Journal of Animal and Feed Sciences, 32, 1, 2023, 26–33 https://doi.org/10.22358/jafs/154037/2022
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Microbial agent spraying in pig housing and slurry can potentially reduce harmful gas emissions – a preliminary study

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KEY WORDS: gas emission, microbial agents, slurry, pigs

Received: 14 June 2022
Revised: 30 August 2022
Accepted: 13 September 2022

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ABSTRACT. This study aimed to evaluate the effects of spraying microbial agents in pig slurry and housing on harmful gas emissions. A total of 300, eight-week-old crossbreed ([Yorkshire × Duroc] × Landrace) growing pigs, with an average body weight of 28.2 ± 0.55 kg were used in this trial lasting 4 weeks (28 days). Experiment 1: pigs were randomly assigned to two treatments and housed in two separate rooms (150 heads/room). Slurry stored in a slurry pit, produced by growing pigs housed in one room, was sprayed with *Bacillus subtilis* (TRT1), while slurry from the second room was sprayed with *Lactobacillus plantarum* (TRT2). The results showed that *L. plantarum* had a better limiting effect on ammonia (NH_3), hydrogen sulphide (H_2S), and carbon dioxide (CO_2) concentrations (P = 0.01, P = 0.03 and P = 0.01 respectively) than *B. subtilis*. After Experiment 1, the pigs were rearranged and transferred to finishing rooms. At this point, they were subdivided and housed in 3 separate rooms consisting of 100 pigs each (Experiment 2). Subsequently, their slurry pits were sprayed with or without a mixture of microbial agents (*B. subtilis* and *B. licheniformisis*) as follows: CON (no microbial agents), BSBL1 (mixed microbial agent spray 1000:1) and BSBL2 (mixed microbial agent spray 1000:2). In Experiment 2, we observed that the gases, i.e. NH_3, H_2S, total mercaptans, acetic acid, and CO_2 were strongly reduced with increasing levels of the microbial agent. Our findings clearly indicated that spraying *L. plantarum* in slurry exerted a greater effect on odorous gas emission compared to spraying *B. subtilis*. Moreover, the microbial spray mixtures provided improved positive outcomes possibly as a combined effect compared to solitary sprays.

Introduction

Atmospheric air is essential for the organism living on Earth. Despite the recognition of anthropogenic activities as the culprits of global warming and air pollutant emissions, there is a need for multi-sectoral action. Multiple reports have shown that air pollution is an enormous burden on health and is considered a major contributor to excessive mortality rate due to respiratory, cardiovascular and other diseases (Hong et al., 2019; Lelieveld et al., 2019). The intensified animal production system is accused of being the leading sector emitting the majority of atmospheric pollutants. In fact, the emitted gases cause the greenhouse effect by trapping infrared radiation and its subsequent emission in the form of reverse thermal radiation, leading to an increase in the Earth's surface temperature (Marszałek et al., 2018). The pig industry has grown rapidly in recent years, with the intensive rearing dominating other production systems. Slurry produced during pig breeding pollutes the environment by emitting

