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Abstract

The purpose of this study is to examine whether spraying an anti-microbial agent into the slurry pit will reduce the noxious odor substances from piggery barns. For this, a total of 200 crossbred [(Landrace × Yorkshire) × Duroc] growing pigs with an initial average body weight (BW) of 23.58 ± 1.47 kg were selected and housed in two different rooms, i.e. control (CON) and treatment (TRT). Each room has 100 pigs (60 gilts and 40 borrows). For a period of 42 days, all pigs were fed with corn-soybean meal-based basal diet. Later the noxious odor substances were measured by the following methods. First, fecal samples were randomly collected and stored in sealed and unsealed containers, and sprayed with the non-anti-microbial agent (NAMA) (saline water) and multi-bacterial spraying (MBS) agent (200:1, mixing ratio-fecal sample: probiotic), Second, the slurry pit of CON and TRT rooms were directly sprayed with NAMA and MBS, respectively. The fecal sample that was stored in sealed and un-sealed containers and sprayed with MBS significantly reduced NH_3 and CO_2 concentration at the end of day 7. However, at the end of day 42, the fecal sample showed a lower H_2S, methyl mercaptans, acetic acid, and CO_2 concentration compared to the unsealed container. Moreover, at the end of days 7, 14, 21, 28, 35, and 42 compared to the CON room and TRT room slurry pit emits lower concentrations of NH_3, acetic acid, H_2S, and methyl mercaptans, and CO_2 into the atmosphere. Based on the current findings, we infer that spraying anti-microbial agents on pig dung would be one of the better approaches to suppress the odor emission from the barn in the future.

Keywords: multi-bacterial spray; slurry odor; gas emission; growing pigs

INTRODUCTION

Livestock farming plays an important role in global food production as it has been transformed from small farms to industrialized enterprises in recent decades. Though industrialized farms have better efficiency in animal management, there is growing anxiety about the release of livestock pollutants, which generates environmental and green gas pollution [1,2]. In terms of green gas pollutants, the emissions such as ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), nitrous oxide (N_2O), and other odors released from livestock production are amenities [3]. Notably, NH_3 emissions are largely responsible for the acidification and eutrophication of nitrogen-limited ecosystems while N_2O and CH_4 contribute considerably to the radiative forcing of the atmosphere [4]. Earlier studies [5], have shown that nitrogen and phosphorus released from livestock manure are considered to be the major source of environmental pollution. Eventually, the South Korean Ministry of Agriculture, Food and Rural Affairs [6] also pointed out that swine manure has accounted for the highest ratio (40.6%) of odor emission compared to other animal facilities. Such noxious odor emission from the piggery not only affects the environment, animals' health, and production but also leads to civil complaints [7] and local and global air pollution [8]. Since 2005, the number of complaints related to the livestock industry has been upsurged by 27% [9]. Consequently, the Korean Ministry of Environment has passed the law on Offensive Odor Control in 2005 [10] to minimize the complaints regarding odor from the vicinity of the piggery barn.

Commercial pig production has been rapidly growing worldwide with a trend towards larger production units thereby utilizing modern production technologies such as modern housing, improved feeding, and better breeding methods to reduce the risk of air pollution [11]. However, measuring and assessing the released odors from livestock manure has become a challenging task for farmers and researchers and thus this subject has been viewed from a global issue perspective. Over the past years, many investigations have been conducted on odor management using physical and chemical methods. Colletti et al. [12] and Rzeznik et al. [13] conducted a study to minimize NH_3, H_2S, CH_4, and CO_2 nuisance through field experiments. Beyond this, several experts have focused their research on using novel technologies like biofilters [14], bio-scrubbers, mechanical ventilation [15], food alterations [4], and feces and urine separation [16] to alleviate odor emission. Although these approaches were effective, they were very expensive and had a short-term impact. Previously, Banskota et al. [17] reported that the oil/water spray technique showed a major impact on pig farm dust control. Similarly, Godbout et al. [18] reported that canola oil sprinkling 2 times per day reduced 27% hydrogen sulfide and 30% ammonia concentrations in the piggery barn. Moreover, Rahman and Borhan, [19] noted that the addition of microorganisms directly to the manure of anaerobic dairy lagoons reduces the solids and nutrient content. Furthermore, Maurer et al. [20] pronounced that context of biochemical agents could be better options to overcome odor issues. However, Young and Yun [21] noted that the application of *Bacillus*-based probiotics complex could be a potential solution to reduce the malodor from the livestock barn. In addition, Sannikova and Kovaleva [22] reported that the application of *Bacillus* genus bacteria substantially reduced the sulfurous, rotten egg-like smell of the industrial wastewater. Also, Kim et al. [23] used microbial additive, soybean oil, and essential oil as spraying agents to reduce the odor emissions from the confinement pig building. In 2013, Bellot et al. [24] reported that animals (horse, guinea pig, and cow) bedding with *Bacillus* strains probiotics reduced the bad smell of animal waste. The research outcome of the above-mentioned studies has highly inspired us to initiate this study to discover whether it is applicable to piggery. To our knowledge, this would be the first report to use mixed probiotics as a multi-bacterial spraying agent on growing pig barn and we hypothesized that direct spraying antimicrobial agent into the slurry pit would be one of the effective methods to reduce the noxious odor smell from the barn in the mere future. Thus, the purpose of this study is to analyze whether spraying anti-microbial agent into the slurry pit under growing pig pen reduces harmful gas emissions.

