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Effect of dietary vanadium supplementation on growth performance, mineral balance and antioxidant activity in male Sahiwal calves

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ABSTRACT

The study was aimed to examine the effect of supplementation of sodium metavandate ($NaVO_3$) as source of vanadium on DMI intake, growth performance, antioxidant activity, level of mineral in plasma and their balance in male Sahiwal calves. The vanadium content in maize (*Zea mays*) and bajra (*Pennisetum glaucum*) grains was 58 ppb and 55 ppb while in berseem (*Trifolium alexandrinum*) and mustard (*Brassica campestris*) fodder it was 8.37 and 7.24 ppm, respectively. Male Sahiwal calves (20) of comparable age (6±0.82 months) and body weight (71±8.06 kg) were randomly allotted to 4 different treatments with replication of 5 animals in each. Supplementation was done with 0, 2, 4 and 8 ppm of vanadium in groups T_1, T_2, T_3 and T_4, respectively, for 120 days. Blood samples were collected at monthly intervals to examine antioxidant activity in blood, plasma and mineral levels. Feed consumption (DM intake, DM intake% BW) and growth rate did not show any significant effect of vanadium supplementation. Glutathione peroxidase activity was higher in groups T_3 and T_4 as compared to T_1 and T_2 whereas, SOD and catalase activity was similar in all the groups. Excretion and absorption patterns of Ca, P, Cu and Fe and their plasma levels were similar in different groups. However, vanadium and Zn balance and their plasma levels increased due to vanadium supplementation. The present study revealed that in growing calves, vanadium supplementation showed enhanced glutathione peroxidise activity, plasma Zn and vanadium levels.

Key words: Growth performance, Mineral balance, Nitrogen balance, Nutrient intake, Sahiwal calves, Vanadium

Vanadium, an ultra trace element, has become a subject of interest amongst nutritionists after its discovery in various marine species as an essential trace element (Almedeida *et al*. 2001). Vanadium inhibits various ATPases, phosphatases, and phosphoryl-transfer enzymes (Nielsen 1984). It has gained acceptance of possibly essential element in humans (Harland *et al.* 1994), goats and rats (Nielson *et al.* 1984). Vanadium-deprived goats showed elevated abortion rates and decreased milk production (Anke *et al*. 2000). Vanadium exists in 6 oxidation states in nature, viz. –1, 0, +2, +3, +4 and +5, while only 3 oxidation states (+3, +4 and +5) are predominant under physiological condition. The pentavalent (VO_3^-) form predominates in extracellular body fluids whereas the quadrivalent (VO^{2+}) is most common intracellularly (Hirano *et al.* 1996). In rats, supplementation of vanadium complexes mitigated effect on reactive oxygen species and increased glutathione peroxidase (GPx) activity (Francik *et al.* 2011). Supplementation of vanadyl sulphate protects the rats from lipid peroxidation and also decreases thiobarbituric acid reactive substances (Harati *et al.* 2006). Liu *et al*. (2015) also reported that vanadyl(IV)-ascorbate complex (VOAsc) would reduce oxidative stress, hyperglycemia and insulin resistance in high fat high-sucrose diet (HFSD)-induced type 2 diabetes in mice. HFSD animals supplemented with VOAsc exhibited a marked increase in catalase activity, SOD, GSH-Px and GSH. Thus, vanadium supplementation can also be explored to enhance the immune status of the animals.

Vanadium absorption, excretion and retention in body depends on its solubility, chemical nature (Trevino *et al.* 2018) and complex formation as chelates (Thompson *et al.* 2006). Studies were conducted on interactions of vanadium with iron (Nielsen *et al.* 1984, Zaparowska *et al.* 1993), zinc (Rossetti *et al.* 1990), aluminium (Wiegmann *et al.* 1982), manganese, cobalt, lithium and molybdenum (Thompson *et al.* 1993) and copper (Rucker *et al.* 2000). The research on role of vanadium in ruminants is lacking and still unclear. The present study was conducted to examine the vanadium and other minerals absorption, retention, their plasma levels and antioxidant activity as affected by different levels of vanadium supplementation in male Sahiwal calves.

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MATERIALS AND METHODS

Ethics approval: Prior to the requisition of the experiment, animal care procedures were approved and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the committee for control and supervision on experiments on animals rules, recommended by the Government of India.