high ammonia (NH_3) and greenhouse gases to the atmosphere (Calvet et al., 2017). In addition, the excessive use of slurry for agricultural fertilisation can lead to eutrophication of lakes and rivers, given that these heat-trapping gases are released into the Earth's atmosphere at various stages of slurry management (Girard et al., 2009). Moreover, nutrients in manure, mainly nitrogen and phosphorous, are a significant component of pollution from agriculture to surface, ground and marine waters, damaging ecosystems through eutrophication and restricting their recreational use. Typically, slurry is defined as a liquid heterogeneous mixture of animal excreta, undigested food residues, and water used for hygienic and cleaning purposes in livestock buildings, characterised by the presence of mineral components easily assimilable to plants (Marszałek et al., 2018). Greenhouse gas (GHG) production by livestock accounts for 14.5% of total anthropogenic emissions (Twine, 2021). In particular, it has been reported that intensive pig rearing generates approx. 10% of GHG emissions from livestock, which is the second highest in this sector (Giraldi-Díaz et al., 2021); in addition, environmental issues associated with pig production concern water and air pollution (Rodhe et al., 2012). Pig slurry contains harmful substances, such as heavy metals, unpleasant odours, parasites, and pathogens that pose potential risks to the environment and public health, especially when improperly treated and applied (Sun et al., 2021). Other studies found that the majority of odour-causing substances are generated by protein degradation and if carbohydrates are limited in pig slurry during the storage period, proteins become the main source of fermentable carbon (Hwang et al., 2016). In the literature, there are various methods described to reduce gas emissions in pig slurries, including storage in hermetically sealed tanks, acidification, separation into solid and liquid fractions, anaerobic digestion and aeration (Marszałek et al., 2018). On the other hand, research has identified animal nutrition as a unique option to reduce these impacts. Initially, the use of microbial agents (probiotics) in livestock was driven by the need for alternative strategies to increase production and health of animals rather than the use of antibiotics. Probiotic supplements containing spores of *Bacillus subtilis* and *B. licheniformis* have been reported to decrease ammonia emissions by about 50%, and inconsistent results among studies are perhaps dependent on the bacterial strains used, type of feed ingredients, environmental conditions, trial duration and host age, but also the lack of sufficiently robust methodologies for determining gaseous emissions (Prenafeta-Boldú et al., 2017). There is limited research on the effects of microbial agents sprayed in to slurry materials on gas emissions in swine manure and pig houses. The present work focused on the effect of an experimental spraying of *B. subtilis* (1.0×10^7 CFU/g) or *Lactobacillus plantarum* (1.0×10^7 CFU/g) during the growing period (Experiment 1), or a mixture of *B. subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g) during the finishing period (Experiment 2) into slurry pits as a strategy to reduce emissions of NH_3, hydrogen sulphide (H_2S), carbon dioxide (CO_2), total mercaptans (R-SH), and total acetic acid (AA) concentrations from slurry and pig house atmosphere.

Material and methods

Ethical declaration

The present study was conducted at the Gongju research unit (Dankook University). The protocol (#DK-2-2106#) for this trial was approved by the Ethics Committee of the Dankook University, Cheonan, South Korea, in accordance with the Animal Care and Use Guidelines. Microbial agents used in the study were provided by a commercial company (Powerzyme, B&B Gyeonggi-do, South Korea). Fermentation was inoculated with *B. subtilis* (1.0×10^9 CFU/g) and *L. plantarum* (1.0×10^7 CFU/g) in Experiment 1 (Exp. 1); and *B. subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g) in Experiment 2 (Exp. 2), and incubated for 48 h. Subsequently, the material was dried at 60 °C for more than 72 h. Wood powder was used as carrier.

Experimental housing, design and sampling procedures

Experiment 1. Exp. 1 was strictly conducted to evaluate two microbial agents, namely *B. subtilis* (1.0×10^7 CFU/g) and *L. plantarum* (1.0×10^7 CFU/g) for their efficacy in reducing gas emissions. A total of 300, eight-week-old crossed ([Yorkshire × Duroc] × Landrace) healthy growing pigs, with an average body weight (BW) of 28.2 ± 0.55 kg were used in this trial for 3 weeks (21 days). Based on body weight and sex, pigs were randomly assigned to two treatment groups and housed in two separate rooms (150 pigs in each room). The pens were uniformly equipped with self-feeders and nipple drinkers to allow unlimited access to feed and water throughout the experiment. The pig room had a 0.45-m deep slurry pit under a 22.8 m^2 of slatted plastic floor divided equally into 4 blocks. The ambient temperature in the facilities was maintained at approximately 25 °C by a ventilation control

system. Slurry stored in the slurry pit produced by growing pigs housed in one room was sprayed with *B. subtilis* 1.0×10^7 CFU/g (at an estimated dilution of 1000:5) and designated TRT1, while slurry stored in the slurry pit, produced by growing pigs housed in another room was sprayed with *L. plantarum* 1.0×10^7 CFU/g (at an estimated dilution of 1000:5) and designated TRT2. The slurry pits under both rooms were manually sprayed with microbial agents every morning (8:00) and evening (18:00) throughout the experiment. All pigs were fed a basal diet formulated according to the recommendations of the National Research Council (NRC, 2012) and all feed components and calculated nutritional values of the basal diet are presented in Table 1.

