



## Effects of Anion Supplementation on Growth Performance, Nutrient Digestibility, Meat Quality and Fecal Noxious Gas Content in Growing-finishing Pigs

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**ABSTRACT :** Forty-eight ((Duroc×Yorkshire)×Landrace) pigs with an average initial body weight (BW) of 48.47±1.13 kg were used in a 12-week growth trial to investigate the influence of Anion (silicate) supplementation on growth performance, nutrient digestibility, meat quality and fecal noxious gas content in growing-finishing pigs. Pigs were allotted into three dietary treatments in a randomized complete block design according to sex and initial BW. Each dietary treatment consisted of four replications with four pigs per pen. Dietary treatments included: i) CON (basal diet), ii) HCl (basal diet+3 g/kg Anion), iii) HClI (basal diet+6 g/kg Anion). No significant difference ( $p>0.05$ ) was detected for average daily gain (ADG), average daily feed intake (ADFI) and gain/feed ratio (G/F) throughout the experiment, although dietary supplementation of Anion numerically increased these characteristics compared with CON. The dietary HCl group significantly ( $p<0.05$ ) increased the coefficient of total tract apparent digestibility (CTTAD) of dry matter (DM), nitrogen (N) and energy compared with the CON group ( $p<0.05$ ). No significant difference was observed in meat quality except that meat firmness was linearly ( $p<0.05$ ) increased by the Anion supplementation, while an increased tendency in meat color and a decreased tendency in 2-thiobarbituric acid reactive substances (TBARS) was also observed ( $p<0.10$ ). Anion supplementation linearly ( $p<0.05$ ) decreased the fecal  $\text{NH}_3$  compared with the CON group. However, dietary Anion supplementation at 3 g/kg decreased the  $\text{H}_2\text{S}$  concentration compared with CON, while no significant difference was detected in the HClI group, although the  $\text{H}_2\text{S}$  emission was numerically decreased compared with CON. In conclusion, supplementation of the diet with 3 g/kg Anion was found to exert a beneficial effect on nutrient digestibility and meat quality of growing-finishing pigs, and concomitantly decreased the noxious gas emission without negative effect on growth performance. (**Key Words :** Anion, Fecal Noxious Gas Content, Digestibility, Pigs)

### INTRODUCTION

As we know, feeds are not only the source of nutrients but also contain a number of contaminants that may enter the food chain via animal products. Therefore, a considerable effort is being made to develop procedures to diminish the penetration of such substances into the animal body and subsequently contamination in animal products. During the last few decades, various studies have suggested that the use of un-convention feed supplement such as feed adsorbents is the most promising and economical approach to address this issue (Chen et al., 2005a, b; Dakovic et al., 2005). Recently studies also suggested that adsorbents (a variety of clays, bentonites, zeolites, phyllosilicates and

synthetic aluminosilicates) can prevent or reduce the mycotoxicosis bioavailability and its detrimental effects on animals because of its binding effect with aflatoxins, zearalenone and the ammonium, (Abdel-Wahhab et al., 1999; Abdel-Wahhab et al., 2002; Abbès et al., 2006). Similarly, our previous study also suggested that an aluminosilicate mineral product (61.90%  $\text{SiO}_2$ , 23.19%  $\text{Al}_2\text{O}_3$ , 3.97%  $\text{Fe}_2\text{O}_3$  and 3.36%  $\text{Na}_2\text{O}$ ) can be used as antibiotic alternative at the levels of 0.5 and 2.0% (Chen et al., 2005a, b). Our study was concerned about one of the aluminosilicate mineral product marketed under the trade name Anion (Eunjinbio system Choongnam Cheonan, Korea), which is mainly comprised of 72.60%  $\text{SiO}_2$ , 8.18%  $\text{Al}_2\text{O}_3$ , 9.42%  $\text{Fe}_2\text{O}_3$ , 5.25%  $\text{K}_2\text{O}$  and 1.41%  $\text{Na}_2\text{O}$  (Manufacturers specifications, provided by Korea chemistry association).