Animal, diets and management: The experiment was conducted at the NDRI, Karnal which is situated at an altitude of 250 m amsl. The maximum ambient temperature in summer goes up to 45°C and minimum temperature in winter goes down to about 4°C with a diurnal variation to the order of 15–20°C. The average annual rainfall is 696 mm most of which is received from early July to mid-September.

Male Sahiwal calves (20) of similar age (6±0.82 months) and body weight (71±8.06 kg) were randomly allotted to 4 treatment groups and each treatment had 5 replicates. Proper deworming and vaccination was done. All the animals were fed rations in form of TMR to meet their nutrient requirements (ICAR 2013). Concentrate mixture and maize fodder (*Zea mays*) were supplied in the ratio of 40:60 (DM basis) in form of total mixed ration (Table 1). Concentrate mixture (21.33% CP and 70% TDN) was procured from Godrej Agrovet Pvt. Ltd., and the chemical composition of total mixed ration is presented in Table 2.

Animals in groups T_1, T_2, T_3 and T_4 were supplemented with 0, 2, 4, and 8 ppm of vanadium from inorganic source ($NaVO_3$, Hi-Media Laboratories Ltd, Mumbai, India). Stock solutions of 2, 4 and 8 ppm vanadium were prepared and required amount (calculated on the basis of daily dry matter of animals) was given to ensure complete intake of vanadium after proper mixing with 50 g concentrate mixture.

Observations recorded and blood collection schedule: Daily feed offered, residue left and DM intake of the animals were recorded. Body weights were recorded at the beginning of experiment and subsequent fortnightly intervals in the morning hours before offering feed or water by computerized weight management system. Sahiwal calves were weighed for 2 consecutive days, the average of 2 days was considered as body mass for that fortnight. Average daily gain (ADG) and DM intake were calculated.

During experimental period, peripheral blood samples were collected from all the animals by jugular puncture in heparinised vaccutainer at the start of experiment and on 30, 60, 90 and 120 days of vanadium supplementation, mixed well by rotating tubes between palms to ensure proper mixing of blood and anticoagulant and brought to the laboratory after placing in ice box. The samples were centrifuged at 3,000 rpm for 15 min to separate the plasma. Plasma samples were analysed for minerals (Ca, P, V, Zn, Cu and Fe).

Chemical composition analysis and trace elements estimation: The contents of DM, OM, CP, EE and total ash in feeds, residues, faeces samples and urine samples (for nitrogen content) were determined (AOAC 2005). The fractions of cell wall constituents (NDF, ADF, cellulose and ADL) were also analysed (Van Soest *et al.* 1991).

For analysis of trace elements, representative samples of feeds, residue and faeces were taken, oven dried (at 80°C for 16 h) and ground enough to 1 mm sieve. About 1 g of feed, residue, faeces, urine (10 ml) and plasma (1 ml) of sample was digested using tri-acid mixture (HNO_3: $HClO_4$: H_2SO_4; 3:2:1) in Kelplus-KES 12L R till it became clear. Properly digested samples were diluted with distilled water and filtered through Whatman filter paper No. 54. The final volume was made to 25 ml in volumetric flask. Vanadium was estimated by Inductively Coupled Argon Plasma Optical Emission Spectrophotometer (ICP-OES) Shimadzu ICAP 9000 series at wavelength 292.4 nm.

Concentration of calcium (Ca), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) in feed, faeces, urine, and plasma samples were determined in an air-acetylene flame

Table 1. Composition of total mixed ration

Ingredient	Content (g/kg DM)
Maize fodder	500
Ground yellow maize	140
Pearl millet	25
Groundnut cake (expeller extracted)	50
Soybean meal	75
Mustard oilcake (expeller extracted)	65
Wheat bran	75
Deoiled rice polish	55
Mineral mixture[a]	10
Common salt	5
$NaVO_3$	Variable[c]

[a]Per kg contained the following: vitamin A, 700,000 IU; vitamin D_3, 70,000 IU; vitamin E, 250 mg; nicotinamide, 3.0 g; Ca, 190 g; P, 90 g; Na, 50 g; Cu, 1200 mg; Zn, 9.6 g; Fe, 1.5 g; Mn, 6.0 g; I, 325 mg; Co,150 mg; Se, 10 mg; Mg, 19.0 g. [c]Two, 4 and 8 mg vanadium/kg DM was supplemented corresponding to varying levels of $NaVO_3$.

