

Effect of dietary vanadium on small intestinal morphology in broilers

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ABSTRACT

The objective of this study was to determine the effects of dietary vanadium on small intestinal morphology of broilers by the methods of light microscopy (LM) and transmission electron microscopy (TEM). A total of 420 one-day-old avian broilers were divided into six groups (seven replicates in each group and ten broilers in each replicate) and fed on a control diet or the same diet supplemented with 5, 15, 30, 45 and 60 mg/kg vanadium in the form of ammonium metavanadate for 42 days. In comparison with those in the control group, the intestinal villus heights were decreased ($P < 0.05$ or $P < 0.01$) in the 30, 45 and 60 mg/kg groups, and crypt depths and villus height/crypt depth ratio were decreased in the 45 and 60 mg/kg groups. Ultrastructurally, the microvilli were apparently sparse and short, and the numbers of lysosomes were increased in abovementioned three intestines in the 45 and 60 mg/kg groups at 42 days of age. In conclusion, dietary vanadium in excess of 30 mg/kg could alter the villus height, crypt depth, villus height/crypt depth ratio and ultrastructure, which might impact the development of small intestines in broilers.

Keywords: Vanadium; Villus; Crypt; Microvillus; Intestine; Broiler

1. INTRODUCTION

Vanadium as a "slightly" toxic has been ratified to be an essential trace element with anti-diabetic and anti-carcinogenic properties during the last few decades [1]. A number of observations have demonstrated that the biological importance of vanadium [2], such as the insulin-like function [3,4], or the antidiabetic agent [5]. Vanadium also is a potent inhibitor of several phospho-

drolases such as Na^+K^+ -ATPase [6] and Ca^{2+} -ATPase [7], an activator of adenylyl cyclase [8] and a potent anti-carcinogenic agent [9]. There are some reports regarding the effects of vanadium on lymphoid organs, liver and kidney [10-13].

Our previous researches have focused on the effect of vanadium in diets on the population of T cells, the content of cytokines and oxidative stress in small intestine [14-16], while the effect of dietary vanadium on small intestinal morphology has been rarely reported at present. The absorptive functions of the intestine are related to its morphology and any alterations in morphology may predispose the intestine to function disorder [17-19]. The objective of this study was to determine the effect of dietary vanadium on intestinal morphology in broilers by the methods of light microscopy (LM) and transmission electron microscopy (TEM), and provided the systematic data about the influences of dietary vanadium on the morphology of small intestines in broilers, and helpful information for the same or similar studies in both human and other animals in the future.

2. MATERIALS AND METHODS

Broilers and Diets. A total of 420 one-day-old healthy avian broilers were randomly divided into six groups with 70 broilers in each group. There were seven replicates in each group and ten broilers in each replicate. Two of seven replicates in each group were used for the clinical observation during the experiment. Broilers were housed in cages with electrically heated units and were provided with water as well as the undermentioned diets *ad libitum* for 42 days.

A corn-soybean basal diet formulated by NRC (1994) was used as a control diet. 11.5, 34.45, 68.87, 103.33, 137.75 mg ammonium metavanadate were mixed into the corn-soybean basal diet to produce experimental diets with 5, 15, 30, 45 and 60 mg/kg of vanadium, respectively.

The use of chickens in our experiments was followed

and all experimental procedures involving animals were approved by Sichuan Agricultural University Animal Care and Use Committee.

Sample Collection and Handling. The experiment lasted for 42 days. Briefly, five broilers in each group were euthanized at 14, 28 and 42 days of age and the small intestinal sections were collected from the duodenum, jejunum and ileum. Small intestinal tissue (1 cm²) were fixed in 4% formalin solution. After rinsing with water, the samples were dehydrated in a graded series of absolute ethanol (50%, 70%, 80%, 90%, 100%), cleared with benzene twice, saturated with and embedded in paraffin. Sections of 7 µm thickness (10 slices of each sample) were stained with haematoxylin/eosin and prepared for observing by the light microscopy.

Histological Observation. A color video camera (Nikon 3 CCD) and a light microscope (Olympus, Japan) were used to observe the morphological changes. The small intestinal villus height and crypt depth were determined by Image Pro Plus analysis program. The villus height/crypt depth ratio was determined as the ratio of villus height to crypt depth.

Ultrastructural Observation. The ultrastructure was examined by the method of transmission electron Microscopy (TEM). The tissue was processed by routine methods for TEM as described by Elbrønd *et al.* [20,21]. The area of interest was framed with silver paint and the tissue block meticulously cut from the stub and transferred to 100% pure ethanol. The block was kept in the alcohol for at least 1 h and then cut into pieces suitable for TEM (1 mm³). These tissue blocks were then prepared following the normal standard embedding procedure. The TEM preparations were examined in a JEOL 1200 EX transmission electron microscope at 60 kV.

Statistical Analysis. The significance of difference among six groups was analyzed by variance analysis, and results presented as means ± standard deviation ($\bar{X} \pm S$). The analysis was performed under SPSS 12.0 for windows.

