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Original Research Article

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

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ABSTRACT

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 × 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 ($P \leq 0.05$) body weight gain (V at 30mg/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, $P \leq 0.05$). Moreover, increasing dietary V significantly increased ($P \leq 0.05$) 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, $P \leq 0.05$). 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 ($P \leq 0.05$). 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.

1. Introduction

Vanadium (V), as a transition element that exists widely on earth (Nriagu, 1998; Habib and Ibrahim, 2011; Sullivan and Leavey, 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

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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, NH_4VO_3) 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 (fat levels × V levels) 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). Table 1 shows the composition of the basal diets with normal and high fat. The analyzed value of V content in each treatment was 0.12, 2.63, 13.9, 28.6, 0.45, 3.06, 14.9, and 29.5 mg/kg by order, respectively. All the diets were provided in a pellet form.

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	
Wheat bran		30.0
Fiber	49.0	49.0
Dicalcium phosphate	28.0	
Limestone		1.5
Mineral premix[1]	24.0	24.0
Vitamin premix[2]	0.3	0.3
L-cysteine	3.0	
DL-methionine		1.0
Choline chloride	2.5	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	7.4
Cysteine	3.9	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, I 0.21 mg, Na 1.3 g.
[2] Vitamin premix provided the following per kilogram of diet: vitamin A 10,000 IU, vitamin D_3 3,000 IU, vitamin E 22.5 IU, vitamin K 3 mg, thiamin 3 mg, riboflavin 7.5 mg, pyridoxine 4.5 mg, vitamin B_{12} 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 Bioengieering 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 HNO_3 and 1 mL H_2O_2, and dissolved with the automatic microwave-heated digestion system (Multiwave 3000). The digestive production 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 $P \leq 0.05$ was used to indicate statistical significance.

3. Results

3.1. Growth performance

Vanadium (more than 15 mg/kg) decreased ($P \leq 0.05$) body weight at wk 2, 4 and 5, whereas high-fat diet alone increased ($P \leq 0.05$) 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, $P \leq 0.05$), 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 R^2 of all equation is more than 0.85 ($P \leq 0.05$).

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

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	0	94.4	104.3^{a}	124.5^{a}	114.8^{ab}	166.7^{a}	188.4^{a}
High	3	93.5	98.1^{ab}	122.5^{a}	148.1^{a}	169.9^{a}	190.7^{a}
High	15	95.7	94.7^{abc}	110.9^{ab}	129.2^{bc}	147.7^{b}	165.6^{b}
High	30	95.6	83.8^{c}	89.2^{d}	101.0^{d}	116.2^{cd}	130.2^{cd}
Normal	0	93.6	95.4^{abc}	103.2^{bcd}	113.8^{cd}	122.2^{cd}	129.6^{cd}
Normal	3	98.9	101.7^{ab}	111.7^{ab}	125.2^{c}	132.2^{bc}	138.7^{c}
Normal	15	94.1	94.5^{abc}	96.6^{cd}	104.2^{d}	108.9^{d}	110.8^{d}
Normal	30	92.0	99.1^{ab}	105.3^{bc}	117.8^{cd}	126.9^{cd}	130.7^{cd}
Pooled SEM		4.39	3.94	4.58	6.62	2.73	7.63
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^{ab}	143.2^{a}	157.3^{a}
	3	95.9	99.7	117.8^{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^{b}	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 × V		0.782	0.032	<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).