Table 2. Chemical composition of total mixed ration

Chemical composition	(g/kg of total mixed ration)
Dry matter	563
Crude protein	143
Ether extract	34
Neutral detergent fibre	456
Acid detergent fibre	276
Calcium	8
Magnesium	3.8
Zn (mg/kg DM)	50.00
Cu (mg/kg DM)	23.35
Fe (mg/kg DM)	304
V (mg/kg DM)	7.40
Total digestible nutrient[b]	6.35
Metabolizable energy (MJ/kg DM[b)]	9.5

[b]Calculated value (NRC 2001).

on an atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) after digestion in tri acid mixture ($HNO_3/HClO_4/H_2SO_4$; 3:2:1). Two blank samples having tri-acid mixture were also analysed. The procedure for preparation of stock and standard solutions, and choice of instrumental conditions were as per AAS Manual (1988). Phosphorus content in various samples was estimated using calorimetric method (AOAC 2005). Standard curve was prepared for each mineral with their working standards.

Estimation of antioxidant status: The activities of antioxidant enzyme SOD (Madesh and Balasubramanian 1998), catalase (Aebi 1984) and glutathion peroxidase (GPx; Paglia and Valentine 1967) were estimated by haemolysates through spectrophotometry method. For hemolysate preparation, 2 ml of whole blood was collected in vials with anticoagulant, acid citrate dextrose 300 µl/2 ml blood and centrifuged at 2,000 rpm for 15 min at 4°C with separation of plasma and buffy coat. The erythrocyte pellet (packed RBC) was washed with phosphate buffer saline (PBS) solution (NaCl, 137 mM; KCl, 2.7 mM; Na_2HPO_4, 10 mM and KH_2PO_4, 1.8 mM; pH adjused to 7.4). The packed RBC was mixed with an equal volume of PBS to form RBC suspension. Haemolysate (1:20 dilution) was prepared by mixing 0.5 ml RBC suspension with 4.5 ml of stabilizing solution (2.7 mM EDTA and 0.7 mM 2-marcaptoethanol).

Statistical analysis: The data were analysed using the general linear model procedure of Statistical Package for the Social Sciences (SPSS for Windows, v21.0; SPSS Inc., Chicago, IL, USA). To estimate the effect of treatment and period, and their interaction, the following model was used:

$Yijk = \mu + Ti + Dj + (T\times D)ij + eijk$

where Yijk, dependent variable; µ, overall mean of the population; Ti, mean effect of the i^{th} treatments; Dj, mean effect of day of sampling with day as a repeated factor (j = 0, 30, 60, 90, 120 and 150 days of dietary treatment); (T × D)ij, effect of the interaction between effects of treatment and day of sampling, and eijk, unexplained residual element assumed to be independent and normally distributed. The individual animal was used as the experimental unit for all data. The pair-wise comparison of means was carried out using Tukey's honest significant difference (HSD) test. Significance was determined at $P<0.05$. The values are expressed as means±SE and differences between the means were compared as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Vanadium in feedstuffs: The vanadium concentrations (Table 3) in maize and bajra grains were 58 ppb and 55 ppb while in berseem and mustard it was 8.37 and 7.24 ppm, respectively. The total vanadium content of the basal diets formulated was determined to be 5.24 mg/kg DM, whereas concentrate mixture, maize and sorghum fodder contained 7.40, 2.80, and 2.61 mg V/kg DM, respectively. Vanadium content of different feed stuffs collected from different locations showed inconsistency in concentration (Anke *et al.* 2004, Barceloux 1999). However, scanty information regarding vanadium content and its chemical form present in different feeds leaves scope for further chemical characterisation. Balos *et al.* (2017) evaluated vanadium content in poultry feed and gave a range for complete layer and chicken feed on fresh basis. These values for vanadium content in feed were comparable to our study on dry matter basis. Vanadium content of complete feed may be influenced by source of phosphate used for feed formulation as phosphates have vanadium as contaminant. Use of phosphate in feed may incorporate up to 120 mg V/kg feed (Henry and Miles 2001). Various researchers had reported range of vanadium for different source of phosphates (Sullivan *et al*. 1994, Limma *et al.* 1995, Balos *et al.* 2017). Our study suggested that green fodder have higher vanadium content in comparison to grain and byproducts, which might be due to leaves which are rich source of vanadium (Barceloux 1999).