MATERIALS AND METHODS

Ethical declaration

The research protocol was permitted by the Animal Care and Use Committee of Dankook University (DK-1-2102), prior to the study.

Animals, experimental design, and feeding regimen

This study was conducted at Dankook University (Cheonan, Republic of Korea) "Experimental swine research unit" located at Jeouni (Sejong-si, Republic of Korea). A total of 200 crossbred [(Landrace × Yorkshire) × Duroc] growing pigs with an average body weight of 23.58 ± 1.47 kg were divided into two groups (control and treatment) in a complete random block design with 20 replicates and 5 pigs (3 gilts and 2 borrows) per pen and housed in two separate rooms. The pig room has 0.45 m deep slurry pit under a slatted plastic floor with 22.8 m^2 surface and partition. The ambient temperature of the facility was maintained at approximately 25℃ by a ventilation control system. Prior to the trial, the slurry pit was emptied. All pigs were allowed to be fed corn soybean-based basal diet twice a day at 09:00 AM and 4:00 PM for 6 weeks that were formulated to meet or exceed the nutrient requirements of NRC [25] (Table 1).

Growth performance

The body weight (BW) of pigs was measured individually on d1 and d 42 to assess average daily gain (ADG), while the feed allowance and remaining in feeders were collected and calculated to determine the daily feed intake (ADFI) and feed efficacy (G: F). The pens were equipped with self-feeders and nipple drinkers that allowed pigs to have *ad libitum* feed and water throughout the trial.

Sampling and Measurements

At initial and day 42, 200 g of fresh fecal samples were collected from (2 pigs/pen) CON and TRT group pigs that were fed with normal basal diet. The collected fecal samples were placed in 20 boxes with a capacity of 5 liters (10 boxes /treatment). Then, 5 boxes of fecal samples (each treatment) were sealed with a plastic tape, while another 5 boxes were left unsealed. Later, sealed and un-sealed boxes were sprayed with a non-anti-microbial (NAMA- saline water) and multi-bacterial spraying (MBS) agents [200:1, a mixing ratio of fecal (200g) and probiotics (1%)] twice a day until the end of the trial.

Correspondingly, from d1 to d 42, slurry pits of CON and TRT rooms (20 pens /treatment) were uniformly sprayed with NAMA and MBS (respectively), twice a day at 9:00 am and 5:00 pm using the manual sprayer for 15 min. The multi-bacterial spraying agent (G-Fresh) employed in this study contains *Bacillus subtilis, Pediococcus acidilactici, lactococcus lactis, Bacillus coagulans*, and *Bacillus carboniphilus* was commercially obtained from TELLUS Co., Ltd. Republic of Korea. The water and anti-microbial agents were diluted according to the manufacture prescribed ratio. Slurry specimens were mixed with a slatted floor mixer (PORCO, Reck Agrartechnik, Germany) at the end of d1,7, 21, 28, 35, and 42. Later, the concentrations of H_2S, methyl mercaptans, CO_2, NH_3, and acetic acid in the fecal sample and pig barn (atmosphere) were determined directly using Multi-RAE Lite-gas search probe (model PGM-6208, RAE, USA). A detailed scheme of the experiment is presented in Figure 1.

