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Running Title (English)	Application of illite mineral in pigs
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6	Effect of illite to growing-finishing pig diets on growth performance, apparent total tract
7	digestibility, and meat-carcass grade quality
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Abstract

This study investigated the impact of clay mineral 'illite' (IL) on the growth performance, apparent total tract digestibility, and meat-carcass grade quality in growing-finishing pigs. One hundred fifty pigs were divided into two groups with fifteen pens/group and five pigs/pen, a control group that was fed with a corn-soybean meal-based diet and illite treated group were fed a meal-based diet supplemented with 0.5% illite. Compared to the control, IL supplementation increased average daily feed intake (ADFI) and reduce G: F in the pigs during days 43 to 70 and 99 to 126 and increased dry matter during days 42 and 126, and backfat thickness on day 98 (P < 0.05). The drip loss was reduced on day 7, meat firmness tended to increase with dietary IL supplementation. In summary, dietary 0.5% IL supplementation improved ADFI, nutrient utilization of dry matter, and firmness and reduced G: F and drip loss of growing-finishing pigs.

- **Key words:** Backfat thickness, Growing-finishing pigs, Growth performance, Illite, Meat
- 33 quality, Nutrient digestibility

35 **INTRODUCTION**

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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, to diminish the penetration of such substances into the animal body and subsequent contamination of animal products. During the last few decades, various studies have suggested that the use of un-convention feed supplements such as feed adsorbents is the most promising and economical approach to address this issue [1-3]. 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 their binding effect with aflatoxins, zearalenone and the ammonium, [4,5]. Clay minerals are a phyllosilicate that joins tetrahedral sheets to octahedral sheets. Clay minerals is swine nutrition are used in particular due to their absorption/adsorption properties significantly contributing to the health of the swine [1]. Clay minerals bind noxious compounds and expel them from the body of animals [2,3]. The most wellknown are montmorillonite, illite (IL), biotite, diatomite, quartz porphyry, and clinoptilolite. Phyllosilicate minerals including zeolite, kaolinite, bentonite, and vermiculite, have positive effects for on livestock [6,7], due to the specific structures of porous layers and high ion-exchange capacity [8]. Clay minerals were shown to reduce mycotoxins, such as aflatoxin B₁ and zearalenone, in pigs diets [9]. The dietary supplementation of clay improved the growth performance and nutrient digestibility of pigs [10]. In addition, dietary anion supplementation showed beneficial effects on growth performance and meat quality in growing-finishing pigs [11, 12]. A commercial phyllosilicate mineral additive is available under the trade name of IL (SeobongBiobestech Co, Ltd, Seoul, Korea). To the best of our knowledge, no information about the impact of IL on growing-finishing pigs was available. In this study, we evaluate the effects of

supplementation of IL on growth performance, total tract digestibility, and meat quality in growing-finishing pigs.

MATERIALS AND METHODS

Animal ethics

The Dankook University Institutional Animal Care and Use Committee approved (DK-4-1516) the protocols for this experiment.

Source of Anion

The IL was prepared by seobongBiobestech Co, Ltd, Seoul, Korea, the main components of this product was shown in Table 1, which is provided by the Korea Chemical Institution.

Experimental design, animals, and housing

One hundred and fifty pigs ((Landrace × Yorkshire × Duroc) with an initial body weight of 25.42 ± 3.23kg) were randomly allocated to two groups with fifteen pens/group and five pigs/pen. The trial dietary groups consisted of a corn-soybean meal-based basal diet 0, (control) 0.5% illite (IL) for 126 days. The basal diet composition was formulated in order to meet the requirements according to the National Research Council recommendation [13] (Table 2). Each pen was equipped with a stainless steel self-feeder and a nipple drinker. Room temperature was kept at 27°C.

Sampling and measurements

Pigs were weighed on days 0, 42, 70, 98, and 126 as well as the feed consumption was also recorded on a pen basis to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F = ADFI/ADG).

The fresh fecal samples were collected (about 300 g) from each pen during the end of the trial and were -dried and finally ground to pass through a 1-mm sieve for the measurement of dry matter, nitrogen, and energy. The two groups diets and feces samples were determined following the procedures of Association of Official Analytical Chemists [14]

Carcass traits

Two pigs per pen were selected randomly from each treatment at days 0, 14, 28, 42, 56, 70, 84, 98, 112, and 126 experiment period to determine the backfat thickness and at week 18 of the experiment, lean meat percentage (LMP) measurements were measured using a real-time ultrasound instrument (Piglog 105, SFK Technology, Herley, Denmark).