3. RESULTS

Clinical Observation. Broilers grew much faster in 5

mg/kg group and much slower in 30, 45 and 60 mg/kg groups than in control group. Broilers in 45 and 60 mg/kg groups showed decreased feed intake and depression. Loss of body weight was observed in 30, 45 and 60 mg/kg groups respectively at the end of the experiment when compared with that of control group. The change of the body weight is shown in **Table 1**.

Gross and Histological Changes in Small Intestines. Gross changes were not observed in the small intestines. Histologically, changes were observed only on villus height, crypt depth and villus height/crypt depth ratio in the small intestines. The villus height, crypt depth and villus height/crypt depth ratio were used to reflect intestinal development. Effect of dietary vanadium on the abovementioned three parameters was found to be not significant in duodenum, jejunum and ileum of the 5 mg/kg and 15 mg/kg groups. However, dietary vanadium in 30, 45 and 60 mg/kg could impact the three parameters in intestines.

Duodenum. In comparison with that of control group, the duodenal villus heights were significantly decreased ($P < 0.05$ or $P < 0.01$) in the 30 mg/kg group at 28 and 42 days of age, and in the 45 and 60 mg/kg groups from 14 to 42 days of age. The crypt depths were significantly lower ($P < 0.05$ or $P < 0.01$) in the 60 mg/kg group from 14 to 42 days of age, and in the 45 mg/kg group at 28 and 42 days of age than those in the control group. The villus height/crypt depth ratio was decreased ($P < 0.05$ or $P < 0.01$) in the 45 and 60 mg/kg groups at 42 days of age. The results were shown in **Figures 1-3**.

Jejunum. Changes of the jejunum were similar to changes of the duodenum. The jejunal villus heights were decreased ($P < 0.05$ or $P < 0.01$) in the 30 mg/kg group at 14 and 42 days of age, and in the 45 and 60 mg/kg groups from 14 to 42 days of age when compared with those of control group. The crypt depths were lower ($P < 0.05$) in the 45 mg/kg group at 14 days of age and in the 60 mg/kg group from 14 to 42 days of age than those in the control group. The villus height/crypt depth ratio was significantly decreased ($P < 0.05$ or $P < 0.01$) in the 45 and 60 mg/kg groups from 14 to 42 days of age except the 45 mg/kg group at 14 days of age. The results

Table 1. Change of the body weight (g) in experimental chickens.

	7 days	14 days	21 days	28 days	35 days	42 days
control	144.00 ± 6.70	308.20 ± 10.56	595.50 ± 11.52	945.00 ± 11.05	1418.10 ± 10.79	1974.70 ± 36.527
5 mg/kg	141.20 ± 13.68	307.50 ± 12.58	628.10 ± 11.13**	969.40 ± 30.95**	1457.90 ± 10.45**	2155.20 ± 47.85**
15 mg/kg	123.90 ± 12.43**	263.60 ± 10.78**	567.50 ± 11.33**	900.10 ± 12.33**	1371.80 ± 9.82**	1871.60 ± 49.40**
30 mg/kg	112.30 ± 12.96**	246.20 ± 10.29**	479.60 ± 12.38**	878.80 ± 10.99**	1261.50 ± 10.61**	1650.30 ± 41.05**
45 mg/kg	120.70 ± 12.13**	191.30 ± 10.73**	358.40 ± 13.21**	557.60 ± 19.31**	814.30 ± 8.60**	1083.90 ± 40.84**
60 mg/kg	98.60 ± 9.01**	170.40 ± 15.07**	310.30 ± 11.98**	514.30 ± 11.60**	774.80 ± 15.12**	1020.40 ± 29.05**

Data are presented with the means ± standard deviation (n = 5); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

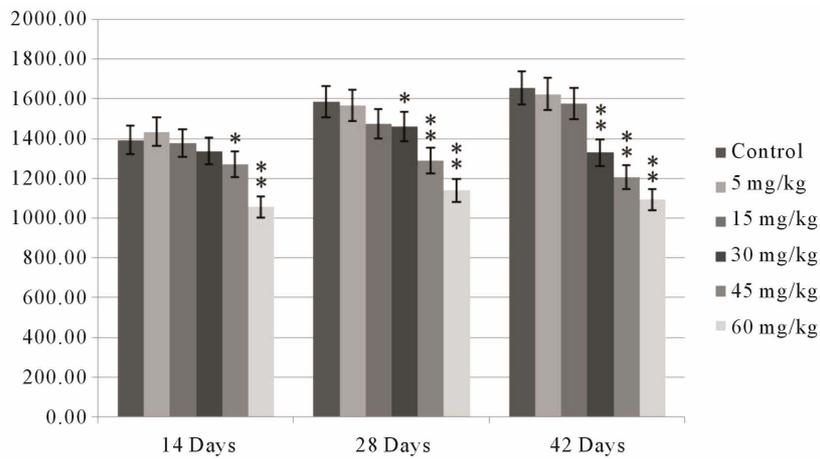


Figure 1. Effect of dietary vanadium on duodenal villus heights (μm); Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