Statistical analysis

The experimental data were analyzed by t-test using the SAS procedure (SAS Inst. Inc., Cary, NC, USA). Growth performance and fecal gas emission were analyzed in a complete random block design using pig as an experimental unit. For slurry odor substances individual room was considered as an experimental unit. The probability value < 0.05 was considered as significant.

RESULTS

The growth performance of growing pigs remains similar throughout the trial (Table 2). Table 3 shows the effect of NAMA and MBS agents in the sealed and un-sealed fecal container. The fecal samples that were stored in the sealed and un-sealed container and sprayed with 200:1 MBS agent reduced (p >0.05) only NH_3 and CO_2 concentration at the end of d7. However, at the end of d 42, the fecal sample that was stored in the sealed container emitted lower H_2S, methyl mercaptans, acetic acid, and CO_2 concentrations compared to the unsealed container. The effect of spraying an anti-microbial agent on growing pig slurry pit is shown in Table 4. At the end of days 7, 14, 21, 28, 35, and 42, the TRT room slurry emits lower NH_3, acetic acid, H_2S, methyl mercaptans, and CO_2 into the atmosphere compared to the CON room.

DISCUSSION

Intensive animal husbandry with a large amount of animal excreta such as urine, feces, undigested feed, etc. may create excessive odors and eventually lead to air pollution problems [19]. The such odor emanating from the swine farms not only elicits a low quality of life but also creates a nuisance in the nearby community [26]. Thus, pollution prevention measures should be carried out from the source according to the livestock farming status. Also, good in-house air quality is very important for animal productivity and for workers safety thus, we anticipate that the future swine industry will largely depend on various technologies that could mitigate the odor nuisance from piggery. Therefore, in this study, we intend to use antimicrobial spraying method to reduce the noxious gas smell from the pig barn.

Livestock production, especially swine facilities become the major cause of malodors [6]. Most importantly they were generated from the incomplete decomposition of organic matter such as proteins, carbohydrates, and fats [19]. Due to the high quantities and/or low odor thresholds, the odorous substances released by cattle dung seem to be volatile fatty acids [27]. In 1999, Sutton et al. [4] stated that incomplete microbial degradation of protein and carbohydrates in manure resulted in high odorous production. Besides, bacterial fermentation in the gastrointestinal tract of pig and the slurry pit beneath pig pen may also contribute to the production of odorous substances from the barn. In 2016, Loyon et al. [28] proposed some techniques to minimize undesired emissions from the manure which include direct-fed microbial products based on carefully selected bacteria that could increase the manure decomposition. Particularly, *Bacillus* species possess spore-forming stability to produce a wide range of hydrolytic enzymes to control malodorous substances [29]. Compared to other odorous compounds nitrogen (N) excretion become the major precursor. In fact, there is a general prediction that growing-finishing pigs typically emit a large amount of noxious gases as a result of feed conversion inefficiencies related to their digestion and metabolism. Apart from this, indoor/outdoor temperature, ventilation rate, animal activity, season, relative humidity, dung depth, pig density, air cleanliness, barn cleanliness, fan size, fan position, pig health, and pit type factors may also affect the performance of pigs [30]. However, there was no adverse result found on the growth performance of growing pigs until the end of the experiment. Earlier studies [31, 32] reported that adjusting diet structure [33] and reducing crude protein ingredient in animals' diet reduce the N excretion and this finding was correlated with current results and the main reason for this outcome are due to proper feed management with the addition of adequate levels of crude protein that suits to the digestive capacity growing pigs.

