

## The Effects of Dietary Biotite V Supplementation on Growth Performance, Nutrients Digestibility and Fecal Noxious Gas Content in Finishing Pigs

Y. J. Chen, O. S. Kwon, B. J. Min, K. S. Shon, J. H. Cho and I. H. Kim\*

Department of Animal Resource and Science, Dankook University, #29 Anseodong, Cheonan  
Choongnam, 330-714, Korea

**ABSTRACT** : Two experiments were conducted to evaluate the effects of dietary Biotite V (BV) supplementation on growth performance, nutrients digestibility and fecal noxious gas content in finishing pigs. In Exp. 1, a total of eighty pigs (initial body weight  $88.0 \pm 1.35$  kg) were used in a 35-d growth trial. Pigs were blocked by weight and allotted to five dietary treatments in a randomized complete block design. There were four pigs per pen and four pens per treatment. Dietary treatments included: 1) Control (CON; basal diet), 2) 200 mesh BV1.0 (basal diet+200 mesh Biotite V 1.0%), 3) 325 mesh BV1.0 (basal diet+325 mesh Biotite V 1.0%), 4) 200 mesh BV2.0 (basal diet+200 mesh Biotite V 2.0%) and 5) 325 mesh BV2.0 (basal diet+325 mesh Biotite V 2.0%). Through the entire experimental period, there were no significant differences in ADG, ADFI and gain/feed among the treatments ( $p > 0.05$ ). With the addition of Biotite V in diet, DM and N digestibilities were increased significantly ( $p < 0.01$ ). Also, Ca and P digestibilities tended to increase in pigs fed Biotite V supplemented diet ( $p < 0.01$ ) compared to pigs fed control diet. Supplementation of Biotite V in diet reduced the fecal  $\text{NH}_3\text{-N}$  and volatile fatty acid (VFA) compared to CON treatment ( $p < 0.01$ ). In Exp. 2, a total of sixty four pigs (initial body weight  $84.0 \pm 1.05$  kg) were used in a 35-d growth trial. Pigs were blocked by weight and allotted to four dietary treatments in a randomized complete block design. There were four pigs per pen and four pens per treatment. Dietary treatments included: 1) LP (low protein diet), 2) HP (high protein diet), 3) LP+BV (low protein diet+325 mesh Biotite V 1.0%) and 4) HP+BV (high protein diet+325 mesh Biotite V 1.0%). Through the entire experimental period, ADG and gain/feed tended to increase in HP and HP+BV treatments, however, there were no significant differences ( $p > 0.05$ ) among the treatments. With the addition of Biotite V in diets, digestibilities of nutrients (DM, N, Ca and P) were increased significantly ( $p < 0.01$ ). The addition of Biotite V in diets reduced the ammonia emissions in feces ( $p < 0.01$ ). Supplementation of Biotite V in diets also reduced the fecal propionic acid, butyric acid and acetic acid ( $p < 0.01$ ) compared to pigs fed diets without Biotite V. In conclusion, supplementation of Biotite V can increase nutrients digestibility and reduce fecal  $\text{NH}_3\text{-N}$  and volatile fatty acid (VFA) concentrations in finishing pigs. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 8 : 1147-1152)

**Key Words** : Biotite V, Digestibility,  $\text{NH}_3\text{-N}$ , VFA, Finishing Pigs

### INTRODUCTION

Nowadays, there are increased concerns about potential hazard and pollution in animal industry. Livestock waste is a main source of environment pollution, it contains several components such as nitrogen, phosphorus and ammonia that have negative effects to the environment. However, it has been known that the excretion of these components can be affected by several aspects, included the digestibility of feed, the amount of these ingredients in diet and the amount of endogenous secretions (Han et al., 2001).

Some researchers reported that a series of silicates including kaolinite, zeolite, bentonite and vermiculite have beneficial effects for livestock (Pond et al., 1988; Hagedorn et al., 1990). Most of silicates are classified as clay minerals. These kinds of minerals are crystalline and have three-dimensional structures creating interconnecting channels and voids capable of binding specific molecules (Shurson et al., 1984). Moon et al. (1991) reported that zeolite might bind ammonium ion in the litter due to the high ion-

exchange capacity.