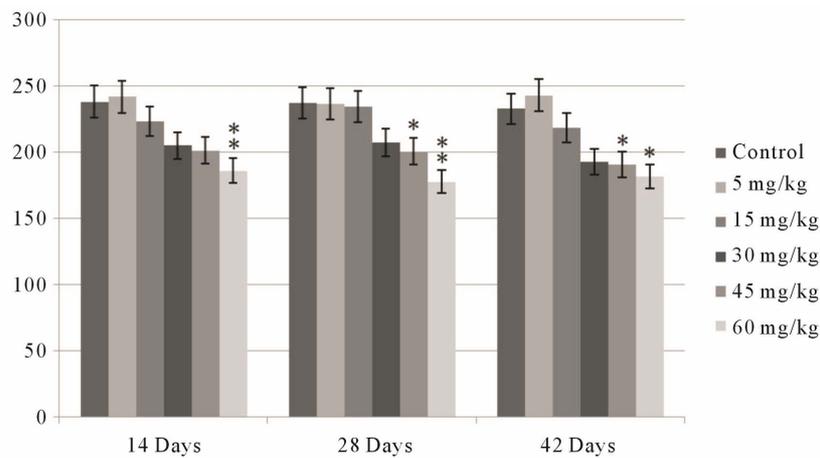


Figure 2. Effect of dietary vanadium on duodenal crypt depths (μm); Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

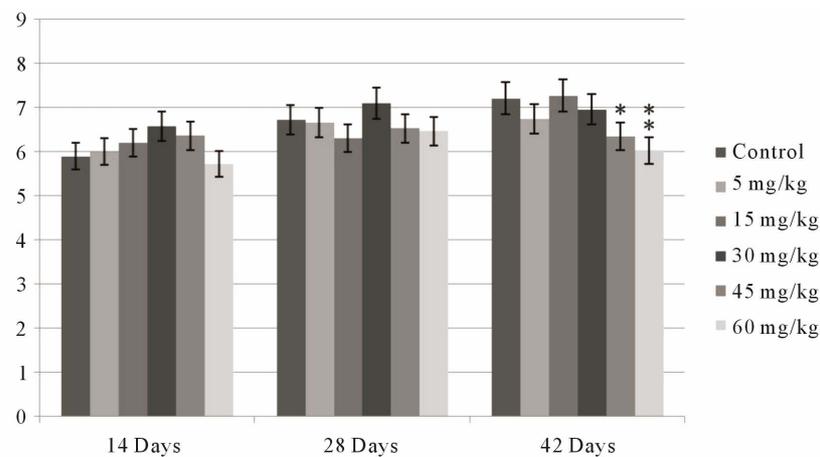


Figure 3. Effect of dietary vanadium on duodenal villus height/crypt depth ratio; Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

were shown in **Figures 4-6**.

Ileum. The ileac villus heights were significantly lower ($P < 0.05$ or $P < 0.01$) in the 30 mg/kg group at 14 and 42 days of age, and in the 45 and 60 mg/kg groups from 14 to 42 days of age than those in the control group. The crypt depth were decreased ($P < 0.05$) in the 60 mg/kg group from 14 to 42 days of age, and in the 45 mg/kg group at 14 and 28 days of age. The villus height/crypt depth ratio was significantly lower ($P < 0.01$) in the 30 mg/kg group at 42 days of age, in the 45 mg/kg at 14 and 42 days of age and in the 60 mg/kg group from 14 to 42 days of age than those in the control group. The results

were shown in **Figures 7-9**.

Ultrastructure. The duodenal microvilli were apparently sparse and short, and the numbers of lysosomes were increased in 45 and 60 mg/kg group at 42 days of age in comparison with those of the control group. The similar situations were observed in the other two intestines. The results of TEM were shown in **Figures 10-15**.

4. DISCUSSION

Changes in the development of enterocytes and in the structure of villi determine the digestive and absorptive capacity of the small intestine [22]. The villus height,

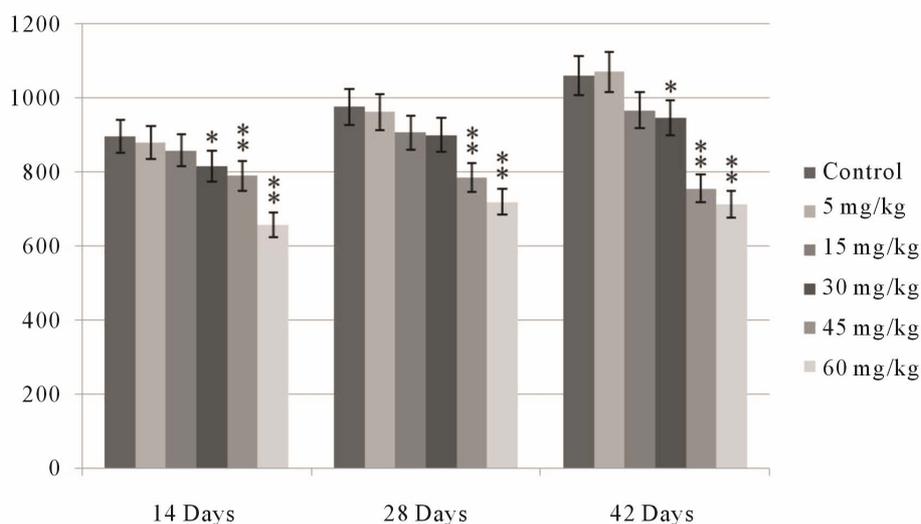


Figure 4. Effect of dietary vanadium on jejunal villus heights (μm); Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