The hazardous gas CH_4 and N_2O emissions from manure are highly linked to environmental pollution. Besides, NH_3, H_2S, and total mercaptans emissions from the livestock production facilities widely affect the quality of air particularly, when produced in large amounts [34]. Previously, Grossi et al. [35] stated that organic matter which was partially decomposed by bacteria in an anaerobic condition had produced more CH_4 and CO_2. Over the past periods, many studies have focused to mitigate these environmental hazards caused by noxious gas emissions through dietary manipulation procedures. For example, Chu et al. [36] reported that the inclusion of probiotics in livestock feed has effectively decreased the concentration of NH_3, fecal pH, and volatile organic matter, also helping to get rid of the toxic odor. On the other hand, Peirson and Nicholson [37] stated that a low-protein diet reduced 40% of odor emission. Generally, unpleasant odors are associated with bad bacteria which can easily grow on the surface to create a terrible smell by breaking down organic contamination. To overcome this subject, several mitigation strategies have been proposed in the millennium, like diet manipulation, vaccines, chemical additives, animal genetic selection, with different efficiencies in reducing enteric CH_4, even studies insist that frequent removal of slurry from the pit storage facility is an effective practice to reduce the odor emission from the barn [35]. In 1999, Jacobson et al. [38] reported that soybean oil sprinkling reduced the odor emissions from the nursery pig building. Similarly, Varel and Miller [39] stated that essential oil as masking agent significantly reduces the odor substance from the barn. Likewise, Bellot et al. [24] report that *B. subtilis* strains 2084 and *B. licheniformis* strain 21 are very effective in controlling the odor substance, especially animal (horse, guinea pig, and cow) bedding with *Bacillus* strains reduced the bad smell from its waste. Over the past decades, activated carbon adsorption, wet scrubbing, masking agents, and various biological additives like essential oils, soybean oils, and microbial additives have been used to control odors from the piggery [40, 41]. For instance, Kim et al. [23] use several effective techniques including tap water, salt water, digested manure, microbial additive, soybean oil, artificial spice, and essential oil to reduce odor creation among those, salt water, artificial spice, and essential oil showed a beneficial impact on suppressing the odor substance from the barn. Previously, Zhu et al. [42] suspected whether the use of microbial additives could mitigate the odor concentration in pig house, fortunately, this study proved that spraying multi-bacterial agent in the slurry pits under growing pig pen significantly reduce the odor substance. The proposed reason for the lower odor emission from the pig barn is due to the reduction of pH in the slurry or due to the natural microbial products that were sprayed into the slurry.

5. Conclusion

Our study demonstrates that spraying anti-microbial agents on pig dung would highly help to control the odor substances from the piggery barns. Also, we believe that the current outcome will provide new insight into the spraying method on piggery to elevate major environmental pollution in the future. As it is a preliminary study we applied 1kg mixed probiotics per 100 heads as an anti-microbial spraying agent, yet our research team has planned to conduct more studies to assess the ideal level of microbial agents to suppress the odor from farmhouse with enduring success.

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Table 1. Basal diet for growing pigs (as fed basis).

Corn	60.32
Soybean meal	16.07
Distillers dried grains with soluble	6.50
Rapeseed meal	2.50
Wheat	6.00
Tallow	3.00
Mallow	300
Dicalcium phosphate	1.08
Limestone	0.65
Salt	0.30
Lysine	0.19
Vitamin premix[a]	0.20
Mineral premix[b]	0.10
Choline, 50%	0.04
Calculated composition	
Crude protein, %	15.50
Crude fat, %	5.78
Lysine, %	0.91
Calcium, %	0.65
Phosphorus, %	0.55
Ash, %	4.59
Crude fibre, %	3.43
Digestible energy (kcal/kg)	3,428,00

[a]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D_3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 µg vitamin B_{12}.[b] Provided per kg of complete diet: 12 mg Cu (as $CuSO_4 \cdot 5H_2O$); 85 mg Zn (as $ZnSO_4$); 8 mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15 mgSe (as $Na_2SeO_3 \cdot 5H_2O$).

Table 2. The effect of basal diet on the growth performance of growing pigs[1].

Items	CON	TRT	SEM[2]	P value
Body weight, kg				
Initial	23.58	23.58	0.02	0.990
Finish	51.98	52.59	0.27	0.457
Overall (d1-d42)				
ADG, g	675[b]	683[a]	9.00	0.210
ADFI, g	1701	1725	16.00	0.335
G: F	2.525	2.490	0.02	0.313

[1]Abbreviation: Control and TRT group pigs were fed with normal basal diet that was formulated according to NRC recommendation. [2]Standard error of means. [a,b] Means in the same row with different superscripts differ significantly ($P<0.05$).

Table 3: Effect of spraying microbial agents on the gas emission of growing pig feces sample stored in the sealed and un-sealed container.