A commercially mineral additive is currently being marketed under the trade name Biotite V (Seobong BioBestech Co., Ltd, Seoul, Korea). Addition of Biotite V to diets for nursery pigs has improved growth performance (Kwon et al., 2002). However, Thacker (2003) observed that there were no effects of Biotite V on growth rate and nutrition digestibility in growing-finishing pigs.

The objectives of this study were 1) to determine the effects of dietary Biotite V supplementation on growth performance, nutrients digestibility and fecal noxious gas content concentration in finishing pigs and 2) to compare the effects of Biotite V supplemented by different particle size and added to different protein level diets.

### MATERIALS AND METHODS

#### Source and composition of Biotite V

The primary compositions of Biotite V include 61.90%  $\text{SiO}_2$ , 23.19%  $\text{Al}_2\text{O}_3$ , 3.97%  $\text{Fe}_2\text{O}_3$  and 3.36%  $\text{Na}_2\text{O}$  (Manufacturers specifications). This product was manufactured by Seobong Biobestech Co., Ltd (Seoul, Korea).

\* Corresponding Author: I. H. Kim. Tel: +82-41-550-3652, Fax: +82-41-553-1618, E-mail: inhokim@dankook.ac.kr  
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**Table 1.** Composition of diets for finishing pigs in Exp. 1

Ingredients (%)	Control	BV 1.0	BV 2.0
Corn	76.23	75.23	74.23
Soybean meal (CP 47.5%)	18.59	18.59	18.59
Molasses	2.50	2.50	2.50
Biotite V	-	1.00	2.00
Tallow	0.81	0.81	0.81
DCP	0.56	0.56	0.56
Limestone	0.80	0.80	0.80
Salt	0.20	0.20	0.20
Vitamin premix <sup>1</sup>	0.12	0.12	0.12
Mineral premix <sup>2</sup>	0.10	0.10	0.10
Antioxidant	0.05	0.05	0.05
L-lysine-HCl	0.04	0.04	0.04
Chemical composition <sup>3</sup>			
ME (kcal/kg)	3,365	3,361	3,358
Crude protein (%)	15.40	15.40	15.40
Lysine (%)	0.76	0.76	0.76
Calcium (%)	0.60	0.60	0.60
Phosphorus (%)	0.50	0.50	0.50

<sup>1</sup> Provided per kg of complete diet: 4,800 IU vitamin A, 960 IU vitamin D<sub>3</sub>, 20 IU vitamin E, 2.4 mg vitamin K, 4.6 mg vitamin B<sub>2</sub>, 1.2 mg vitamin B<sub>6</sub>, 13 mg pantothenic acid, 23.5 mg niacin and 0.02 mg biotin.

<sup>2</sup> Provided per Kg of complete diet: 220 mg Cu, 175 mg Fe, 191 mg Zn, 89 mg Mn, 0.32 mg I, 0.5 mg Co and 0.35 mg Se.

<sup>3</sup> Calculated values.

### Experiment 1

A total of eighty [(Duroc×Yorkshire)×Landrace] pigs with an average initial body weight of 88.0±1.35 kg were used in this 35 days experiment. Pigs were allotted to five treatments on the basis of weight in a randomized complete block design. There were four pigs per pen and four pens per treatment. The five dietary treatments were as follows: 1) Control (CON; basal diet), 2) 200 mesh BV1.0 (basal diet+200 mesh Biotite V 1.0%), 3) 325 mesh BV 1.0 (basal diet+325 mesh Biotite V 1.0%), 4) 200 mesh BV2.0 (basal diet+200 mesh Biotite V 2.0%) and 5) 325 mesh BV2.0 (basal diet+325 mesh Biotite V 2.0%). The basal diet compositions are shown in Table 1.

All diets were provided by meal form. Pigs were allowed *ad libitum* access to feed and water throughout the experiment. Body weight and feed intake were measured at the end of experiment to determine ADG, ADFI and gain/feed. Chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) was added at 0.20% of the diets as an indigestible marker on day-28 and fresh fecal samples were collected on day-33 and 34. Feed and feces samples were analyzed for DM, N, Ca and P concentrations (AOAC, 1995). Chromium was measured by UV absorption spectrophotometer (Shimadzu, UV-1201, Japan) and apparent digestibilities of nutrients (DM, N, Ca and P) were calculated using the indirect-ratio method. NH<sub>3</sub>-N concentration was determined according to the methods of Chaney and Marbach (1962). VFA concentration was analyzed as follows: 2 g fecal samples were obtained and diluted with 8 mL of distilled water. After added 2 drops of concentrated HCl, samples were centrifuged at 17,400×g