Meat quality traits

One pig per pen was selected for slaughter based on the average body weight. One piece of the right loin sample was removed between 10th and 11th ribs after chilling at 2°C for at least 24 h. The meat samples were thawed at ambient temperature prior to evaluation. The carcass was placed at 4°C for a 24-h chilling period. The meat color containing L*, a*, and b* were determined by Chenet al [1]. Minolta colorimeter (CR- 410, Konica Minolta Sensing Inc., Osaka, Japan); firmness, color, and marbling values were assessed as reported by NPPC standards [15]. Drip loss was evaluated utilizing about 5g of meat sample as reported by the plastic bag method described by Honikel [16]. The weight of each sample was taken before and after cooking to determine cooking loss, which was defined as the cooked weight divided by uncooked weight multiplied by Sullivan et al [17]. The thiobarbituric acid reactive substances (TBARS), was determined according to Witte et al [18]. The quality of pork carcasses was graded using the method described by Muniyappan et al [19].

Statistical Analysis

All data were analyzed by the student's t-test for unpaired data with pen as trial unit (SAS software version 9.2; SAS Inst. Inc., Cary, NC). Variability in the data is expressed as the standard error of the means, and a probability level of P < 0.05 were considered to be statistically significant and the tendency was declared at $0.05 \le P < 0.10$.

RESULTS

Growth performance

The effect of supplementation of IL to basal diet on growth performance of growing-finishing pig is presented in Table 3. Dietary inclusion of IL increased (P < 0.05) ADFI and decreased G:F was observed during 43 to 70 and 99-126 days compared with the pigs fed control diet. However, BW and ADG were similar among the diet groups.

Apparent total tract digestibility

The effect of IL supplementation on the nutrition digestibility of growing- finishing pig is presented in Table 4. On days 48 and 126, dietary inclusion of IL increased (P < 0.05) dry matter digestibility compared with the control. Dietary treatments did not influence nitrogen and energy.

Carcass traits

The data presented in Table 5 shows the result of backfat thickness and LMP. Dietary supplementation of IL in diets had shown significant (P < 0.05) improvement in backfat thickness during the day 98. However, LMP were not significantly different among the diet groups.

Meat quality

A decrease (P < 0.05) drip loss of pigs supplemented with IL was observed compared with the control. Furthermore, the supplementation of IL resulted in trends to increase of the firmness (P < 0.10). No impact of dietary treatments was found on other meat quality parameters (TBARS, meat color, cooking loss, sensory evaluation, Marbling) (Table 6).

Carcass grade

Table 7 shows the results of the effects of dietary inclusion of IL on carcass grade. We observed no significant difference (P > 0.05) in carcass weight and backfat thickness among treatments. We found that 1% carcass grade was higher in pigs fed supplemented with IL.

DISCUSSION

Previous studies which investigated supplementation of non-conventional feed additives like clays, synthetic aluminosilicates, phyllosilicates, bentonites, and zeolites have decreased mycotoxicosis impact on livestock farms due to its binding capability with aflatoxins, zearalenone, and ammonium [11, 20]. Dietary inclusion of zeolite particles might stimulate the lining of the gut and gastrointestinal tract and afterward promote swine health conditions [8]. In the current study, the inclusion of IL significantly improved ADFI and decreased G: F compared with the control, and this finding was similar to previous reports [11]. Kwon et al [21] showed that Biotite V supplementation to pigs' diets had significantly increased ADFI and G: F. Thacker [6] also reported that dietary inclusion of zeolite increased ADG and ADFI of finishing pigs. Trckova et al [22] found that adding 1% of kaolin enhanced pigs' growth performance. Similarly, Song et al [7] and Xia et al [23] reported a significant increase in the growth performance due to dietary supplementation of 0.15%, 0.3%, or 0.6% silicate minerals. The improvement in the growth performance of pigs due to the aluminosilicate minerals, clay has been proven to have anti-

bacterial and anti-inflammatory activities [8, 12]. In addition, it has been reported that aluminosilicate mineral was beneficial to the gut health of animals [10]. In the present study, supplementation of 0.5% IL significantly increased digestibility of dry matter which was consistent with the report of Li and Kim [24] who reported that dietary inclusion aluminosilicate minerals increased apparent total tract retention of dry matter in pigs. The positive impacts of aluminosilicate minerals on nutrient digestibility may be attributed to the effects of aluminosilicate minerals on gastrointestinal development and the concomitant increase in digestive enzyme secretion [20,25]. As reported by Chen et al [1] and Yan et al [11] in which pigs fed clay minerals (sericite and Biotite V) had greater apparent total tract digestibility of dry matter, and energy in pigs compared with those fed the control dietary treatment. In contrast, Chen et al [2] reported a did not observe significant effects on nutrient utilization due to the inclusion of clay minerals [2]. The contradictory results were due to diet complexity, inclusion level of minerals, age, and the health status of the pigs.