Table 1. Composition of the experimental grower pig diets (as-fed basis)

Items	Composition
Corn	74.99
Soybean meal (48%)	21.31
Tallow	1.78
Dicalcium phosphate	1.24
Limestone	0.75
Salt	0.20
Lysine (78%)	0.42
Methionine (99%)	0.06
Vitamin premix[1]	0.12
Mineral premix[2]	0.10
Choline (25%)	0.03
Total	100.00
Calculated value	
crude protein, %	16.50
metabolizable energy, kcal/kg	3300
lysine, %	1.12
methionine, %	0.32
Ca	0.66
P	0.56

[1] provided per kilogram of complete diet: mg: vit. A (retinol) 1.3, vit. D_3 (cholecalciferol) 0.022, vit. E (tocotrienol) 45, vit. K_3 (menadione) 4.2, vit. B_5 (calcium D-pantothenate) 24.6, vit. B_2 (riboflavin) 8.6, vit. B_{12} (cobalamins) 0.04; [2] provided per kilogram of complete diet: mg: Cu 15, Fe 80, Zn 56, Mn 73, I 0.3, Co 0.5, Se 0.4

Initially, fresh slurry samples were collected from the pits for analysis of odorous compounds on days 1, 7, 14, and 21. Slurry samples were collected from 4 quadrants of each room and homogenized using a slatted floor mixer (Porco, Betzenweiler, Germany), transferred to 2.6-l plastic containers, and incubated for 24 h at room temperature (25 °C) for further fermentation before analysis. Subsequently, NH_3, H_2S, R-SH, AA and CO_2 gases were determined automatically using a multi-gas monitor (Multi-RAE Lite, RAE Systems, San Jose, CA, USA) in both slurry and pig house atmosphere. To determine gas emissions, room fans were turned off overnight (12 h), and gases were analysed the following morning directly in the room using the same apparatus.

Experiment 2. After Exp. 1, the pigs were regrouped and transferred to finishing rooms. At this point, they were divided and housed in 3 separate rooms with 100 pigs each, and fed a basal diet formulated according to the NRC (2012) recommendations for finishing pigs (Table 2). Their slurry pit was then sprayed with/without microbial agents mixture of *B. subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g) as follows: CON (no microbial agents), BSBL1 (mixed microbial agents at 1000:1 dilution) and BSBL2 (mixed microbial agents at 1000:2 dilution). Slurry samples were collected on days 7, 14, 21, and 28 of the experiment. The sampling and analysis procedures of gas emissions in slurry samples and pig housing atmosphere were similar for Exp. 1 and Exp. 2.

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

Item	Composition
Corn	45.06
Wheat	13.00
Soybean meal	23.00
Rapeseed meal	2.20
Dried distillers' grains with soluble, corn	5.00
Dicalcium phosphate	1.06
Limestone	1.00
Salt	0.30
L-lysine·SO_4 (51%)	0.24
DL-methionine (50%)	0.12
L-tryptophan (10%)	0.01
L-threonine (98.5%)	0.13
Animal fat	5.30
Molasses	3.20
Choline (50%)	0.08
Vitamin premix[1]	0.15
Mineral premix[2]	0.15
Calculated composition	
metabolizable energy, kcal/kg	3400
lysine, %	0.95
methionine, %	0.30
Ca, %	0.76
P, %	0.28