**Table 2.** Composition of diets for finishing pigs in Exp. 2

Ingredients (%)	HP	LP
Corn	59.93	67.45
Soybean meal (CP 47.5%)	23.75	18.14
Rice bran	5.00	5.00
Molasses	4.00	5.00
Animal fat	2.61	2.00
Rapeseed meal	2.00	-
Defl. phosphorus	1.16	1.12
Calcium carbonate	0.44	0.68
L-lysine (78%)	0.34	0.20
Vitamin premix <sup>1</sup>	0.10	0.05
Mineral premix <sup>2</sup>	0.25	0.15
Salt	0.15	0.15
L-threonine (98%)	0.09	0.02
DL-methionine (98%)	0.10	-
Choline chloride (60%)	0.08	0.04
Chemical composition <sup>3</sup>		
ME (kcal/kg)	3,447	3,365
Crude protein (%)	17.72	14.80
Lysine (%)	1.02	0.89
Calcium (%)	0.70	0.70
Phosphorus (%)	0.59	0.60

<sup>1</sup> Supplied per kg diet: 4,000 IU vitamin A, 800 IU vitamin D<sub>3</sub>, 171 IU vitamin E, 2 mg vitamin K, 4 mg vitamin B<sub>2</sub>, 1 mg vitamin B<sub>6</sub>, 16 mg vitamin B<sub>12</sub>, 11 mg pantothenic acid, 20 mg niacin and 0.02 mg biotin.

<sup>2</sup> Supplied per kg diet: 220 mg Cu, 175 mg Fe, 191 mg Zn, 89 mg Mn, 0.3 mg I, 0.5 mg Co and 0.4 mg Se.

<sup>3</sup> Calculated values.

for 10 min. The supernatant were filtered by 0.22-µm filter (Millipore Co., Bedford, MA) and analyzed using a gas chromatography (Hewlett Packard 6890 Plus, USA).

### Experiment 2

Sixty four [(Duroc×Yorkshire)×Landrace] pigs with an average initial body weight of 84.0±1.05 kg were used to evaluate the effects of dietary Biotite V supplementation on growth performance, nutrients digestibility and fecal noxious gas concentration in finishing pigs. The experiment was carried out for 35 days. Pigs were blocked by body weight and allotted randomly to four dietary treatments in a randomized complete block design. Dietary treatments included: 1) LP (low protein diet), 2) HP (high protein diet), 3) LP+BV (low protein diet+325 mesh Biotite V 1.0%) and 4) HP+BV (high protein diet+325 mesh Biotite V 1.0%). There were four pigs per pen and four pens per treatment. All diets were provided by meal form. Pigs were allowed *ad libitum* access to feed and water throughout the experiment. At the end of experiment, body weight and feed intake were measured. ADG, ADFI, gain/feed, nutrients digestibility, NH<sub>3</sub>-N and volatile fatty acid (VFA) were determined. All of these analysis methods and procedures were the same as Exp 1.

### Statistical analyses

All the data were analyzed by the GLM procedure of

**Table 3.** Effects of dietary Biotite V on growth performance in Exp. 1<sup>1</sup>

Items	CON	200 mesh		325 mesh		SE <sup>3</sup>	Contrast <sup>4</sup>		
		BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>	BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>		1	2	3
ADG (kg)	0.714	0.690	0.712	0.710	0.730	0.02	0.940	0.200	0.240
ADFI (kg)	2.812	2.920	2.891	2.773	2.893	0.05	0.290	0.350	0.150
Gain/feed	0.253	0.247	0.252	0.258	0.252	0.01	0.560	0.690	0.060

<sup>1</sup> Eighty pigs with an average initial body weight of 88.0±1.35 kg.<sup>2</sup> BV1.0, added 1.0% of Biotite V; BV2.0, added 2.0% of Biotite V.<sup>3</sup> Standard error.<sup>4</sup> Contrast: 1) CON vs. mean of others; 2) 200 mesh vs. 325 mesh and 3) BV 1.0% vs. BV 2.0%.**Table 4.** Effects of dietary Biotite V on nutrients digestibility in Exp. 1<sup>1</sup>