Items, ppm	CON	TRT	SEM[2]	P- value
Sealed container (Initial)				
NH_3	97.10[a]	30.20[b]	5.18	<0.001
H_2S	99.90[a]	77.49[b]	6.13	0.330
Methyl mercaptans	0.00	0.00	0.00	
Acetic acid	0.00	0.00	0.00	
CO_2	2990[a]	1240[b]	295.00	<0.001
Day-42				
NH_3	13.30	10.60	1.10	0.1572
H_2S	72.08[a]	31.53[b]	5.11	<0.001
Methyl mercaptans	10.50[a]	3.00[b]	1.23	0.008
Acetic acid	6.30[a]	2.50[b]	0.68	0.004
CO_2	12450[a]	8200[b]	1296	0.010
Unsealed container (Initial)				
NH_3	91.90[a]	54.10[b]	3.78	<0.001
H_2S	99.83	99.90	0.49	0.335
Methyl mercaptans	0.00	0.00	-	
Acetic acid	0.00	0.00	-	
CO_2	1600[a]	700[b]	114	<0.001
Day- 42				
NH_3	8.70[a]	3.10[b]	0.37	<0.001
H_2S	15.18[a]	0.06[b]	0.78	<0.001
Methyl mercaptans	0.00	0.00	-	
Acetic acid	0.00	0.00	-	
CO_2	1030[a]	540[b]	110	0.003

[1]Abbreviation: CON and TRT groups fecal samples were collected, stored in sealed and un-sealed containers, and sprayed with: Non-anti-microbial agent (NAMA, saline water) and anti-microbial agent (G-Fresh, 200 gm fecal sample :1 mixed probiotic). [2]Standard error of means. [a,b] Means in the same row with different superscripts differ significantly.

Table 4. Effect of spraying anti-microbial agent on growing pig slurry pit.

Items, ppm	CON	TRT	SEM[2]	P value
Initial				
NH_3	3.75	4.00	0.34	0.620
H_2S	0.48	0.45	0.14	0.874
Methyl mercaptans	6.00	6.00	0.29	1
Acetic acid	4.00	3.50	0.94	0.670
CO_2	3400	3425	217.00	0.913
Day 7				
NH_3	4.25^{a}	2.50^{b}	0.41	0.003
H_2S	0.53^{a}	0.35^{b}	0.07	0.044
Methyl mercaptans	6.50^{a}	4.00^{b}	0.58	0.002
Acetic acid	4.25^{a}	3.50^{b}	0.53	0.046
CO_2	3550^{a}	2775^{b}	137.00	0.001
Day 14				
NH_3	4.75^{a}	2.50^{b}	0.18	0.006
H2S	0.53^{a}	0.35^{b}	0.02	0.003
Methyl mercaptans	6.75^{a}	3.75^{b}	0.58	0.004
Acetic acid	4.50^{a}	3.25^{b}	0.18	0.017
CO_2	3575^{a}	2650^{b}	88	0.000
Day 21				
NH_3	4.75^{a}	2.75^{b}	0.29	0.011
H_2S	0.60^{a}	0.40^{b}	0.03	0.032
Methyl mercaptans	6.75^{a}	3.50^{b}	0.34	0.016
Acetic acid	4.75^{a}	3.00^{b}	0.34	0.026
CO_2	3750^{a}	2775^{b}	34.00	<0.001
Day 28				
NH_3	5.25^{a}	2.75^{b}	0.35	0.010

H_2S	0.63[a]	0.40[b]	0.04	0.003
Methyl mercaptans	6.25[a]	3.25[b]	0.50	0.001
Acetic acid	5.00[a]	2.75[b]	0.34	0.003
CO_2	3800[a]	2700[b]	129.00	0.001
Day 35				
NH_3	5.00[a]	3.25[b]	0.18	<0.001
H_2S	0.68[a]	0.43[b]	0.05	0.003
Methyl mercaptans	6.50[a]	3.50[b]	0.29	0.005
Acetic acid	5.00[a]	2.75[b]	0.34	0.011
CO_2	3950[a]	2800[b]	110	0.001
Day 42				
NH_3	5.50[a]	3.50[b]	0.29	0.003
H_2S	0.73[a]	0.50[b]	0.03	0.003
Methyl mercaptans	6.75[a]	3.50[b]	0.34	0.001
Acetic acid	5.25[a]	2.50[b]	0.34	<0.001
CO_2	4075[a]	3050[b]	109.00	0.009

[1]Abbreviation: CON and TRT pen of growing pig's slurry pit were sprayed: Non-anti-microbial agent (NAMA, saline water) and anti-microbial agent (G-Fresh, 100 heads/1kg mixed probiotics), respectively. [2]Standard error of means. [a,b] Means in the same row with different superscripts differ significantly (P<0.05).