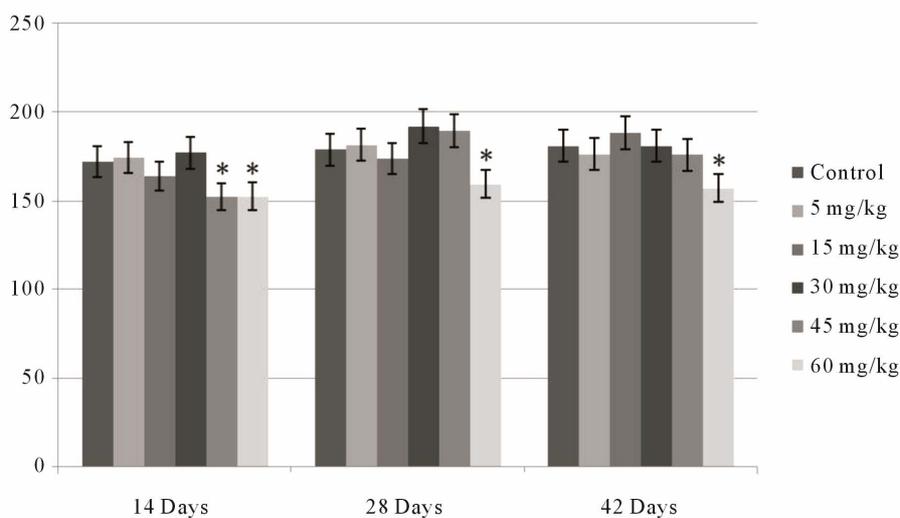


Figure 5 Effect of dietary vanadium on jejunal crypt depths (μm); Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

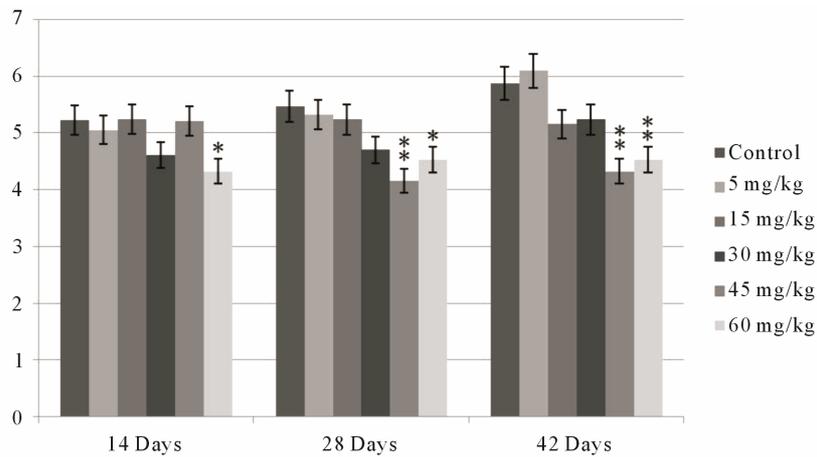


Figure 6. Effect of dietary vanadium on jejunal villus height/crypt depth ratio; Data are presented with the means \pm standard deviation (n = 5); *P < 0.05, compared with the control group; **P < 0.01, compared with the control group.

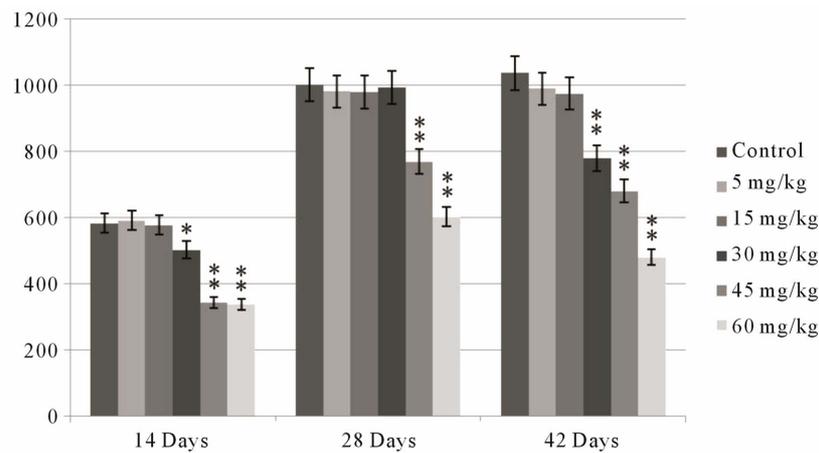


Figure 7. Effect of dietary vanadium on ileac villus heights (μm); Data are presented with the means \pm standard deviation (n = 5); *P < 0.05, compared with the control group; **P < 0.01, compared with the control group.