Items (%)	CON	200 mesh		325 mesh		SE <sup>3</sup>	Contrast <sup>4</sup>		
		BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>	BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>		1	2	3
Dry matter	68.65	71.84	70.38	70.04	71.04	0.16	0.01	0.01	0.19
Nitrogen	61.43	67.66	65.70	60.51	62.44	0.60	0.01	0.01	0.99
Calcium	23.21	46.80	43.85	41.77	46.13	0.77	0.01	0.10	0.38
Phosphorus	22.73	42.04	33.61	32.72	36.55	1.18	0.01	0.02	0.07

<sup>1</sup> Eighty pigs with an average initial body weight of 88.0±1.35 kg.<sup>2</sup> BV1.0, added 1.0% of Biotite V; BV2.0, added 2.0% of Biotite V.<sup>3</sup> Standard error.<sup>4</sup> Contrast: 1) CON vs. mean of others; 2) 200 mesh vs. 325 mesh and 3) BV 1.0% vs. BV 2.0%.**Table 5.** Effects of dietary Biotite V on fecal noxious gas content in Exp. 1<sup>1</sup>

Items (ppm)	CON	200 mesh		325 mesh		SE <sup>3</sup>	Contrast <sup>4</sup>		
		BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>	BV1.0 <sup>2</sup>	BV2.0 <sup>2</sup>		1	2	3
NH <sub>3</sub> -N	725.53	694.29	680.16	593.11	557.11	5.56	0.01	0.01	0.01
Propionic acid	6,873.75	5,986.42	5,583.52	5,629.75	5,279.45	120.49	0.01	0.03	0.01
Butyric acid	2,470.30	2,228.05	2,167.08	2,294.26	1,979.84	69.79	0.01	0.41	0.03
Acetic acid	4,937.60	4,509.97	4,220.66	4,300.57	4,043.53	104.48	0.01	0.10	0.03

<sup>1</sup> Eighty pigs with an average initial body weight of 88.0±1.35 kg.<sup>2</sup> BV1.0; added 1.0% of Biotite V, BV2.0; added 2.0% of Biotite V.<sup>3</sup> Standard error.<sup>4</sup> Contrast: 1) CON vs. mean of others; 2) 200 mesh vs. 325 mesh and 3) BV 1.0% vs. BV 2.0%.

SAS (1996). In Exp. 1, orthogonal contrasts were used to separate treatment means and consisted of 1) control group vs. BV supplementation groups, 2) 200 mesh vs. 325 mesh and 3) BV 1.0% vs. BV 2.0%. In Exp. 2, orthogonal comparisons were 1) LP vs. HP, 2) -BV vs. +BV and 3) interaction.

## RESULTS

### Experiment 1

The growth performance of each treatment is presented in Table 3. No significant differences ( $p>0.05$ ) were observed by addition of Biotite V on ADG, ADFI and gain/feed. Among the treatments, addition of Biotite V with different particle size and dose also had no significant differences ( $p>0.05$ ).

Effects of dietary Biotite V on nutrients digestibility are shown in Table 4. DM and N digestibilities were increased significantly ( $p<0.01$ ) in Biotite V supplementation groups compared to CON group. Among the treatment groups, as the addition of 200 mesh Biotite V in diets, digestibilities of DM and N were higher than those of added 325 mesh Biotite V in diets ( $p<0.01$ ). Digestibility of Ca was

increased in Biotite V supplementation groups ( $p<0.01$ ) compared to CON group. Digestibility of P had the same trend as DM and N ( $p<0.05$ ).

Effects of dietary Biotite V on fecal noxious gas content are shown in Table 5. Fecal NH<sub>3</sub>-N and VFA (propionic acid, butyric acid and acetic acid) were reduced ( $p<0.01$ ) when pigs fed basal diet with Biotite V compared to pigs fed basal diet without Biotite V. Among the treatments, NH<sub>3</sub>-N and propionic acid were decreased significantly ( $p<0.01$ ) when pigs fed 325 mesh Biotite V compared to pigs fed 200 mesh Biotite V. However, no significant differences ( $p>0.05$ ) were found in butyric acid and acetic acid between different particle size treatments. Fecal NH<sub>3</sub>-N and VFA (propionic acid, butyric acid and acetic acid) tended to be reduced ( $p<0.05$ ) when diets added 2% Biotite V compared to diets added 1% Biotite V.