[1] provided per kilogram of complete diet: IU: vit. A 10 000, vit. D_3 2 000, vit. E 48; mg: vit. K_3 1.5, riboflavin 6, niacin 40, D-pantothenic acid 17, biotin 0.2, folic acid 2, choline 166, vit. B_6 2, vit. B_{12} 28; [2] provided per kilogram of complete diet: mg: Fe (as $FeSO_4 \cdot 7H_2O$) 90, Cu (as $CuSO_4 \cdot 5H_2O$) 15, Zn (as $ZnSO_4$) 50, Mn (as MnO_2) 54, I (as KI) 0.99, Se (as $Na_2SeO_3 \cdot 5H_2O$) 0.25

Statistical analysis

The obtained experimental data were statistically analysed using Student's *t*-test in Exp. 1

and the GLM procedure of SAS version 9.0 (SAS Institute, 2002) in Exp. 2. The pig room served as the experimental unit and Duncan's multiple range test (Exp. 2) was applied to determine the effect of microbial agent spraying in pig housing air and slurry; microbial agents were considered as a fixed variable. Data are presented as means ± standard error of the mean (SEM). The $P < 0.05$ value was adopted as statistical significance, while the *P*-value between 0.05 and 0.10 was considered a trend.

Results

Experiment 1

Table 3 shows the effect of microbial agent spraying on odorous substances in the slurry. At the beginning of the trial, spraying with *L. plantarum* in the TRT2 room significantly reduced the concentrations of odorous substances, i.e. NH_3, H_2S, and CO_2 compared to the TRT1 room sprayed with *B. subtilis* ($P = 0.01$, $P = 0.03$ and $P = 0.01$, respectively). At the end of week 1, 2, and 3, there was a markedly higher reduction of H_2S, CO_2, R-SH, and total AA levels in TRT2 compared to TRT1 ($P < 0.05$).

Table 3. Effect of microbial agent spray on gas-emission in slurry

Items, ppm	TRT1	TRT2	SEM	*P*-value
Initial (day 1)				
NH_3	96.21[a]	88.98[b]	5.20	0.01
H_2S	99.01[a]	96.46[b]	6.09	0.03
R-SH	0.00	0.00	–	
AA	0.00	0.00	–	
CO_2	2900[a]	1250[b]	295	0.01
Week-1 (day 7)				
NH_3	12.27[a]	9.44[b]	1.56	0.01
H_2S	71.04[a]	30.48[b]	5.15	0.01
R-SH	11.00	3.10	1.64	0.07
AA	5.90[a]	2.50[b]	0.75	0.04
CO_2	12460[a]	8260[b]	1280	0.04
Week-2 (day 14)				
NH_3	8.13	5.70	1.14	0.15
H_2S	40.38[a]	19.48[b]	4.99	0.01
R-SH	11.40	9.70	1.63	0.11
AA	6.50[a]	2.90[b]	0.86	0.05
CO_2	14510[a]	10340[b]	1329	0.04
Week-3 (day 21)				
NH_3	5.26	2.97	0.95	0.10
H_2S	20.10[a]	9.68[b]	1.90	0.011
R-SH	9.80	8.90	1.37	0.41
AA	5.70[a]	2.10[b]	0.85	0.03
CO_2	11460[a]	7400[b]	1231	0.04

TRT1 – *Bacillus subtilis* [1.0×10^7 CFU/g (500 g/1000 kg dilution)], TRT2 – *Lactobacillus plantarum* [1.0×10^7 CFU/g (500 g/1000 kg dilution)]; NH_3 – ammonia, H_2S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO_2 – carbon dioxide, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 4 presents the effect of microbial agent spraying on slurry odour substances in pig houses. At the beginning of the study, there was no significant difference between TRT1 and TRT2 rooms in terms of reduction of NH_3, H_2S, CO_2, R-SH, and total AA emissions ($P > 0.05$). By the end of the first week, NH_3, H_2S, and R-SH levels were not significantly decreased in TRT1 compared to TRT2; however, acetic acid and CO_2 levels were highly reduced in TRT2 compared to TRT1. At the end of week 2, the concentrations of NH_3, AA and CO_2 significantly declined in TRT2 in comparison to TRT1 ($P > 0.05$); however, R-SH and H_2S levels showed no differences between treatments. At the end of week 3, a significant reduction in AA and CO_2 concentrations were recorded in TRT2 compared to TRT1 ($P = 0.01$, 0.01, respectively), in contrast to NH_3, H_2S and R-SH levels, for which no significant differences were found between treatments.