Dry matter intake and average daily gain: The diet had adequate crude protein (CP) and total digestible nutrients (TDN) to support maintenance and growth of Sahiwal calves (ICAR 2013). Dry matter intake (kg/100 kg BW) varied from 3.29 to 3.55 in different treatment group (Table 4). Average daily gain (g/d) was 57, 57, 60 and 59 in groups T_1, T_2, T_3 and T_4. The level of vanadium in the diet did not influence feed consumption (DM intake, DM intake% BW) and average daily gain. Requirement of vanadium in dairy animals is yet to be determined. However, importance of vanadium in higher animals is supported by biologist and biomedical scientists. Similar to our study,

Table 3. Vanadium content in some commonly available feedstuffs

Item	Vanadium content
Grains (ppb)	
Maize (*Zea mays*)	58.00±5.2
Bajra (*Pennisetum glaucum*)	55.00±7.5
By-products (ppb)	
Maize bran (*Zea mays*)	1.90±0.05
Rice bran (*Oryza sativa*)	1.20±0.11
Wheat bran (*Triticum astivum*)	4.24±0.12
De-oiled rice bran (*Oryza sativa*)	2.50±0.21
Oil seed cakes (ppm)	
Soybean meal (*Glycine max*)	5.08±0.13
Mustard cake (*Brassica camprestris*)	4.25±0.17
Guar meal (*Cyamipsis tertagonoloba*)	5.34±0.12
Cotton seed cake (*Gossypium herbaceum*)	1.73±0.02
Groundnut cake (*Arachis hypogaea*)	0.53±0.01
Non-leguminous fodders (ppm)	
Maize (*Zea mays*)	2.80±0.02
Sorghum (*Sorgum bicolor*)	2.61±0.01
Oats (*Avena sativa*)	2.38±0.04
Leguminous fodders (ppm)	
Berseem (*Trifolium alexandrinum*)	8.37±0.21
Mustard (*Brassica campestris*)	7.24±0.18
Other feeds (ppm)	
Concentrate mixture	7.40±0.14
Mineral mixture	196±85.21

Kumar *et al*. (2017) reported that vanadium supplementation did not have any significant effect on DM, CP and TDN intake, and digestibility of nutrients up to 9 ppm level. Pal *et al.* (2017) reported that supplementation of crossbred calves with inorganic source of vanadium did not change the dry matter intake, growth performance and feed efficiency. Similarly, Heidari *et al.* (2016) reported that dietary supplementation of 0.04, 0.08 and 0.12 mg of V as vanadyl sulphate per kilogram of metabolic body weight did not affect body weight, body condition score, dry matter intake and energy balance in multiparous periparturient Holstein cows. In contrast to our findings, Bonomi *et al.* (2003) reported the addition of vanadium in milk replacer fed to lamb kids at the dose of 5 and 10 ppm for 30 d improved the weight gain, efficiency of feed utilisation and dressing percentage. Gurtler *et al.* (1999) reported that vanadium deficiency did not affect the growth of female or male calves.