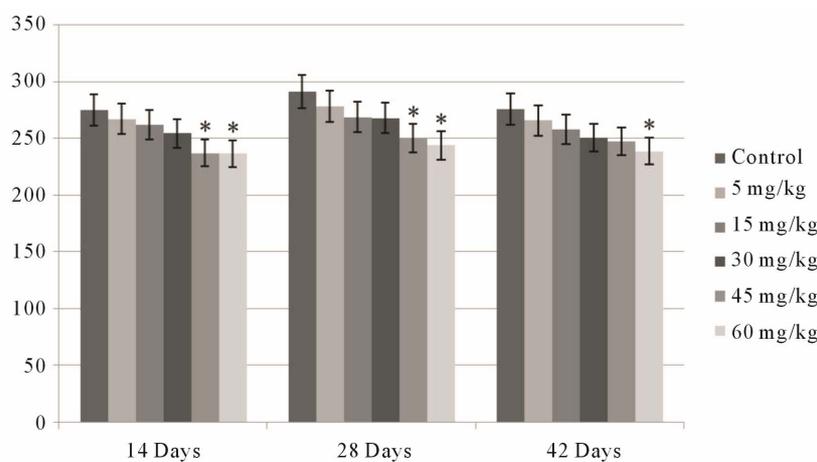


Figure 8. Effect of dietary vanadium on ileac crypt depths (μm); Data are presented with the means \pm standard deviation (n = 5); *P < 0.05, compared with the control group; **P < 0.01, compared with the control group.

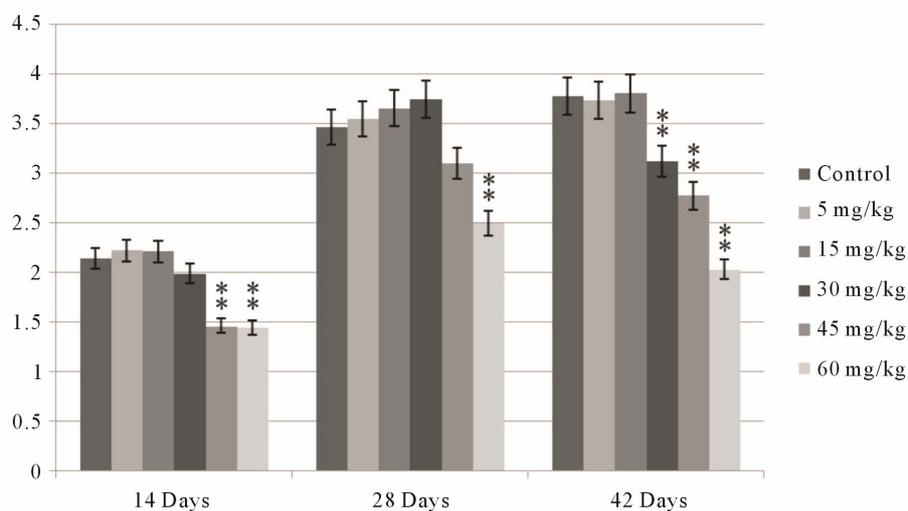


Figure 9. Effect of dietary vanadium on ileac villus height/crypt depth ratio; Data are presented with the means \pm standard deviation ($n = 5$); * $P < 0.05$, compared with the control group; ** $P < 0.01$, compared with the control group.

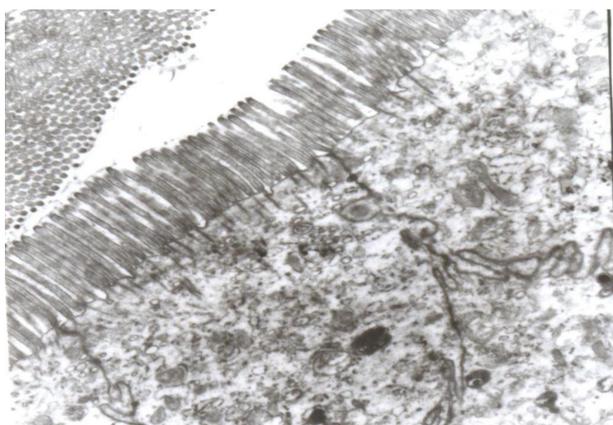


Figure 10. Duodenal villus ultrastructure in control group at 42 days of age (TEM $\times 10,000$).