Table 4. Effect of microbial agent sprays on gas emissions in pig room

Items, ppm	TRT1	TRT2	SEM	*P*-value
Initial (day 1)				
NH_3	3.50	3.25	0.18	0.67
H_2S	0.45	0.48	0.02	0.73
R-SH	5.75	6.00	0.73	0.78
AA	4.00	3.50	0.61	0.59
CO_2	3350	3400	68	0.64
Week-1 (day 7)				
NH_3	4.00	3.00	0.08	0.28
H_2S	0.50	0.40	0.06	0.46
R-SH	6.00	5.50	0.46	0.55
AA	4.75[a]	2.75[b]	0.29	0.04
CO_2	3500[a]	3250[b]	46	0.02
Week-2 (day 14)				
NH_3	4.25	3.75	0.20	0.21
H_2S	0.60	0.58	0.13	0.06
R-SH	6.50	5.00	0.35	0.10
AA	5.00[a]	2.25[b]	0.44	0.01
CO_2	3600[a]	3150[b]	61	<0.01
Week-3 (day 21)				
NH_3	4.75	2.50	0.44	0.35
H_2S	0.63	0.23	0.08	0.21
R-SH	6.50	4.75	0.18	0.11
AA	5.25[a]	2.00[b]	0.44	0.01
CO_2	3700[a]	3075[b]	44	0.01

TRT1 – *Bacillus subtilis* [1.0×10^7 CFU/g (500 g/1000 kg dilution)], TRT2 – *Lactobacillus plantarum* [1.0×10^7 CFU/g (500 g/1000 kg dilution)]; NH_3 – ammonia, H_2S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO_2 – carbon dioxide, SEM – standard error of the mean; [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

Experiment 2

Table 5 shows the effect of spraying with probiotic mixtures on gas emissions in pig slurry.

Table 5. Effect of mixed microbial agent sprays on gas emission in slurry

Items	CON	BSBL1	BSBL2	SEM	*P*-value
Week-1 (day 7)					
NH_3	19.7	10.3	9.3	0.8	<0.001
H_2S	5.43	2.30	0.13	2.70	0.45
R-SH	0.0	0.0	0.0	0.0	–
AA	3.3	3.3	2.0	1.1	0.66
CO_2	21133.3	10033.3	6833.3	1888.9	0.01
Week-2 (day 14)					
NH_3	15.3	7.3	6.0	0.8	<0.001
H_2S	7.23	3.4	0.80	0.50	0.02
R-SH	9.0	0.0	0.0	0.0	<0.01
AA	4.6	0.0	4.0	0.0	0.66
CO_2	46333.3	9466.7	6500.0	1754.7	0.04
Week-3 (day 21)					
NH_3	20.7	0.7	0.3	0.4	0.15
H_2S	12.00	4.00	0.00	0.00	0.01
R-SH	7.30	2.0	0.0	0.0	<0.01
AA	7.7	1.0	0.0	0.0	<0.01
CO_2	966.7	366.7	0.0	419.4	0.36
Week-4 (day 28)					
NH_3	23.0	0.0	0.0	0.0	<0.01
H_2S	8.00	0.0	0.0	0.0	<0.01
R-SH	5.56	0.0	0.0	0.0	<0.01
AA	2.0	0.0	0.0	0.0	<0.01
CO_2	1002.8	432.7	0.0	0.0	0.01