Blood antioxidant enzymes and plasma mineral levels: Blood glutathione peroxidase activity was 13.90, 14.58, 23.73 and 21.05 µmole NADPH oxidized/g Hb/min in groups T_1, T_2, T_3 and T_4, respectively, which increased (P<0.05) by 70.71 and 51.43% in groups T_3 and T_4 (Table 5). Similar to our observations, Francik *et al.* (2011) reported that vanadium increased plasma glutathione peroxidise levels in treated rats. Kim *et al*. (2011) reported that vanadyl sulphate supplementation increased the nuclear translocation of Nrf2 and the accumulation of phosphorylated Nrf2 which leads to increase in expression and activity of glutathione peroxidise in human liver cells. While blood catalase and SOD enzymes activities were not affected by vanadium supplementation. Similarly, Liu *et al*. (2015) also reported that vanadyl (IV)-ascorbate complex (VOAsc) would reduce oxidative stress, hyperglycemia and insulin resistance in high fat high-sucrose diet (HFSD)-induced type 2 diabetes in mice. HFSD animals supplemented with VOAsc exhibited a marked (P<0.01) increase in catalase activity (44.8%), SOD (15.3%), GSH-Px (16.9%) and GSH (43.2%) in HFSD animals supplemented with VOAsc relative to the HFSD group. Pal *et al*. (2017) reported that supplementation of inorganic source of vanadium in crossbred calves increased plasma glutathione peroxidise antioxidant enzyme activity. Overall plasma Ca, P, Cu and Fe levels were similar in all the groups whereas plasma Zn level was higher (P<0.05) in group T_4 than other groups which might be due to increased level of Zn retention found in 8 ppm of vanadium supplemented group. Similarly, Kroaniak *et al.* (2013) reported that Zn concentration in pancreas increased in response to administration of vanadium complexes in diabetic rats. Some other studies reported the interactions of vanadium with iron (Nielsen *et al.* 1984, Zaparowska *et al.* 1993), Zn (Rossetti *et al.* 1990), Al (Wiegmann *et al.* 1982), Mn, Co, Li and Mo (Thompson *et al.* 1993) and copper (Rucker *et al*. 2000). The differences in results could be due to differences in form of vanadium used. Plasma vanadium level showed significant (P<0.05) differences among the groups which might be because of response of supplementation. Plasma vanadium level showed linear increase in response to dose of supplementation. Vanadium concentration in the blood of mammals was reported to be about 0.2–0.5 ng/ml out of which nearly 80% present in plasma as a component of proteins such as transferrin and albumins (Thompson *et al.* 2006).

Mineral balance: Vanadium balance (Table 6) increased (P<0.05) with increasing dietary vanadium level. Similarly, faecal and urinary excretion of vanadium (mg/d) also increased with increase in the level of supplemental vanadium. Retention of vanadium was significantly

Table 4. Effect of supplementation of different levels of vanadium on dry matter intake and growth performance

Parameter	Group			
	T_1	T_2	T_3	T_4
Initial BW (kg)	71.15± 11.25	71.74± 11.13	71.50± 10.16	68.22± 9.88
Final BW (kg)	139.4± 6.24	139.09± 5.21	141.70± 7.24	139.02± 6.24
Gain (kg)	68.25	67.35	70.20	70.75
ADG (kg)	0.57± 0.03	0.57± 0.04	0.60±. 0.030	59± 0.03
DMI (kg/d)	3.14± 0.16	3.25± 0.18	3.10± 0.15	3.17± 0.18
DMI (% BW)	3.18± 0.07	3.05± 0.08	3.05± 0.10	3.09± 0.14
V intake (mg/d)	18.05± 0.86	24.05± 1.02	30.05± 0.80	42.05± 0.87

Table 5. Effect of vanadium supplementation on antioxidant status and plasma mineral levels

Parameter	Group			
	T_1	T_2	T_3	T_4
Antioxidant enzyme activity				
SOD (U/mg Hb)	57.48± 0.93	56.42± 1.34	58.79± 1.14	56.65± 1.66
Catalase (µmol of H_2O_2 consumed/min/g Hb)	141.12± 19.13	147.54± 11.08	141.36± 09.14	148.23± 13.45
GPx (NADPH oxidized/g Hb/min)	13.90^a± 1.23	14.58^a± 1.08	23.73^c± 1.50	21.05^b± 1.20
Plasma mineral content				
V (mg/l)	0.5^a± 0.00	0.68^b± 0.01	0.77^c± 0.01	1.8^d± 0.07
Ca (mg/dl)	10.10± 0.30	9.64± 0.28	10.07± 0.25	10.11± 0.27
Mg (mg/dl)	5.63± 0.23	5.52± 0.33	5.25± 0.33	5.79± 0.30
Fe (mg/dl)	1.36± 1.17	1.65± 2.14	1.59± 0.76	1.68± 1.59
Cu (mg/dl)	0.93± 0.21	0.99± 0.17	1.03± 0.21	1.03± 0.21
Zn (mg/dl)	1.03^a± 0.05	1.09^a± 0.10	1.13^a± 0.06	1.75^b± 0.07

Means bearing different superscripts in a row differ significantly (P<0.05). SOD, superoxide dismutase; GPx, glutathione peroxidise.