Therefore, the objective of the current study was to evaluate the effect of Anion with more than 70% of silicon dioxide (organic origin) on growth performance, nutrient

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digestibility, meat quality and fecal noxious gas content in growing-finishing pigs.

## MATERIALS AND METHODS

The experiment was performed at the Experimental Unit of the Dankook University, and the protocol of the current experiment was approved by the Animal Care and Use Committee of Dankook University.

### Preparation of Anion

The Anion was prepared by Eunjinbio system Choongnam Cheonan, Korea, The main components of this product was shown in Table 1, which is provided by the Korea Chemical Institution.

### Experimental design, animal and diets

Forty-eight ((Duroc×Yorkshire)×Landrace)) pigs with an average initial BW of 48.47±1.13 kg were used in this 12-week growth trial. Pigs were allotted by the initial BW into three dietary treatments in a randomized complete block design. Each dietary treatment was consisted of four replications with four pigs per pen. The experimental dietary treatments included: i) CON (basal diet), ii) HCI (basal diet +3 g/kg Anion), iii) HCII (basal diet +6 g/kg Anion). The diets used in this experiment were formulated to meet or exceed NRC (1998) recommendations for all nutrients (Table 2), regardless of the treatments. Treatment additives were included in the diet by replacing the same amount of corn and each treatment was made isolysin and isocaloric by manipulation of soybean meal and fat source. All diets were dried at 100°C for 12 h to determine DM and

**Table 1.** Composition for extract Anion supplementation<sup>1</sup>

Ingredients	% <sup>2</sup>
SiO <sub>2</sub>	72.60
Al <sub>2</sub> O <sub>3</sub>	8.18
Fe <sub>2</sub> O <sub>3</sub>	9.42
K <sub>2</sub> O	5.25
Na <sub>2</sub> O	1.41
CaO	0.62
TiO <sub>2</sub>	0.42
P <sub>2</sub> O <sub>5</sub>	0.40
ZrO <sub>2</sub>	0.10
MgO	0.06
MnO	0.04
BaO	0.03
Ge	1.00
Ig.loss	0.47

<sup>1</sup> Provided by Ejunbio system Eunjinbio system Choongnam Cheonan, Korea.

<sup>2</sup> Provided by the Korea Chemical Institution.

ground through a 1-mm screen in a Wiley mill before analyzing for CP, P and Ca (AOAC, 2000). Pigs were housed in an environmental controlled, slatted-floor facility in 24 adjacent pens (1.80×1.80 m) and were allowed *ad libitum* access to feed and water through a self-feeder and nipple waterer throughout the experimental period.

### Data and sampling collection

*Growth performance and nutrients digestibility* : Body weight (BW) and feed consumption were measured weekly to monitor ADG, ADFI and G/F. Chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) was added to the diet at 0.20% level as an indigestible marker at the beginning of 6<sup>th</sup> week and 16<sup>th</sup> week to calculate the digestibility coefficient. Fecal grab samples were then collected randomly from at least two pigs in each pen. Feed and fecal samples were dried for 72 h at 70°C, after which they were finely ground to be able to pass

**Table 2.** Formula and chemical compositions of diets (as-fed basis)

Ingredients (g/kg)	
Ground corn	599.3
Soybean meal	237.5
Rice bran	50.0
Molasses	40.0
Animal fat	26.1
Rapeseed meal	21.0
Phosphorus defluorinated	11.6
Calcium carbonate	4.4
L-lysine·HCl	3.4
Salt	1.5
Vitamin premix <sup>1</sup>	1.0
Mineral premix <sup>2</sup>	1.5
DL-methionine, 98%	1.0
Choline chloride, 60%	0.8
L-Threonine, 98%	0.9
Calculated chemical composition <sup>3</sup>	
DE (MJ/kg)	14.4
CP (g/kg)	177.2
Lys (g/kg)	10.2
Ca (g/kg)	7.0
P (g/kg)	5.9
Analyzed composition	
DE (MJ/kg)	14.3
CP (g/kg)	178.3
Ca (g/kg)	6.9
P (g/kg)	5.8

<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 µg vitamin B<sub>12</sub>, 11 mg pantothenic acid, 20 mg of niacin and 0.08 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> All calculated values are based on NRC (1998) tabular values.

through a 1-mm screen and then frozen and stored in a refrigerator at  $-20^{\circ}\text{C}$  until analysis. DM and N concentrations were analyzed according to AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the coefficient of CTTAD of DM and N was calculated using indirect-ratio methods. Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL).