CON – normal slurry without microbial agent, BSBL1 – mixed probiotic spray (*Bacillus subtilis* 1.0 × 10^9 CFU/g and *B. licheniformis* 1.0 × 10^9 CFU/g in dilution 1000:1), BSBL2 – mixed probiotic spray (B. subtilis 1.0 × 10^9 CFU/g and *B. licheniformis* 1.0 × 10^9 CFU/g in dilution 1000:2); NH_3 – ammonia, H_2S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO_2 – carbon dioxide, SEM – standard error of the mean; $P < 0.05$ denotes statistical significance

Table 6. Effect of microbial agent sprays on gas emissions in pig room atmosphere

Items, ppm	CON	BSBL1	BSBL2	SEM	*P*-value
Initial (day 1)					
NH_3	3.75	4.00	7.3	0.34	0.620
H_2S	0.48	0.45	4.30	0.14	0.874
R-SH	0.00	0.00	0.0	0.00	–
AA	2.00	1.50	3.3	0.64	0.034
CO_2	3400	3025	1033.3	117	0.013
Week-1 (day 7)					
NH_3	4.25	2.50	0.73	0.41	0.003
H_2S	0.53	0.35	0.4	0.07	0.044
R-SH	6.50	4.00	0.0	0.58	0.002
AA	4.25	3.50	0.0	0.53	0.046
CO_2	3550	2275	466.7	137	0.001
Week-2 (day 14)					
NH_3	4.75	2.50	0.7	0.18	0.006
H_2S	0.53	0.35	0.00	0.02	0.003
R-SH	6.75	3.75	2.0	0.58	0.004
AA	4.50	3.25	1.0	0.18	0.017
CO_2	3975	2650	566.7	88	0.001
Week-3 (day 21)					
NH_3	4.75	0.5	0.0	0.00	0.001
H_2S	0.40	0.0	0.0	0.03	<0.001
R-SH	6.15	0.0	0.0	0.00	<0.001
AA	4.05	0.00	0.0	0.00	<0.001
CO_2	3750	1775	432.7	34	0.01

CON – normal slurry without microbial agent, BSBL1 – mixed probiotic spray (*Bacillus subtilis* 1.0 × 10^9 CFU/g and *B. licheniformis* 1.0 × 10^9 CFU/g in dilution 1000:1), BSBL2 – mixed probiotic spray (*B. subtilis* 1.0 × 10^9 CFU/g and *B. licheniformis* 1.0 × 10^9 CFU/g in dilution 1000:2); NH_3 – ammonia, H_2S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO_2 – carbon dioxide, SEM – standard error of the mean; $P < 0.05$ denotes statistical significances

At the end of week 1, NH_3 and CO_2 emissions were significantly reduced by increasing doses of microbial agents ($P < 0.01$ and $P = 0.01$, respectively), but there was no significant difference in H_2S, AA and R-SH emissions. At the end of week 2, NH_3, H_2S, R-SH and CO_2 emissions ($P < 0.01$, $P = 0.02$, $P < 0.01$ and $P = 0.04$, respectively) were markedly reduced by increasing doses of microbial agents, while AA levels did not differ between the treatments. At the end of week 3, R-SH, H_2S and AA levels were highly reduced by spraying different amounts of microbial agents ($P < 0.01$, $P = 0.01$ and $P = 0.01$, respectively) to the extent that BSBL1 and BSBL2 were free of these gases at the end of week 4. Nevertheless, no significant reduction was recorded for NH_3 and CO_2 at the same time. Interestingly, the production of NH_3, H_2S, MM, AA and CO_2 was significantly reduced by the increasing doses of microbial spray at the end of week 4. Promising results were also obtained for the pig room (Table 6). To illustrate this, AA and CO_2 concentrations were significantly lower in BSBL2 than in BSBL1 in the first week, and similarly, there was a significant reduction in NH_3, H_2S, AA, R-SH and CO_2 levels from week 2 to 4.