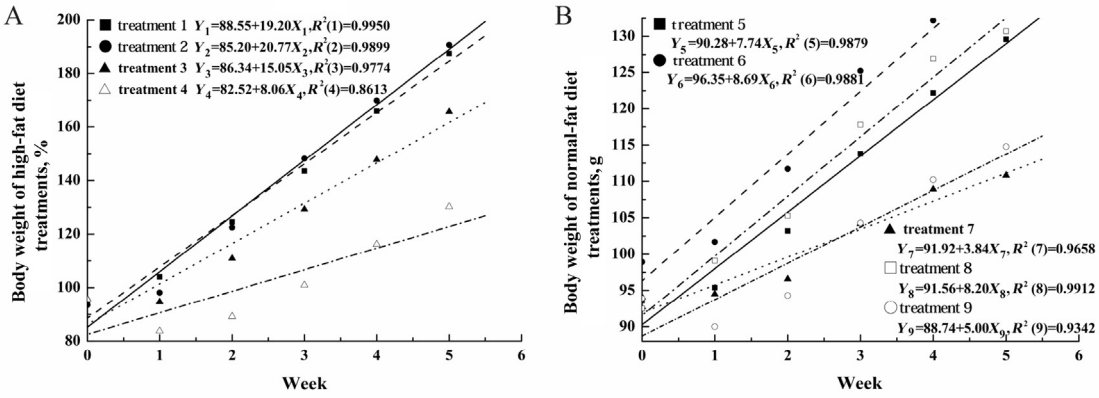

Fig. 1. The polynomial regression equation between body weight and experiment time. (A) The linear regression equation between body weight in rats fed high-fat diet and experiment time; (B) The linear relationship of body weight in rats fed normal-fat diet and experiment time. Treatment 1: high-fat diet; 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.

was observed that body weight gain was decreased ($P \leq 0.05$) 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 ($P \leq 0.05$) from 2 to 5 wk and the overall phase.

As shown in Table 4, V (30 mg/kg) decreased ($P \leq 0.05$) feed intake during wk 3 and the overall phase, V at 15 and 30 mg/kg decreased ($P \leq 0.05$) feed intake during wk 4. On the other hand, high-fat diet enhanced ($P \leq 0.05$) feed intake of rats in spite of V level during wk 4, 5 and the overall phase. An interaction ($P \leq 0.05$) 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 ($P \leq 0.05$) the feed conversion rate (FCR) since wk 2, whereas inclusion of V decreased (more than 15 mg/kg; $P \leq 0.05$) the FCR in spite of the fat addition during wk 1, 5 and the overall phase, and V at 30 mg/kg decreased ($P \leq 0.05$) the FCR (Table 5). Also, adding V in normal-fat diet was found to have lower (interaction, $P \leq 0.05$) 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 ($P \leq 0.05$) the relative weight of

Table 3
Effect of vanadium (V) and fat level on body weight gain of Wistar rat.[1]

Item		Week					Overall phase
		1	2	3	4	5	
Fat level	V, mg/kg						
High	0	9.9^{a}	20.1^{ab}	20.4^{ab}	21.9^{a}	21.7^{a}	94.0^{a}
High	3	4.5^{ab}	24.5^{a}	25.6^{a}	21.8^{a}	20.7^{ab}	97.1^{a}
High	15	−1.0^{bc}	16.1^{bc}	18.4^{abc}	18.4^{a}	17.9^{ab}	69.9^{b}
High	30	−11.8^{d}	5.4^{d}	11.8^{cd}	15.2^{ab}	14.0^{b}	34.6^{cd}
Normal	0	1.8^{bc}	7.8^{d}	10.6^{cd}	8.4^{bc}	7.4^{c}	36.0^{cd}
Normal	3	2.8^{abc}	10.0^{cd}	13.5^{bcd}	7.1^{bc}	6.5^{c}	39.8^{c}
Normal	15	0.5^{bc}	2.2^{d}	7.5^{d}	4.1^{c}	1.1^{c}	15.6^{d}
Normal	30	6.6^{ab}	6.2^{d}	12.4^{cd}	9.1^{bc}	3.8^{c}	38.2^{c}
Pooled SEM		2.55	2.66	2.71	2.57	2.3	7.05
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Main effect							
Fat level	High	0.6	16.8^{a}	19.3^{a}	19.4^{a}	18.7^{a}	74.8^{a}
	Normal	2.8	6.6^{b}	11.0^{b}	7.4^{b}	5.0^{b}	33.2^{b}
Pooled SEM		1.3	1.28	1.34	1.34	1.05	3.45
V, mg/kg	0	5.6^{a}	13.6^{ab}	15.2^{ab}	14.7	14.1^{ab}	63.3^{a}
	3	3.8^{a}	18.1^{a}	20.3^{a}	15.3	14.5^{a}	72.1^{a}
	15	−0.3^{ab}	9.2^{bc}	13.0^{b}	11.6	10.1^{ab}	44.8^{b}
	30	−3.2^{b}	5.8^{c}	12.1^{b}	12.4	9.2^{b}	36.3^{b}
Pooled SEM		1.84	1.81	1.90	1.90	1.50	4.94
P-value[2]							
Fat		0.259	<0.001	<0.001	<0.001	<0.001	<0.001
V		0.005	<0.001	0.001	0.445	0.026	<0.001
Fat × V		<0.001	0.0013	0.085	0.367	0.500	<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.[1]