Table 6. Effect of vanadium supplementation on trace mineral utilization and plasma trace mineral level

Parameter	Group			
	T_1	T_2	T_3	T_4
V intake (mg/d)	18.05± 0.86	24.05± 1.02	30.05± 0.80	42.05± 0.87
V outgo in faeces (mg/d)	16.80± 0.22	18.33± 0.29	20.97± 0.32	23.77± 0.49
Apparent absorption (% of intake)	06.92^{a}± 0.37	10.83^{b}± 0.75	08.34^{b}± 0.94	08.82^{b}± 0.99
V outgo in urine (mg/d)	1.07± 0.07	1.46± 0.05	1.16± 0.07	1.23± 0.09
Retention (% of intake)	0.94^{a}± 0.01	03.72^{b}± 0.06	05.04^{b}± 0.03	04.10^{b}± 0.04
Ca intake (g/d)	15.27± 1.80	15.12± 0.93	16.60± 2.21	17.37± 1.99
Apparent absorption (% of intake)	31.20± 0.83	30.61± 0.79	31.55± 0.91	30.36± 0.86
Retention (% of intake)	25.47± 0.83	25.46± 0.91	27.83± 0.86	25.1± 0.85
P intake (g/d)	20.75± 0.90	17.72± 0.97	18.88± 0.82	19.68± 0.99
Apparent absorption (% of intake)	51.78± 0.83	51.36± 0.79	49.55± 0.91	49.13± 0.86
Retention (% of intake)	19.95± 0.85	21.11± 0.83	18.11± 0.91	19.68± 0.86
Cu intake (mg/d)	56.12± 3.13	56.20± 3.68	59.40± 2.96	57.66± 2.09
Apparent absorption (% of intake)	08.85± 1.04	09.37± 1.27	10.67± 1.50	08.75± 1.95
Retention (% of intake)	1.18±. 0.18	1.26± 0.160	1.43± 0.13	1.17± 0.22
Fe intake (mg/d)	2150.12± 58.13	2077.20± 30.68	2223.40± 25.96	2222.82± 29.09
Apparent absorption (% of intake)	17.86± 2.04	17.50± 1.27	17.04± 1.10	17.84± 1.95
Retention (% of intake)	16.49± 0.80	15.93± 0.33	15.93± 0.13	16.79± 0.22
Zn intake (mg/d)	147.69± 7.20	148.20± 8.35	148.80± 6.90	144.01± 5.49
Apparent absorption	28.73^{a}± 2.75	40.36^{b}± 1.04	38.55^{b}± 1.02	44.17^{c}± 1.32
Retention (% of intake)	25.70± 2.66	38.53^{b}± 1.19	37.62^{b}± 1.02	42.47^{c}± 1.36

^{a,b,c}Means with a different superscripts in a row differ significantly (P<0.05).

(P<0.05) different among the groups and the maximum retention was in group T_3. Retention of Ca, P, Cu, Mg and Fe was similar in all the groups. While Zn absorption increased by 40.48, 34.18 and 53.74% in 2, 4 and 8 ppm vanadium supplemented groups. Zn retention and absorption was (P<0.05) higher in vanadium supplemented groups as compared to control group, and was maximum in 8 ppm vanadium supplemented group. Information about vanadium absorption, retention and excretion is still lacking. The dietary vanadium requirement in growing calves has not been established explaining its semi-essential nature. Findings of the present study suggested that most of the vanadium was excreted through faeces, the absorption coefficient was around 6%, which increased in a dose-dependent manner of vanadium supplementation. Similar to present study, Harris *et al.* (1984) reported that vanadium is absorbed from digestive tract at efficiency less than 5% of ingested vanadium, therefore, most of the ingested vanadium was found in the faeces. Absorbed vanadate was converted to the vanadyl cation, which can complex with ferritin and transferrin in plasma and body fluids. Contrary to this, NRC (2005) report suggested that rate of absorption for vanadium was 1% or less and most of the vanadium is excreted by kidney through urine and a minor amount was in faeces.

Hence, the present study revealed that, vanadium supplementation to growing calves enhanced glutathione peroxidase activity, plasma Zn and vanadium levels in Sahiwal calves.

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