Discussion

Microbial agents have been proposed as a suitable strategy for reducing undesirable environmental emissions from manure (Prenafeta-Boldú et al., 2017). The same author stated that *Bacillus* spp. (spore-forming bacteria) were best suited for this role due to their stability potential and ability to produce various hydrolytic enzymes. Studies have shown that housing, stored manure and exercise areas emit about 69–80% of total NH_3 in Europe (Sommer et al., 2006). Normally, NH_3 in livestock

facilities is mainly derived from urea. Urea in urine is relatively stable; however, when it comes in contact with urease, NH_3 is produced, which can subsequently be volatilised. Urease is ubiquitous in faeces, and thus contact between urea and urease readily occurs in production facilities (van Kempen, 2001). H_2S is a chemically unstable reducing agent, easily oxidised, and produces toxic sulphuric by-products upon combustion (Habeeb et al., 2017). It has been reported that NH_3 and H_2S emissions can pose a health risk, given their malodorous and hazardous properties, contributing to ecosystem acidification (Wu et al., 2020). Our findings in Exp. 1 showed that the TRT2 room sprayed with *L. plantarum* had significantly reduced levels of odorous substances, i.e. NH_3, H_2S, and CO_2 compared to the TRT1 room sprayed with *B. subtilis*. As this is a preliminary trial, there was little evidence that could explain this outcome. However, it is known that feeding a high protein diet may increase the amount of NH_3 and volatile organic compounds, and lower dietary crude protein concentrations decrease NH_4^+ concentrations in both fresh and stored manure (Otto et al., 2003). Previous studies focused on probiotics as feed additives in pigs and demonstrated their beneficial effects in reducing noxious gas emissions. For instance, it was reported that *Bacillus*-based probiotics potentially decreased harmful gas emissions in finishing pigs (Chen et al., 2006). Moreover, a study by Nguyen et al. (2019) reported a high reduction in gas emissions by a mixture of probiotic supplements in the diet of weaned pigs. It is normal that carbohydrates are catabolised to various compounds, such as CO_2, CH_4, H_2, short chain fatty acid, its precursors – branched chain fatty acid, as well as phenols, indoles, sulphur, ammonia, and amine, but it has been reported that odorous compounds are mainly produced during protein degradation rather than carbohydrates or a large amount of dietary protein (Cho et al., 2015). However, Otto et al. (2003) also found that restricting dietary crude proteins from 15 to 9% reduced total NH_3 emissions by almost 80%, and a reduction of dietary CP *in vitro* from 16 to 12% declined total NH_3 emissions nearly by 79%. This implies that dietary manipulation of crude protein content may have a direct impact on NH_3 and other harmful gas substances. It is reasonable to note that N excretion has a higher proportion of odorous compounds, given the fact that they are generated from microbial nutrient degradation under anaerobic condition, and N excretion causes incomplete microbial degradation. Therefore, decreasing nutrient excretion is crucial for reducing those emissions (Cho et al., 2015). It is clear that control of odour generation and suppression of nutrient excretion depends on the basic formulation and balance of nutrients in the animal diet. The mechanism of action of microbial agents is not completely understood given the available methodologies, but a correlation has been made with the effect of microbial fermentation of food residues in the slurry. Usually, when probiotics (microbial agents) were fed to pigs, especially *Bacillus* and *Lactobacillus* spp.), they enhanced microbial fermentation in the gut. Planned comparisons were carried out with probiotics composed of the same microbial agent, as sprayed on the slurry in the present study, and there were some previous results that show benefits when probiotics used in pig diet lowered gas emissions; in addition, it is generally believed that *Bacillus* species are capable of hydrolysing proteins, considering that they produce a number of hydrolytic enzymes to degrade various substrates. However, most studies on probiotics were conducted *in vivo*, which means that we were not able to spot the mechanism of action outside the organism. Although ideal conditions of microbial activity were considered to simulate the mode of action of microbial agent in slurry, more researches are needed to further investigate the microbial activity and related factors in pig manure. Park et al. (2020) reported that fermentable carbohydrates (FC) are a promising material for reducing odour emissions from pig manure. Effective microbial products are generally utilised to reduce odour and promote fermentation in agricultural fields, and they include actinomycetes, *B. subtilis*, lactic acid bacteria, yeasts, etc. (Kim et al., 2022). The latter authors also commended the microbial agent for reducing pH, NH_3 concentration, and urease activity, which are part of emission factors. Kim et al. (2005) reported that the inclusion of 0.3% of a microbial agent mixture had a definite inhibitory effect on NH_3 and sulphide dioxide emissions. Moreover, the authors also reported that dietary probiotic supplements containing *B. subtilis* and *B. licheniformis* spores decreased ammonia emissions by approx. 50%. These differences in results between studies are possibly due to host age, environmental conditions, feed types and bacterial strains used. Meanwhile, about 50% of total sulphur is lost in the form of volatile sulphur compounds (VSCs), and common VSCs mainly include hydrogen sulphide, methyl mercaptans (R-SH) and others. It should be noted that H_2S is the most released VSCs, accounting for about 39–43% of emissions. Generally, NH_3 and VSCs are the predominant odours on the pig farm, yet they are cor-