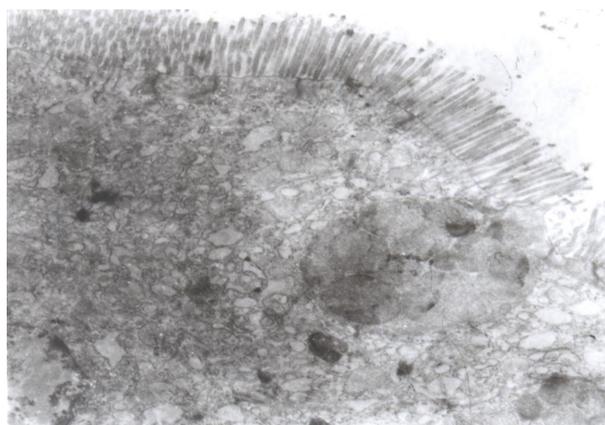


Figure 12. Jejunal villus ultrastructure in control group at 42 days of age (TEM $\times 10,000$).

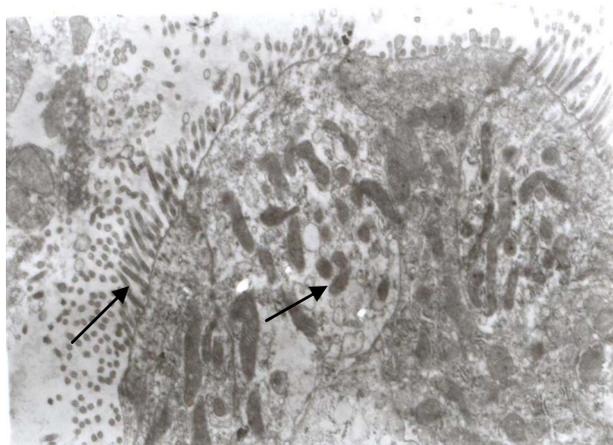


Figure 11. Duodenal villus ultrastructure in 60mg/kg group at 42 days of age. The microvilli are apparently sparse and short and the numbers of lysosomes are increased (TEM $\times 10,000$).

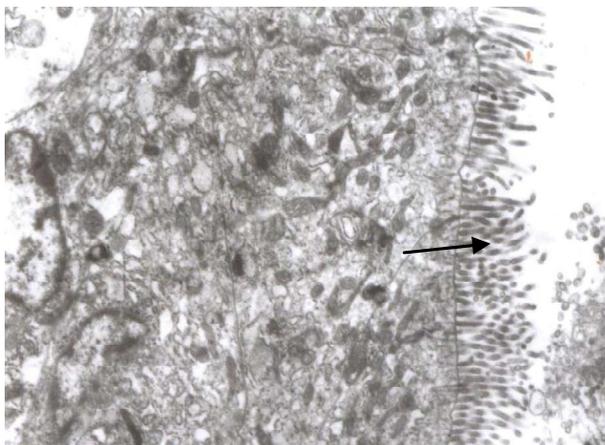


Figure 13. Jejunal villus ultrastructure in 60mg/kg group at 42 days of age. The microvilli are apparently sparse and short (TEM $\times 10,000$).

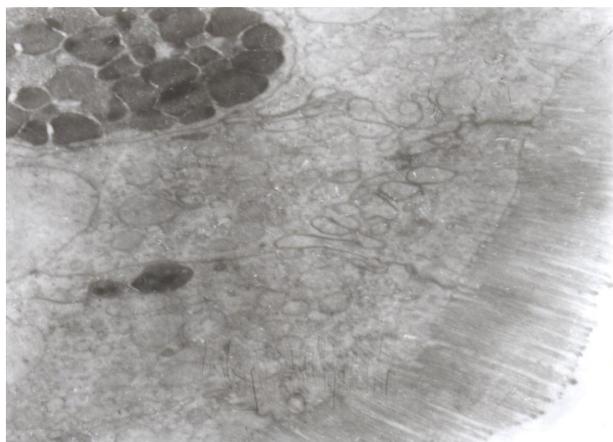


Figure 14. Ileac villus ultrastructure in control group at 42 days of age (TEM \times 10,000).

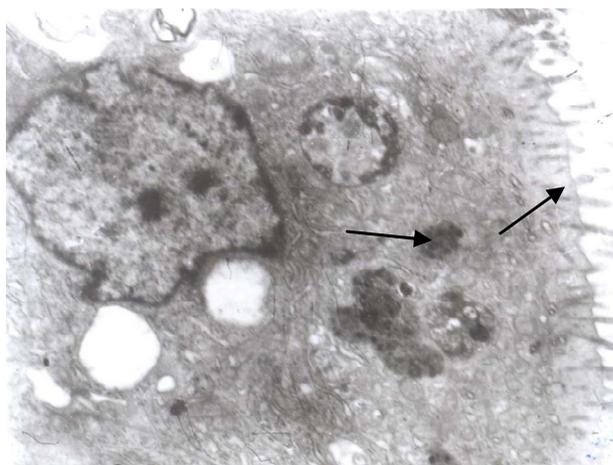


Figure 15. Ileac villus ultrastructure in 60 mg/kg group at 42 days of age. The microvilli are apparently sparse and short and the numbers of lysosomes are increased (TEM \times 10,000).

crypt depth and villus height/crypt depth ratio are direct representation of the intestinal environment and may be used as indicators of intestinal health. Previous studies have proved that villus height/crypt depth ratio was an end point in assessing the developmental state of an individual's intestine [23].