**Meat quality:** All pigs were slaughtered at a local commercial slaughterhouse at the end of the experiment. One piece of the right loin sample was removed between 10<sup>th</sup> and 11<sup>th</sup> ribs after chilling at  $2^{\circ}\text{C}$  for at least 24 h. The meat samples were thawed at ambient temperature prior to evaluation. Sensory evaluation (Color, marbling and firmness scores) was conducted according to the National Pork Producers Council Standards (NPPC, 1991). Immediately after the subjective tests were conducted, the L\* (lightness), a\* (redness) and b\* (yellowness) values were measured at 3 locations on the surface of each sample using a Model CR-410 Chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). At the same time, duplicate pH values of each sample were directly measured using a pH meter (Pittsburgh, PA, USA). The longissimus muscle area (LMA) was measured by tracing the longissimus muscle surface at the 10<sup>th</sup> rib, which was also conducted using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). Drip loss was measured using approximately 2 g of meat sample according to the plastic bag method described by Honikel (1998). The TBARS were measured using the method described by Witte et al. (1970), in which the TBARS were expressed in terms of milligrams of malonaldehyde (MDA) per kilogram of muscle. Trichloroacetic acid solution (TCA, 20% wt/vol) was utilized for the extraction. UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) was employed for the spectrophotometric analyses.

**Fecal noxious gas content :** Fresh feces were collected from two pigs in each pen on the last 2 d of 5<sup>th</sup> week, all the feces samples were stored immediately at  $-20^{\circ}\text{C}$  until analysis. The total sampled from each pig was thawed at then homogenized, the stock slurries were then stored in 2.6-Liter plastic boxed with a small hole in the middle of one side wall that was sealed with adhesive plaster. The samples were allowed to ferment for 5 d at room temperature ( $25^{\circ}\text{C}$ ), after which the gas was sampled using a Gastec (model GV-100) gas sampling pump (Gastec Corp., Japan; Gastec detector tube No. 3M and 3 La for  $\text{NH}_3$ ; No. 4 LL and 4 LK for  $\text{H}_2\text{S}$ ). Prior to measurement, the slurry samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the slurry

sample and to homogenize them. The adhesive plaster was then punctured and 100 ml of the headspace air was sampled approximately 2.0 cm above the feces or slurry surface, after which each box was re-sealed with adhesive plaster. The headspace measurement was repeated at 10<sup>th</sup> and 15<sup>th</sup> d after the initial measurement and the concentrations of gas were determined using the average of the 3 head space measurements.

### Statistical analyses

All data were subjected to the GLM procedures of SAS as a randomized complete block design. Each pen was considered as the experimental unit. The initial BW was used as a covariate for ADFI and ADG. Duncan's multiple range test was adopted to compare the means of the treatments (Duncan, 1955). Orthogonal polynomials were employed to assess the linear and quadratic effect of the dosage of Anion supplementation. Variability in the data was expressed as the pooled standard error (SE) and a  $p < 0.05$  was considered to be statistically significant.

## RESULTS

### Pig health

All pigs grew normally and showed no signs of ill health that could have been attributed to the ingestion of high dosage used in herein study.

### Growth performance

In the current experiment, no significant difference was detected for ADG, ADFI and G/F throughout the experiment (Table 3), although dietary Anion numerically increased these characteristics compared with CON group.

### DM, N and energy digestibility

Digestibility (DM, N and Energy) was increased (quadratic effect,  $p < 0.05$ ) by Anion supplementation, where digestibility (DM, N and energy) was higher in HCI group (5.84%, 4.47% and 2.94%) compared with CON group, respectively (Table 4).