rosive and toxic to human health. Returning to our results, there were fluctuation in gas emissions in the course of Exp. 1, but we proved that spraying with *L. plantarum* was more effective than spraying with *B. subtilis* in reducing harmful gas emissions. A recent publication by Hu and Kim (2022) found that dietary *B. subtilis* supplementation in weaning piglets had a significant effect on R-SH emissions, but not on NH_3 and H_2S levels. In contrast, our results in Exp. 1 showed that R-SH and NH_3 levels were not significant either in the slurry or the pig house. Moreover, we demonstrated that H_2S levels significantly differed between TRT1 and TRT2 from week 1 until the end of the trial. In Exp. 2, we observed a synergistic positive effect of a mixture of microbial agents on harmful gas emissions at different levels of inclusion. A possible reason for this finding could be the action of the *Bacillus* strain, which stimulates secretion of enzymes, such as cellulase, amylase, and protease, and the activity of the enzymes might explain the effects on manure decomposition and reduction of gas emissions. Our results are consistent with the study of Davis et al. (2008), who have found that *Bacillus* spp. have the ability to produce spore coatings that are resistant to heat, enzymatic degradation and acidic environment in the gut. In addition, dietary supplementation with manure-degrading microorganisms would provide a convenient and continuous inoculation strategy for manure storage facilities. Upadhaya et al. (2015) reported that NH_3 emissions from slurry were significantly reduced when *Bacillus*-based feed additive was applied. Moreover, it seems that gas emissions were more pronounced in slurry samples compared to pig house, indicating that there could be an amount of gas that remained condensed in the slurry, but perhaps did not vaporise in the pig room atmosphere. The amount of gas in the pig room atmosphere was significantly reduced ($P < 0.05$) by microbial agent spraying and according to our results, increasing levels (dilutions) of the microbial agent mixtures provided better effects, but with the present methodology, we were not able to determine the optimal level of the agents.

Conclusions

The results of this study clearly suggested that spraying *L. plantarum* in the slurry exerted a more potent inhibitory effect on odorous gas emissions than spraying *B. subtilis*. Moreover, the mixture of microbial agents seems to have a synergic reducing effect on gas emissions. Thanks to these findings, we can assume that the mixture of microbial agents has an collective effect on the production of noxious gases, which provides better results than solitary microbial agents. Considering the fact that these findings regarding the effect of microbial agents on gas emissions in the slurry are preliminary, our team is developing a robust methodology that will address all aspects of the problem, including optimal levels of microbial agents, mechanism of microbial action in slurry, microbial activity changes with seasons (summer and winter), exposure time, and possibly provide recommendations on the integration and application procedures to be considered among other management practices.

Acknowledgements

The Department of Animal Resource & Science was supported through the Research-Focused Department Promotion & Interdisciplinary Convergence Research Projects as a part of the University Innovation Support Program for Dankook University in 2022.

Conflict of interest

The Authors declare that there is no conflict of interest.

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