### Experiment 2

The results of dietary treatments on growth performance are shown in Table 6. Although ADG and gain/feed tended to be increased when pigs fed high level protein diets compared to pigs fed low level protein diets, there were not statistical differences ( $p>0.05$ ). With the addition of Biotite

**Table 6.** Effects of dietary Biotite V on growth performance in Exp. 2<sup>1</sup>

Items	LP <sup>2</sup>		HP <sup>2</sup>		SE	Contrast <sup>3</sup>		
	-BV	+BV	-BV	+BV		1	2	3
ADG (kg)	0.682	0.700	0.706	0.709	0.02	0.36	0.65	0.77
ADFI (kg)	2.915	2.881	2.800	2.739	0.07	0.12	0.54	0.86
Gain/feed	0.234	0.241	0.252	0.260	0.01	0.06	0.37	1.00

<sup>1</sup> Sixty four pigs with an average initial body weight of 84.0±1.05 kg.

<sup>2</sup> LP, low protein level diets; HP, high protein level diets.

<sup>3</sup> Contrast were: 1) LP vs. HP; 2) -BV vs. +BV and 3) Interaction (LP vs. HP×-BV vs. +BV).

**Table 7.** Effects of Biotite V on nutrients digestibility in Exp. 2

Items (%)	LP <sup>1</sup>		HP <sup>1</sup>		SE	Contrast <sup>2</sup>		
	-BV	+BV	-BV	+BV		1	2	3
Dry matter	63.91	67.80	66.15	70.18	0.36	0.01	0.01	0.85
Nitrogen	56.90	66.70	63.49	68.28	0.51	0.01	0.01	0.01
Calcium	33.72	40.38	35.28	43.78	1.10	0.05	0.01	0.43
Phosphorus	27.55	36.79	27.87	40.64	1.38	0.16	0.01	0.23

<sup>1</sup> LP, low protein level diets; HP, high protein level diets.

<sup>2</sup> Contrast: 1) LP vs. HP; 2) -BV vs. +BV and 3) Interaction (LP vs. HP×-BV vs. +BV).

**Table 8.** Effects of dietary Biotite V on fecal noxious gas content in Exp. 2

Item (ppm)	LP <sup>1</sup>		HP <sup>1</sup>		SE	Contrast <sup>2</sup>		
	-BV	+BV	-BV	+BV		1	2	3
NH <sub>3</sub> -N	688.13	685.89	703.8	652.27	7.76	0.29	0.01	0.02
Propionic acid	5,488.55	5,374.70	6,514.25	5,169.48	124.88	0.02	0.01	0.01
Butyric acid	2,242.91	2,168.79	2,793.79	1,839.44	85.97	0.25	0.01	0.01
Acetic acid	4,027.79	4,059.44	4,909.40	3,534.31	80.31	0.07	0.01	0.01

<sup>1</sup> LP, low protein level diets; HP, high protein level diets.

<sup>2</sup> Contrast: 1) LP vs. HP; 2) -BV vs. +BV and 3) Interaction (LP vs. HP×-BV vs. +BV).

V to the high level protein diets, ADG and gain/feed also tended to be increased, however, no statistical difference ( $p>0.05$ ) was observed compared to low level protein diets added Biotite V.

Effects of dietary treatments on nutrients digestibility are presented in Table 7. With the addition of Biotite V in diets, digestibilities of DM, Ca, P and N were increased significantly ( $p<0.01$ ) compared to pigs fed diets without Biotite V. With the exception of P, digestibilities of DM, N and Ca were increased ( $p<0.05$ ) when pigs fed high level protein diets compared to pigs fed low level protein diets.

Effects of dietary treatments on fecal noxious gas content are shown in Table 8. The addition of Biotite V to either high level protein diets or low level protein diets decreased significantly fecal NH<sub>3</sub>-N, propionic acid, butyric acid and acetic acid concentrations ( $p<0.01$ ). Fecal NH<sub>3</sub>-N, butyric acid and acetic acid concentrations were not affected ( $p>0.05$ ) by feeding different protein level diets. An interaction of LP vs. HP×-BV vs. +BV was also observed among the treatments.