### Meat quality

No significant difference was observed on meat quality except the meat firmness was linearly ( $p < 0.05$ ) increased by the Anion supplementation (Table 5), meat firmness was increased by 20.22% in the HCII treatment compared with the CON. In addition, a linearly increase tendency ( $p < 0.10$ ) were observed on meat color. TBARS showed a decreased tendency with increasing Anion supplementation ( $p < 0.10$ ).

### Fecal noxious gas content

Feeding diets with Anion supplementation at 3 g/kg and 6 g/kg linearly ( $p < 0.05$ ) decreased the fecal  $\text{NH}_3$  by 16.44%

**Table 3.** Effects of anion supplementation on growth performance in growing-finishing pigs

Items	CON <sup>1</sup>	HCI <sup>1</sup>	HCII <sup>1</sup>	SE <sup>2</sup>	p-value	
					Linear	Quadratic
0 to 6 week						
ADG (kg)	0.718	0.755	0.733	0.021	0.78	0.22
ADFI (kg)	1.965	1.936	2.033	0.076	0.22	0.30
gain/feed	0.365	0.390	0.361	0.016	0.34	0.19
6 to 12 week						
ADG (kg)	0.881	0.895	0.884	0.024	0.93	0.60
ADFI (kg)	2.632	2.725	2.644	0.067	0.65	0.12
gain/feed	0.335	0.328	0.334	0.013	0.74	0.40
Overall						
ADG (kg)	0.800	0.825	0.809	0.023	0.93	0.39
ADFI (kg)	2.279	2.311	2.319	0.059	0.20	0.36
gain/feed	0.351	0.357	0.349	0.014	0.48	0.95

<sup>1</sup> Each value represents 4 pens of four pigs. CON = Basal diet; HCI = Basal diet with Anion supplementation 3 g/kg; HCII = Basal diet with Anion supplementation 6 g/kg.

<sup>2</sup> Pooled Standard error.

**Table 4.** Effects of anion supplementation on nutrient digestibility in growing-finishing pigs

Items	CON <sup>1</sup>	HCI <sup>1</sup>	HCII <sup>1</sup>	SE <sup>2</sup>	p-value	
					Linear	Quadratic
DM	0.774 <sup>b</sup>	0.817 <sup>a</sup>	0.773 <sup>b</sup>	0.009	0.907	0.0017
N	0.761 <sup>b</sup>	0.792 <sup>a</sup>	0.744 <sup>b</sup>	0.006	0.053	<0.0001
Energy	0.806 <sup>ab</sup>	0.827 <sup>a</sup>	0.777 <sup>b</sup>	0.013	0.132	0.037

<sup>1</sup> Each value represents 4 pens of two pigs. CON = Basal diet; HCI = Basal diet with Anion supplementation 3 g/kg; HCII = Basal diet with Anion supplementation 6 g/kg.

<sup>2</sup> Pooled Standard error.

<sup>ab</sup> Means in the same row with different superscripts differ (p<0.05).

**Table 5.** Effects of anion supplementation on meat quality in growing-finishing pigs

Items	CON <sup>1</sup>	HCI <sup>1</sup>	HCII <sup>1</sup>	SE <sup>2</sup>	p-value	
					Linear	Quadratic
pH	5.73	5.69	5.73	0.44	0.958	0.481
LMA (cm <sup>2</sup> )	34.53	37.14	34.53	1.50	0.587	0.301
Sensory evaluation						
Color	2.23	2.36	2.52	0.108	0.067	0.913
Marbling	1.88	1.69	1.80	0.085	0.557	0.148
Firmness	1.81 <sup>b</sup>	1.83 <sup>b</sup>	2.20 <sup>a</sup>	0.055	0.015	0.109
Cook loss (%)	38.64	37.68	37.66	1.23	0.580	0.802
TBARS (mg. MA/kg)	0.012	0.010	0.009	<0.001	0.051	0.687
Meat color						
L*	54.69	54.98	54.71	1.03	0.990	0.830
a*	18.22	16.98	17.49	0.42	0.240	0.110
b*	6.98	7.32	6.94	0.39	0.940	0.470
Drip loss (%)	6.23	5.94	5.61	0.53	0.880	0.320

<sup>1</sup> Each value represents 4 pens of four pigs. CON = Basal diet; HCI = Basal diet with Anion supplementation 3 g/kg; HCII = Basal diet with Anion supplementation 6 g/kg; LMA = Longissimus muscle area at the 10<sup>th</sup>-rib.