Item		Week					Overall phase
		1	2	3	4	5	
Fat level	V, mg/kg						
High	0	7.5^{abc}	8.0^{a}	9.4^{ab}	10.6^{a}	10.8^{a}	323.6^{a}
High	3	7.8^{ab}	8.4^{a}	9.6^{a}	10.7^{a}	11.0^{a}	332.6^{a}
High	15	6.9^{abc}	7.8^{a}	8.6^{abc}	9.6^{ab}	10.6^{a}	304.1^{ab}
High	30	6.0^{c}	6.6^{b}	6.8^{d}	7.6^{cd}	8.0^{bc}	244.8^{de}
Normal	0	6.9^{abc}	7.5^{ab}	8.1^{bcd}	8.1^{bcd}	7.2^{bc}	264.2^{bcde}
Normal	3	8.1^{a}	7.9^{a}	8.8^{abc}	8.9^{abc}	8.5^{b}	295.2^{abc}
Normal	15	6.4^{bc}	7.0^{b}	7.1^{d}	6.5^{d}	6.2^{c}	230.3^{e}
Normal	30	7.2^{abc}	7.9^{a}	8.1^{bcd}	8.2^{bcd}	7.8^{bc}	274.3^{bcd}
Pooled SEM		0.48	0.40	0.46	0.60	0.58	13.87
P-value		0.062	0.017	<0.001	<0.001	<0.001	<0.001
Main effect							
Fat level	High	7.1	7.7	8.7	9.6^{a}	10.1^{a}	302.2^{a}
	Normal	7.1	7.5	8.0	7.9^{b}	7.5^{b}	267.1^{b}
Pooled SEM		0.23	0.2	0.23	0.29	0.28	6.77
V, mg/kg	0	7.2^{ab}	7.7^{ab}	8.7^{ab}	9.3^{a}	8.6^{ab}	292.2^{ab}
	3	8.0^{a}	8.2^{a}	9.3^{a}	9.9^{a}	9.9^{a}	316.2^{a}
	15	6.6^{b}	7.2^{b}	7.9^{bc}	8.1^{b}	8.6^{b}	270.0^{bc}
	30	6.6^{b}	7.2^{b}	7.4^{c}	7.9^{b}	8.0^{b}	258.6^{c}
Pooled SEM		0.34	0.28	0.32	0.42	0.40	9.56
P-value[2]							
Fat		0.728	0.394	0.060	<0.001	<0.001	<0.001
V		0.018	0.048	<0.001	0.002	0.080	<0.001
Fat × V		0.226	0.012	0.076	0.021	0.004	0.002

$^{a, b, c}$ 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).