Pigs backfat thickness is one of the important forecasts of meat-carcass lean and quality [26]. The impact of BFT has a reflection on LMP and helps in evaluating meat quality [27]. Furthermore, Boyd et al. [28] found that the BFT is a significant parameter to determine enough energy in pig within a short span of time. The inclusion of anion did not influence on LMP and backfat thickness in finishing pigs [11]. In this study, the inclusion of IL could improve backfat thickness in growing-finishing pigs during day 98. These results agree with Chen et al [6] report that dietary supplementation of aluminosilicate minerals increases BFT in growing-finishing pigs.

Recently, different meat characteristics (pH, color, and firmness) have been employed to separate fresh pork into quality groups in the pork industry. Previous studies also suggested that supplementation of zeolites could alter tissue mineral concentration in poultry and pigs because of

its ability to absorb metal ions [1]. Meat color is a major indicator of pork quality as it influences customers' choices [29]. Therefore, it is proper to suggest that the improved firmness observed in the current study might be due to its liking of the metabolism or interaction with those metal ions and subsequently alter the mineral distribution in tissue [30-32]. Similarly, Yan et al [14] reported that pigs fed IL had significant effects on firmness and drip loss than those fed diets compared with the control. In contrast, Thacker [8] found that dietary clays did not influence meat quality and carcass grade of pigs fed diets compared with the control. In our study, IL supplementation had no significant effects on carcass weight and BFT compared with control. However, in this current study, we found that "1%" carcass grade increased in pigs fed with IL inclusion compared with the control. Therefore, as limited studies were conducted to evaluate the effects of illite clay series on meat quality and carcass grade of pigs, no related reference is available to compare this effect. Further study is still necessary to evaluate its effect on meat quality.

CONCLUSION

Our study demonstrated that pigs fed diet supplementation with 0.5% illite could be beneficial to enhance the daily feed intake, nutrient utilization of dry matter, and to reduce G:F and meat quality of drip loss in growing-finishing pigs.

Conflicts of Interest

There is no conflict of interest related to this publication

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 efficiency associated with altered fecal microbiota and bile acid composition in pigs. Front.
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Table 1. Composition for illite supplementation¹

Ingredients	% ²
SiO ₂	64.60
Al_2O_3	20.80
Fe_2O_3	3.05
FeO	0.46
MgO	2.30
CaO	0.53
Na ₂ O	2.60
K ₂ O	0.40
${ m TiO_2}$	0.15
Ignition loss	4.74

¹Provided by SeobongBiobestech Co, Ltd, Seoul, Korea

²Provided by the Korea Chemical Institution.

Table 2. Composition of finishing pig diets (as fed basis)

Ingredients (%)	Content
Corn	76.73
Soybean meal (48%)	15.32
Tallow	2.52
Molasses	2.00
Dicalcium phosphate	1.15
Limestone	0.81
Salt	0.30
Methionine (99%)	0.07
Lysine	0.48
Threonine (99%)	0.14
Tryptophan (99%)	0.05
Mineral mix ¹	0,20
Vitamin mix ²	0.20
Choline (25%)	0.03
Total	100.00
Calculated value	
Crude protein, %	14.00
Calcium, %	0.60
Phosphorus, %	0.55
Lysine, %	1.00
Methionine, %	0.30
Threonine, %	0.65
Tryptophan, %	0.20
Metabolizable energy, kcal/kg	3300
Crude fat, %	5.38
Fiber, %	2.34
Ash, %	3.26

¹Provided per kg diet: Fe, 115 mg as ferrous sulfate; Cu, 70 mg as copper sulfate; Mn, 20 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

 $^{^2}$ Provided per kilograms of diet: vitamin A, 13,000 IU; vitamin D₃, 1,700 IU; vitamin E, 60 IU; vitamin K₃, 5 mg; vitamin B₁, 4.2 mg; vitamin B₂, 19 mg; vitamin B₆, 6.7 mg; vitamin B₁₂, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg.