<sup>2</sup> Pooled Standard error.

<sup>ab</sup> Means in the same row with different superscripts differ (p<0.05).

**Table 6.** Effects of anion supplementation on fecal noxious gas content in growing-finishing pigs

Items (mg/kg)	CON <sup>1</sup>	HCI <sup>1</sup>	HCII <sup>1</sup>	SE <sup>2</sup>	p-value	
					Linear	Quadratic
H <sub>2</sub> S	4.64 <sup>a</sup>	3.88 <sup>b</sup>	4.01 <sup>ab</sup>	0.19	0.060	0.113
NH <sub>3</sub>	2.19 <sup>a</sup>	1.83 <sup>b</sup>	1.73 <sup>b</sup>	0.06	0.002	0.534

<sup>1</sup> Each value represents 4 pens of two pigs. CON = Basal diet; HCI = Basal diet with Anion supplementation 3 g/kg; HCII = Basal diet with Anion supplementation 6 g/kg.

<sup>2</sup> Pooled Standard error.

<sup>a,b</sup> Means in the same row with different superscripts differ ( $p < 0.05$ ).

and 21.00% respectively, when compared with CON (Table 6). Fecal H<sub>2</sub>S concentration was 16.38% lower ( $p < 0.05$ ) in diets with Anion supplemented at 3 g/kg than CON group. Anion supplementation at 6 g/kg decreased ( $p > 0.05$ ) the H<sub>2</sub>S by 13.58% compared with CON group.

## DISCUSSION

### Growth performance and nutrient digestibility

As we known, various studies have documented that non-conventional feed supplements such as adsorbents (a variety of clays, bentonites, zeolites, phyllosilicates and synthetic aluminosilicates) are capable of preventing or reducing the mycotoxicosis effects on farm animals because of its binding ability with aflatoxins, zearalenone and the ammonium (Abdel-Wahhab et al., 1999; Denli et al., 2009). Previous study conducted by Mumpton and Fishman (1977) also suggested that effect of zeolite particles might stimulate the lining of the stomach and intestinal tract and subsequently promote animal health condition. Similarly, our lab previous study reported a kind of a commercially mineral additive named Biotite V (61.90% SiO<sub>2</sub>) can have some antibacterial and antimycotic activity because of its three-dimensional structure and ion-ex-change capacity (Chen et al., 2005a). Therefore, it is appropriate to postulate that Anion supplementation could exert somewhat beneficial effects to animals due to its high concentration of silica dioxide in herein study. However, results of current study were out of anticipation and detected no difference for growth performance throughout the experiment, which is partially in agreement with Chen et al. (2005a, b), who reported that the growth performance was unaffected by Biotite V supplementation at the levels of 0.5% to 2.0%. Moreover, Denli et al. (2009) also suggested that diatomaceous (60% silica dioxide) supplemented at the range from 1 g/kg to 5 g/kg can not affect the growth performance in broilers. Conversely, positive effects were observed by Kwon et al. (2003) and Papaioannou et al. (2004), who suggested the feed conversion was significantly increased after clinoptilolite feeding (3% and 2% respectively) in weaning pigs, which is higher than the studies without effect. Therefore, the variations may properly be due to the different adsorbents, the