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 5
Effect of vanadium (V) and fat level on weekly and overall phase feed conversion rate in Wistar rat.[1]

Item		Week					Overall phase
		1	2	3	4	5	
Fat level	V, mg/kg						
High	0	0.19^{a}	0.36^{a}	0.30^{ab}	0.30^{a}	0.29^{a}	0.29^{a}
High	3	0.08^{abc}	0.42^{a}	0.38^{a}	0.29^{a}	0.27^{ab}	0.29^{a}
High	15	−0.05^{bc}	030^{ab}	0.29^{ab}	0.28^{a}	0.24^{ab}	0.23^{b}
High	30	−0.33^{d}	0.11^{c}	0.25^{bc}	0.27^{a}	0.23^{ab}	0.14^{c}
Normal	0	0.04^{abc}	0.14^{c}	0.17^{bc}	0.14^{b}	0.15^{bc}	0.13^{c}
Normal	3	0.05^{abc}	0.18^{bc}	0.21^{bc}	0.07^{b}	0.10^{cd}	0.13^{c}
Normal	15	0.01^{abc}	0.03^{c}	0.15^{c}	0.09^{b}	0.02^{d}	0.06^{d}
Normal	30	0.10^{ab}	0.10^{c}	0.22^{bc}	0.15^{b}	0.05^{bc}	0.13^{c}
Pooled SEM		0.06	0.05	0.04	0.04	0.04	0.02
P-value		<0.001	<0.001	0.002	<0.001	<0.001	<0.001
Main effect							
Fat level	High	−0.02	0.30^{a}	0.31^{a}	0.28^{a}	0.26^{a}	0.24^{a}
	Normal	0.04	0.11^{b}	0.19^{b}	0.12^{b}	0.08^{b}	0.12^{b}
Pooled SEM		0.03	0.02	0.02	0.02	0.02	0.01
V, mg/kg	0	0.11^{a}	0.25^{ab}	0.23	0.28	0.22^{a}	0.21^{a}
	3	0.07^{ab}	0.32^{a}	0.31	0.22	0.19^{ab}	0.22^{a}
	15	−0.02^{bc}	0.16^{bc}	0.22	0.19	0.13^{b}	0.15^{b}
	30	−0.13^{c}	0.11^{c}	0.23	0.18	0.15^{b}	0.13^{b}
Pooled SEM		0.04	0.03	0.03	0.02	0.02	0.01
P-value[2]							
Fat		0.140	<0.001	<0.001	<0.001	<0.001	<0.001
V		0.001	<0.001	0.118	0.842	0.036	<0.001
Fat × V		<0.001	0.050	0.254	0.812	0.457	<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).

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.

Table 6
Effect of dietary vanadium (V) and fat level on relative organ weight (%).[1]

Item		Relative weight				
		Liver	Kidney	Lung	Spleen	Heart
Fat level	V, mg/kg					
High	0	3.48^{b}	1.02^{b}	0.75	0.37	0.50
High	3	3.78^{b}	0.98^{b}	0.77	0.28	0.48
High	15	3.82^{b}	1.00^{b}	0.65	0.31	0.54
High	30	4.75^{a}	1.20^{b}	0.71	0.35	0.57
Normal	0	3.89^{b}	1.97^{a}	0.81	0.36	0.59
Normal	3	3.69^{b}	1.62^{a}	0.87	0.34	0.60
Normal	15	3.51^{b}	1.93^{a}	0.78	0.35	0.60
Normal	30	3.93^{b}	1.87^{a}	0.76	0.40	0.53
Pooled SEM		0.16	0.12	0.07	0.04	0.05
Main effect						
Fat level	High	3.96	1.05^{b}	0.81	0.33	0.52
	Normal	3.77	1.85^{a}	0.72	0.37	0.58
Pooled SEM		0.08	0.06	0.04	0.02	0.02
V, mg/kg	0	3.67^{b}	1.47	0.78	0.37	0.54
	3	3.74^{b}	1.26	0.81	0.30	0.53
	15	3.68^{b}	1.43	0.71	0.33	0.57
	30	4.37^{a}	1.51	0.73	0.38	0.55
Pooled SEM		0.11	0.08	0.05	0.03	0.03
P-value[2]						
Fat		0.085	<0.001	0.091	0.235	0.094
V		<0.001	0.158	0.456	0.302	0.921
Fat × V		0.003	0.413	0.945	0.810	0.359

a, b, c Means in the same column without common superscripts differ significantly ($P < 0.05$).
[1] Results are presented as arithmetic means of animals in each group and expressed in percentage of body weight. Each mean represents 9 replicates, with one 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).