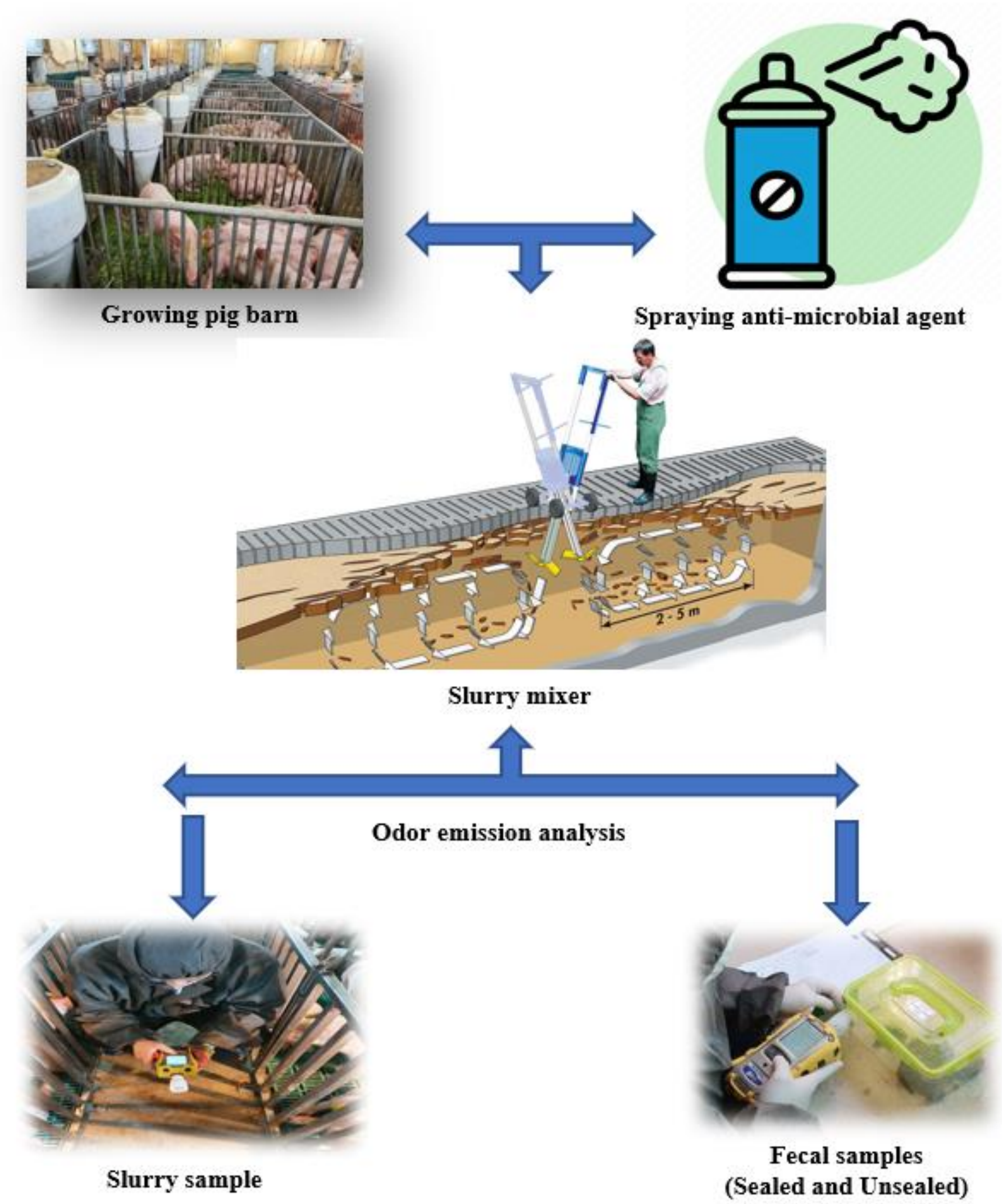

Figure 1. Schematic view of the experiment.