supplemental levels as well as different animal and environment conditions employed in each study. However interestingly, digestibility of DM and N was quadratic increased by the Anion supplementation in herein study indicated that the Anion indeed exert a positive effect to the animal at the level of 3 g/kg, which is supported by Mumpton (1999), who suggested that zeolites may support the growth of nitrogen-loving bacteria that contribute to the health of the animal by absorption of the NH<sub>4</sub><sup>+</sup> and regulating pH in the gut system. Similarly, Our previous study also suggested that Biotite V supplemented at 5 g/kg and 20 g/kg can increase the nutrient digestibility in growing and finishing pigs due to its beneficial effect on intestine tract Chen et al. (2005a, b). Therefore, the decreased digestibility observed with the increasing addition levels may be attributed to the different proportion between the main ingredient such as (SiO<sub>2</sub>:Al<sub>2</sub>O<sub>3</sub>) in herein study (8.87) compared with our previous study (2.66), indicating the excess amount of SiO<sub>2</sub> may prevent the absorption of amino acids or other nutrients. Moreover, the mineral toxicity introduced by other additional highly dosage mineral complex (Fe<sub>2</sub>O<sub>3</sub> 0.56 g/kg, K<sub>2</sub>O 0.315 g/kg and Na<sub>2</sub>O 0.085 g/kg diet) in the Anion may also explain this effect, which is supported by Muhle and Mangelsdorf (2002), who suggested poorly soluble particles in the crystalline silica may not act as a molecular form but act as a whole particle and subsequently lead to various particle toxicity

### Meat quality

Recently, a variety of different meat characteristics (pH, color, and firmness) have been employed to separate fresh pork into quality groups in pork industry. Approaches have also been investigated in animal nutrition to improve the meat quality in recent years. It has been widely documented that minerals such as Fe and Cu have beneficial effects on animals (Stahly et al., 1980; Apply et al., 2007). Previous studies also suggested that supplementation of zeolites could alter tissue mineral concentration in poultry and pigs because of its ability of absorbing metal ions (Pond and Yen, 1983; Watkins et al., 1989). Therefore, it is properly to suggest that the improved firmness observed in the current study might be due to its affection of the metabolism or

interaction with those metal ions and subsequently alter the mineral distribution in tissue. Moreover, the tendency on the TBARS and meat color may also be attributed to the additional mineral supplementation such as  $\text{Fe}_2\text{O}_3$ ,  $\text{K}_2\text{O}$  and  $\text{Na}_2\text{O}$  in Anion. This hypothesis is supported by Apple et al. (2007), who suggested that the Fe supplementation can increase the meat color and decrease the TBARS during retail display. Moreover, Davis (1980) also reviewed and stated that the interaction between Zn, Cu and Fe may be in the feed, lumen of the intestine, within the cells of various organs, related to the transport mechanisms; therefore, it is suitable to postulate the tendency observed in the current study may be due to the additional mineral supplementation in Anion. However, as limited studies were conducted to evaluate the effects of aluminosilicate clay series on meat quality of pigs, no related reference is available to compare this effect. Further study is still necessary to evaluate its effect on meat quality.

### Fecal noxious gas content

Recently, various natural zeolites are widely focused their studies on the evaluation of the effect of the dietary utilization on environmental stressors such as aerial  $\text{NH}_3$  and  $\text{H}_2\text{S}$ , which are considered the main components of pig manure contributing to environmental pollution (Zahn et al., 1997). For example, Mumpton and Fishman (1977) reported that the zeolites are capable of adsorbing up to about 30% of a gas because of the well known "molecular sieving" property. Similarly, Melenova et al. (2003) and Meisinger et al. (2001) also suggested the zeolite application can remove the excess ammonia both in the digestive tract and bedding. In the current study, both the  $\text{H}_2\text{S}$  and  $\text{NH}_3$  are significantly decreased with the increasing Anion supplementation, which was in agreement with our previous study conducted by Chen et al. (2005b), who suggested the Biotite V supplementation at 2% can decrease the fecal noxious gas content in finishing pigs. Therefore, it is properly to postulate that the effect observed in the current study may possibly attribute to the reasons as follow: i) The improved nutrients digestibility caused by the Anion may have benefit from the environmental, ii) Nitrification of sludge is accelerated by the use of Anion, which selectively exchanges  $\text{NH}_4^+$  from wastewater and provides an ideal growth medium for nitrifying bacteria, which then oxidize  $\text{NH}_4^+$  to nitrate, iii) The control of the viscosity and nutrient retention of the manure.

### CONCLUSION

In conclusion, supplementation of the diet with 3 g/kg Anion was found to exert a beneficial effect to the nutrients digestibility and meat quality of finishing pigs, concomitantly decreasing the noxious gas emission without

negative effect on growth performance.

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