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, the 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;

Table 7
The effect of dietary vanadium (V) in high-fat on blood characteristics.[1]

Item		AST, U/L	ALT, U/L	MDA, nmol/mL	TG, mmol/L	BUN, mmol/L
Fat level	V, mg/kg					
High	0	16.98^{d}	11.84^{c}	15.07^{c}	2.78^{a}	14.78^{c}
High	3	18.20^{cd}	13.27^{bc}	15.76^{bc}	1.81^{b}	18.11^{c}
High	15	19.66^{bc}	14.26^{ab}	16.78^{ab}	1.60^{b}	18.39^{bc}
High	30	21.48^{a}	15.70^{a}	17.87^{a}	1.51^{b}	25.20^{a}
Normal	0	18.66^{bc}	12.71^{bc}	15.21^{bc}	1.54^{b}	22.11^{ab}
Normal	3	18.96^{bc}	12.94^{bc}	15.77^{bc}	1.56^{b}	23.74^{a}
Normal	15	19.21^{bc}	13.17^{bc}	15.94^{bc}	1.54^{b}	24.70^{a}
Normal	30	20.07^{ab}	13.81^{b}	16.17^{bc}	1.60^{b}	24.50^{a}
Pooled SEM		0.55	0.59	0.50	0.10	1.33
P-value		<0.001	0.005	0.019	<0.001	<0.001
Main effect						
Fat level	High	19.08	13.76	16.37	1.92^{a}	19.31^{b}
	Normal	19.32	13.16	15.77	1.56^{b}	23.76^{a}
Pooled SEM		0.27	0.29	0.25	0.05	0.66
V, mg/kg	0	17.97^{c}	12.27^{c}	15.14^{c}	2.16^{a}	18.77^{b}
	3	18.62^{bc}	13.11^{bc}	15.76^{bc}	1.68^{b}	20.92^{b}
	15	19.43^{b}	13.72^{ab}	16.36^{ab}	1.57^{b}	21.55^{b}
	30	20.78^{a}	14.75^{a}	17.02^{a}	1.55^{b}	24.85^{a}
Pooled SEM		0.39	0.42	0.35	0.07	0.94
P-value[2]						
Fat		0.541	0.153	0.098	<0.001	<0.001
V		<0.001	0.001	0.004	<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).

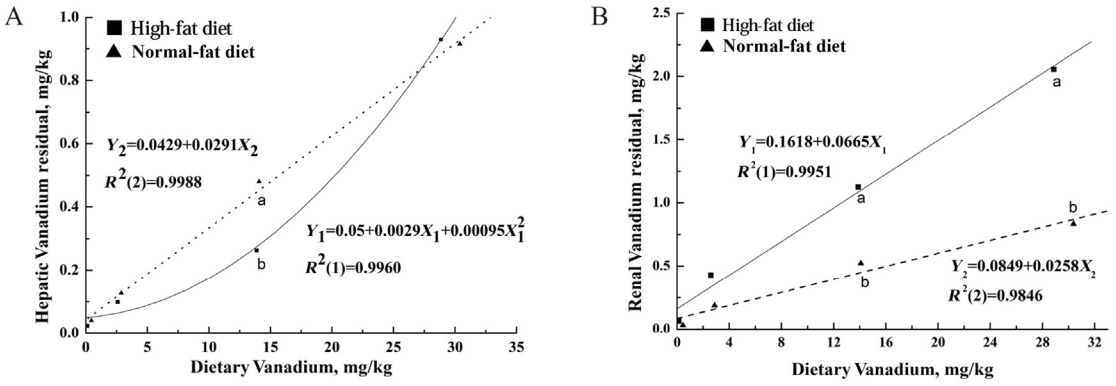

Fig. 2. The polynomial regression equation between vanadium (V) residual in liver and kidney and supplementation level of V. (A) The relationship between hepatic V residual in both normal- and high-fat groups and dietary V content. (B) The relationship between renal V residual in both high and normal-fat groups and dietary V content.

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 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 other people 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.

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