In the present study, we found that dietary vanadium in the range of 30 - 60 mg/kg caused marked effect on the small intestinal villi (duodenum, jejunum and ileum), including the shortened villus height and crypt depth and the decreased villus height/crypt depth ratio in the small intestines. The negative influences were found to be most severe in the 45 and 60 mg/kg groups, implying that dietary vanadium in range of 45 - 60 mg/kg could impact the growth and development of small intestines.

Ultrastructure examination revealed that the microvillus height was shorter and the numbers of lysosomes in enterocytes were increased in the 45 and 60 mg/kg group at 42 days of age. The increased number of lysosomes

may indicate an increase in the amount of denatured protein and damaged organelles [24], which is consistent with results in the present study. Increased denatured protein and damage organelles may impact the microvillus growth and then impact the intestinal villus growth.

Our previous studies have proven that dietary vanadium in 30, 45 and 60 mg/kg can impact the local mucosal immune function of the intestine in broilers [14] and lead to a body weight loss [16], which is consistent with the negative effect of vanadium on the small intestines in broilers in the present study.

In conclusion, it is clear that dietary vanadium in excess of 30 mg/kg can restrain the growth of villus and crypt, reduce the villus height/crypt depth ratio, and alter the ultrastructure in the small intestines. The abovementioned changes may impact the intestinal development in broilers. However, dietary vanadium in 5 mg/kg may promote the growth of broilers.

5. ACKNOWLEDGEMENTS

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REFERENCES

- [1] Mukherjee, B., Patra, B. and Mahapatra, S. (2004) Vanadium—An element of atypical biological significance. *Toxicology Letters*, **150**, 135-143. [doi:10.1016/j.toxlet.2004.01.009](https://doi.org/10.1016/j.toxlet.2004.01.009)
- [2] Milena, J.S., Snezana, U.M., Ivanka, H.A., Marija, T. and Predrag, D. (2007) Compounds of Mo, V and W in biochemistry and their biomedical activity. *Journal of Trace Elements in Medicine and Biology*, **21**, 8-16. [doi:10.1016/j.jtemb.2006.11.004](https://doi.org/10.1016/j.jtemb.2006.11.004)
- [3] Dubyak, G.R. and Kleinzeller, A. (1980) Insulin-mimetic effects of vanadate in isolated rat adipocytes. *Journal of Biological Chemistry*, **255**, 5306-5312.
- [4] Madsen, K.L., Arisno, D. and Fedorak, R.N. (1995) Vanadate treatment rapidly improves glucose transport and activates 6-phosphofructo-1-kinase in diabetic rat intestine. *Diabetologia*, **38**, 403-412. [doi:10.1007/BF00410277](https://doi.org/10.1007/BF00410277)
- [5] Domingo, J.L. (2002) Vanadium and tungsten derivatives as antidiabetic agents. *Biological Trace Element Research*, **88**, 97-112. [doi:10.1385/BTER:88:2:097](https://doi.org/10.1385/BTER:88:2:097)
- [6] Norgaard, A., Kjeldsev, K. and Hansen, O. (1983) A simple and rapid method for the determination of the number of ^3H -ouabain binding sites in biopsies of skeletal muscle. *Biochemical and Biophysical Research Communications*, **111**, 319-325. [doi:10.1016/S0006-291X\(83\)80154-5](https://doi.org/10.1016/S0006-291X(83)80154-5)
- [7] Bahdan, R.N. (1984) Mechanisms of action of Vanadium. *Annual Review of Pharmacology and Toxicology*, **24**, 501-507. [doi:10.1146/annurev.pa.24.040184.002441](https://doi.org/10.1146/annurev.pa.24.040184.002441)
- [8] Schmitz, W. and Scholz, H. (1982) Effect of Vanadium in the +5, +4 and +3 oxidation states on cardiac force of

