Sichuan Province (2014NZ0043, 2013NZ0054), and Department of Science and Technology Project of Sichuan Province (2014NZ0002, 2013NZ0054).

References

Aarati M, Ani M. Vanadyl sulfate ameliorates insulin resistance and restores plasma dehydroepiandrosterone-sulfate levels in fructose-fed, insulin-resistant rats. Clin Biochem 2004;37:694–7.
Blondel O, Baleille D, Porthe B. In vivo insulin resistance in streptozotocin-diabetic rats-evidence for reversal following oral vanadate treatment. Diabetologia 1989;32:185–90.
Borges G, Mendonça P, Joaquim N, Coucelo J, Aureliano M. Acute effects of vanadate on the histological and sperm parameters of male Guinea pigs. J Appl Sci Environ Manag 2003;7:297–302.
Hosseini MJ, Shafi K, Ghazikhansari M, Pourmahmud J. Toxicity of vanadium on isolated rat liver mitochondria: a new mechanistic approach. Metallomics 2013;5:552–66.
Imura H, Shimada A, Naota M, Morita T, Togawa M, Hasegawa T, et al. Vanadium toxicity in mice: possible impairment of lipid metabolism and mucosal epithelial cell necrosis in the small intestine. Toxicol Pathol 2013;41:842–56.
Kamal M, Tamara S, Shaban D. Investigation of antioxidant system activity in rats liver exposed to ammonium metavanadate and/or nickel sulfate. Adv Environ Biol 2012;6:24–32.
Kurt G, Ozden TY, Ozsoy N, Tunali S, Can A, Akev N, et al. Influence of vanadium supplementation on oxidative stress factors in the muscle of streptozotocin-diabetic rats. Biomolecules 2011;24:943–9.
Lissner L, Levitsky DA, Strupp BJ, Kallikwar HJ, Roe DA. Dietary fat and the regulation of energy intake in human subjects. Am J Clin Nutr 1987;46:886–92.
Liu J, Cui H, Liu X, Peng X, Deng J, Zuo Z, et al. Dietary high vanadium causes oxidative damage-induced renal and hepatic toxicity in broilers. Biol Trace Elem Res 2012;145:189–200.
Marzban L, McNeill JH. Insulin-like actions of vanadium: potential as a therapeutic agent. J Trace Elem Exp Med 2003;16:253–67.
Nanjee AA, Griniuviene B, Sadzadeh SM. Effect of type of dietary fat and ethanol on antioxidant enzyme mRNA induction in rat liver. J Lipid Res 1995;36:736–44.
Ng S, Lin RC, Laybhart DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs [heterogenic] dysfunction in female rat offspring. Nature 2010;467:963–6.
Nriagu JO. Vanadium in the environment (part 1, chemistry and biochemistry). [B]. New York, NY, 1998.
Park SR, Sharma RP. Accumulation and depletion of vanadium in selected tissues of rats treated with vanadyl sulfate and sodium orthovanadate. J Environ Pathol Toxicol 1977;2:235–45.
Schwarz K, Milne DB. Growth effects of vanadium in the rat. Science 1971;174:426–8.
Sekar N, Williams S, Balasubramaniyam N, Kamarajan P, Govindasamy S. Optimization of sodium orthovanadate to treat streptozotocin-induced diabetic rats. J Biosci 1990;15:67–75.
Sekar N, Li J, Shechter Y. Vanadium salts as insulin substitutes: mechanisms of action, a scientific and therapeutic tool in diabetes mellitus research. Crit Rev Biochem Mol 1996;31:339–59.
Sharma R, Oberg S, Parker R. Vanadium retention in rat tissues following acute exposures to different dose levels. J Environ Expos Health A 1980:6:45–54.
Shyamala MP, Venukumar MR, Latha MS. Antioxidant of the Syzygium aromaticum (Gaertn..) Linn. (CLOVES) in rats fed with high fat diet. Indian J Pharm 2003;35:99–103.
Srinivasan K, Viswanad B, Asrati L, Knul CI, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharm Res 2005;32:313–20.
Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 1987;237:885–8.
Strasas CA. Vanadium: essentiality and toxicity in the laboratory rat. Lafayette: Purdue University; 1971.
Sullivan MJ, Leavy S. Heavy metals in bottled natural spring water. J Environ Health 2011;73:8–13.
Uchii T, Oba-Shimizu AW, Gogo-Abite M. Effects of vanadium pentoxide on the histological and sperm parameters of male guinea pigs. J Appl Sci Environ Manag 2008;12:107–15.
Valko M, Morris H, Cronin M. Metals, toxicity and oxidative stress. Curr Med Chem 2005;12:1161–208.
Varga I, Szabeni A, Szoboszlai N, Szoboszlai N, Kovacs B. Determination of trace elements in human liver biopsy samples by ICP–MS and TXRF: hepatic steatosis and nickel accumulation. Anal Bioanal Chem 2005;383:476–82.
Wang JP, Cui RY, Zhang KY, Ding XM, Luo YH, Bai SP, et al. High-fat diet increased renal and hepatic oxidative stress induced by vanadium of Wistar rat. Biol Trace Elem Res 2016;170(2):415–23.
Wang JP, He KR, Ding XM, Bai SP, Zeng QF, Zhang KY. Effect of feeding and withdrawal of vanadium and vitamin C on egg quality and vanadium residual over time in laying hens. Biol Trace Elem Res 2017;171:367–75.
Wei CL, Al Bayati MA, Culbertson MR, Rosenblatt LS, Hansen LD. Acute toxicity of ammonium metavanadate in mice. J Toxicol Environ Health 1982;10:673–87.
Yuan ZH, Zhang KY, Ding DM, Luo YH, Bai SP, Zeng QF, et al. Effect of tea polyphenols on production performance, egg quality, and hepatic antioxidant status of laying hens in vanadium-containing diets. Poultry Sci 2016;95(7):1709–17.