## DISCUSSION

Previous reports about different silicates supplementation in pig's diet were various. Castro and Iglesias (1989) suggested that with addition of zeolite, growth performance could be improved significantly in pre-

fattening pigs. Kwon et al. (2003) reported that dietary supplementation of Biotite V improved finishing pig's weight gain and feed conversion. However, in our study, the results from two experiments indicated that no significant differences were observed on growth performance by the addition of Biotite V. The present findings are in agreement with the findings of Thacker (2003). More studies should be conducted to evaluate this effect.

In another trial conducted by Thacker (2003), using barley and soybean meal based diets observed that no effect on digestibility when pigs fed diet with Biotite V, similar results were observed by Kwon et al. (2003). However, results from our experiments indicated that nutrients digestibilities were increased significantly by addition of Biotite V. In Exp. 2, nutrients digestibilities in HP+BV treatment were higher than other treatments (Table 7). This result indicated that the effects of Biotite V in high protein level diets were better than low protein level diets. No obvious effects were observed by adding different particle size and dose of Biotite V (Table 4). Whether there is an interaction between particle size and addition dose is unclear from present study.

It is known that ammonia N is a major source of environment pollution. The primary objective of present study was to determine fecal NH<sub>3</sub>-N and VFA concentrations when pigs fed diet with Biotite V. Our experiments confirmed that supplementation of Biotite V

decreased fecal  $\text{NH}_3\text{-N}$  and VFA concentrations significantly. Similar findings were observed by Kwon et al. (2003) who reported fecal ammonia gas concentration was reduced by addition of Biotite V in finishing pigs. However, contrary to the present results, Thacker (2003) suggested that fecal N was unaffected by addition of Biotite V. In Exp. 1, total N intake was the same in each treatment, reduced N excretion might be due to improvement of N digestibility. Another mechanism of this effect might associate with the properties of Biotite V. Highly ion exchange and adsorption capacity might bind ammonium ions, so that the fecal  $\text{NH}_3\text{-N}$  excretion was decreased.

Several reports suggested that fecal  $\text{NH}_3\text{-N}$  concentration of swine waste could be reduced by using lower CP (Crude Protein) concentration of diet with AA (Amino Acid) supplementation (Cromwell and Coffey, 1993; Carter et al., 1996). In Exp. 2, diets with different protein level were used. Fecal  $\text{NH}_3\text{-N}$  in LP treatments was lower than HP treatments when pigs fed diet without Biotite V (Table 8). This result was in agreement with previous reports. Fecal  $\text{NH}_3\text{-N}$  in HP+BV treatment was lower than other treatments, also an interaction of LP vs. HP $\times$ -GB vs. +GB was observed in this trial. All those results from Exp. 2 indicated that addition of Biotite V in diets could decrease fecal  $\text{NH}_3\text{-N}$  and this effect was more dramatic in diet with high level protein compared to diet with low level protein. However, why more effect was observed in high protein level diet can't be fully explained by present study.

It has been know that volatile fatty acids are responsible for a significant proportion of odor from swine production facilities (Zahn et al., 2001). The results in our study indicated that with the supplementation of Biotite V in diets, fecal propionic acid, butyric acid and acetic acid of finishing pigs decreased significantly, also more dramatic effect was observed in high protein level diets. This effect might be due to digestibilities of nutrients were improved by the addition of Biotite V. Mackie et al. (1998) reported that volatile fatty acids originate in part from AA deamination by anaerobic bacteria in the gastrointestinal tract and feces. Therefore, increasing digestibility of nutrients might be decrease the synthesis of VFA.

In conclusion, Biotite V supplementation to corn-soybean meal based diet improved nutrients digestibility and decreased fecal  $\text{NH}_3\text{-N}$  and VFA concentrations in finishing pigs.

### IMPLICATIONS

The public concern about pollution in animal industry demands that more effective methods should be observed in the future. From our study, Biotite V offers a potential method to better the odor of swine production facilities and

reduce the pollution of feces. Also, this possible method was proved without any effect on the growth performance of pigs. Obtained data suggest that Biotite V can decrease fecal VFA concentration. However, we collected fresh fecal samples at the end of experiment period, therefore, this result may not be accurate enough to evaluate the status of VFA during all experiment period. Further research is needed to determine fecal VFA concentration throughout all experiment period.

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