- contraction, adenylate cyclase and (Na⁺, K⁺)-ATPase activity. *Biochemical Pharmacology*, **31**, 3853-3860. [doi:10.1016/0006-2952\(82\)90302-1](https://doi.org/10.1016/0006-2952(82)90302-1)
- [9] Bishayee, A. and Chatterjee, M. (1995) Time course effects of vanadium supplement on cytosolic reduced glutathione level and glutathione S-transferase activity. *Biological Trace Element Research*, **48**, 275-285. [doi:10.1007/BF02789409](https://doi.org/10.1007/BF02789409)
- [10] Cui, W., Cui, H., Peng, X., Fang, J., Zuo, Z., Liu, X. and Wu, B. (2011) Dietary excess vanadium induces lesions and changes of cell cycle of spleen in broilers. *Biological Trace Element Research*, **143**, 949-956. [doi:10.1007/s12011-010-8938-0](https://doi.org/10.1007/s12011-010-8938-0)
- [11] Liu, J., Cui, H., Liu, X., Peng, X., Deng, J., Zuo, Z., Cui, W., Deng, Y. and Wang, K. (2012) Dietary high Vanadium causes Oxidative damage-induced renal and hepatic toxicity in broilers. *Biological Trace Element Research*, **145**, 189-200. [doi:10.1007/s12011-011-9185-8](https://doi.org/10.1007/s12011-011-9185-8)
- [12] Cui, W., Cui, H., Peng, X., Fang, J., Zuo, Z., Liu, X. and Wu, B. (2012) Dietary vanadium induces lymphocyte apoptosis in the bursa of Fabricius of broilers. *Biological Trace Element Research*, **146**, 59-67. [doi:10.1007/s12011-011-9215-6](https://doi.org/10.1007/s12011-011-9215-6)
- [13] Liu, X., Cui, H., Peng, X., Fang, J., Cui, W. and Wu, B. (2012) Suppression of renal cell proliferation, induction of apoptosis and cell cycle arrest: Cytotoxicity of vanadium in broilers. *Health*, **4**, 101-107. [doi:10.4236/health.2012.42016](https://doi.org/10.4236/health.2012.42016)
- [14] Wang, K., Cui, H., Deng, Y., Peng, X., Fang, J., Zuo, Z. and Cui, W. (2012) Effect of dietary Vanadium on the Ileac T-cells and contents of cytokines in broilers. *Biological Trace Element Research*, **147**, 113-119. [doi:10.1007/s12011-011-9274-8](https://doi.org/10.1007/s12011-011-9274-8)
- [15] Deng, Y., Cui, H., Peng, X., Fang, J., Wang, K., Cui, W. and Liu, X. (2012) Dietary vanadium induces oxidative stress in the intestine of broilers. *Biological Trace Element Research*, **145**, 52-58. [doi:10.1007/s12011-011-9163-1](https://doi.org/10.1007/s12011-011-9163-1)
- [16] Deng, Y., Cui, H., Peng, X., Fang, J., Wang, K., Cui, W. and Liu, X. (2011) Effect of dietary Vanadium on cecal tonsil T-cell Subsets and IL-2 contents in broilers. *Biological Trace Element Research*, **144**, 647-656. [doi:10.1007/s12011-011-9018-9](https://doi.org/10.1007/s12011-011-9018-9)
- [17] Buraczewska, L., Świąch, E., Tuśnio, A., Taciak, M., Ceregrzyn, M. and Korczyński, W. (2007) The effect of pectin on amino acid digestibility and digesta viscosity, motility and morphology of the small intestine, and on N-balance and performance of young pigs. *Livestock Science*, **109**, 53-56. [doi:10.1016/j.livsci.2007.01.058](https://doi.org/10.1016/j.livsci.2007.01.058)
- [18] Mette, S., Lise, D. and Bent, B. (2007) Pre-weaning eating activity and morphological parameters in the small and large intestine of piglets. *Livestock Science*, **108**, 128-131. [doi:10.1016/j.livsci.2007.01.017](https://doi.org/10.1016/j.livsci.2007.01.017)
- [19] Jin, Y., Peng, Y., Fenghua, L., Cheng, G., Guo, K., Lu, An., Zhu, X., Luan, W. and Xu, J. (2010) Effect of heat stress on the porcine small intestine: A morphological and gene expression study. *Comparative Biochemistry and Physiology*, **156**, 119-128. [doi:10.1016/j.cbpa.2010.01.008](https://doi.org/10.1016/j.cbpa.2010.01.008)
- [20] Elbrønd, V., Skadhauge, E., Thomsen, L. and Dantzer, V. (1998) Morphological adaptations to induced changes in transepithelial Na-transport in chicken lower intestine, coprodeum: A study of resalination, aldosterone stimulation, and epithelial turn over. *Cell Tissue Research*, **292**, 543-552. [doi:10.1007/s004410051083](https://doi.org/10.1007/s004410051083)
- [21] Elbrønd, V., Dantzer, V., and Skadhauge, E. (1999) Differences in epithelial morphology correlate to Na-transport. A study of the proximal, mid and distal region of the coprodeum from hens on high and low NaCl diet. *Journal of Morphology*, **239**, 75-86. [doi:10.1002/\(SICI\)1097-4687\(199901\)239:1<75::AID-JMOR5>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1097-4687(199901)239:1<75::AID-JMOR5>3.0.CO;2-D)
- [22] Zitnan, R., Kuhla, S., Nurnberg, K. and Schonhusen, U. (2003) Influence of the diet on the morphology of ruminal and intestinal mucosa and on intestinal carbohydrate levels in cattle. *Veterinarni Medicina*, **48**, 177-182.
- [23] Wang, Y., Xu, M., Wang, F., Yu, Z.P., Yao, J.H., Zan, L.S. and Yang, F.X. (2009) Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. *Livestock Science*, **122**, 48-52. [doi:10.1016/j.livsci.2008.07.024](https://doi.org/10.1016/j.livsci.2008.07.024)
- [24] Sonna, L., Fujita, J., Gaffin, S., Gaffin, S.L. and Craig M.L. (2002) Invited review: Effects of heat and cold stress on mammalian gene expression. *Journal of Applied Physiology*, **92**, 1725-1742.