Table 3. Effect of dietary illite supplementation on growth performance in growing - finishing pig^1

Items	CON	IL	SEM ²	P- value
Body weight, kg				
Initial	24.5	24.5	0.01	0.988
Day 42	50.4	50.9	0.30	0.767
Day 70	70.5	71.3	0.20	0.711
Day 98	92.4	93.4	0.30	0.822
Day 126	115.9	116.7	0.60	0.822
Day 0 to 42				
ADG, g	616	629	7.00	0.555
ADFI, g	1588 ^b	1642 ^a	18.00	0.222
G:F	0.388	0.383	0.002	0.511
Day 43 to 70				
ADG, g	718	729	8.00	0.581
ADFI, g	2141 ^b	2287 ^a	33.00	0.022
G:F	0.336^{a}	0.319 ^b	0.003	<.0001
Day 71 to 98				
ADG, g	782	788	10.00	0.850
ADFI, g	2538	2524	22.00	0.844
G:F	0.308	0.312	0.004	0.563
Day 99 to126				
ADG, g	839	834	12.00	0.872
ADFI, g	2735 ^b	2843 ^a	35.00	0.012
G:F	0.305^{a}	0.295^{b}	0.002	0.001
Overall				
ADG, g	725	732	5.00	0.766
ADFI, g	2201	2224	13.00	0.677
G:F	0.330	0.329	0.001	0.810

¹Abbreviation: CON, basal diet; IL, 0.5% illite.ADG, average daily gain; ADFI, average daily feed intake, G:F; gain to feed ratio.

²Standard error of means.

 $^{^{}a,b}$ Means in the same row with different superscripts differ (P < 0.05).

Table 4. Effect of dietary illite supplementation on apparent total tract nutrient digestibility in growing - finishing pig¹

Items, %	CON	IL	SEM ²	P- value
Day 42				
Dry matter	76.61	77.95	1.02	0.018
Nitrogen	74.58	74.50	1.41	0.980
Energy	75.40	75.10	1.41	0.903
Day 126				
Dry matter	73.04	74.99	0.65	0.043
Nitrogen	72.28	72.38	0.76	0.871
Energy	73.88	74.36	0.50	0.620

¹Abbreviation: CON, basal diet; IL, 0.5% illite.

²Standard error of means.

Table 5. Effect of dietary illite supplementation on backfat thickness in growing - finishing pig¹

Items, mm	CON	IL	SEM ²	P- value
· · · · · · · · · · · · · · · · · · ·	COIV	IL .	SLW	1 - value
Back fat thickness, mm				
Initial	6.00	6.10	0.07	0.563
Day 14	6.50	6.70	0.09	0.394
Day 28	7.20	7.30	0.08	0.465
Day 42	7.80	8.00	0.08	0.369
Day 56	9.30	9.50	0.09	0.393
Day 70	11.10	11.10	0.09	0.953
Day 84	12.80	13.10	0.12	0.207
Day 98	14.40^{b}	15.00^{a}	0.13	0.020
Day 112	16.30	16.70	0.15	0.162
Day 126	18.70	18.90	0.24	0.655
Lean muscle percentage, %				7
Day 126	55.9	55.4	0.30	0.240

¹Abbreviation: CON, basal diet; IL, 0.5% illite.

²Standard error of means.

 $^{^{}a,b}$ Means in the same row with different superscripts differ (P < 0.05).

Table 6. Effect of dietary illite supplementation on the meat quality of growing-finishing pigs¹

Item	CON	IL	SEM ²	P- value
TBARS	3.69	3.74	0.29	0.925
Meat color				
L*	55.16	55.69	1.58	0.766
a*	16.05	16.52	0.72	0.584
b*	6.14	6.23	0.66	0.903
Sensory evaluation				
Color	3.2	2.9	0.2	0.189
Firmness	3.1	3.3	0.2	0.088
Marbling	2.6	2.3	0.2	0.435
Cooking loss, %	30.3	30.7	0.9	0.795
Drip loss,%				
d1	1.39	1.75	0.66	0.616
d3	2.61	2.98	0.59	0.591
d5	4.07	3.94	0.50	0.853
d7	5.75 ^a	5.56 ^b	0.01	<.0001

¹Abbreviation: CON, basal diet; IL, 0.5% illite. TBARS, thiobarbituric acid reactive substances

²Standard error of means.

 $^{^{}a,b}$ Means in the same row with different superscripts differ (P < 0.05).

Table 7. Effect of dietary illite supplementation on carcass grade in growing - finishing pig¹

Items	CON	IL	SEM ²	P- value
Back fat thickness, mm	19.2	19.5	0.9	0.574
Carcass weight, kg	87.7	86.8	0.4	0.447
Carcass grade				
1+	21	22	-	-
1	38	41	-	-
2	16	12		-

¹Abbreviation: CON, basal diet; IL, 0.5% illite.

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300

301

²Standard error of means.