

Article

Effects of Manure Removal Frequencies and Deodorants on Ammonia and GHG Concentrations in Livestock House

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Abstract: In order to mitigate the concentration of NH₃ and greenhouse gases (GHGs: CO₂, N₂O, CH₄) in livestock houses, two experiments, one determining the ideal manure removal frequency by cleaning the feces from a livestock house once, twice, three, and four times a day, and one in which microbial deodorant and VenaZn deodorant were sprayed, were conducted in a rabbit breeding house. The NH₃, CO₂, N₂O, and CH₄ concentrations were monitored continuously with an Innova 1512 photoacoustic gas monitor during the experiments. The results were as follows: the manure removal frequency had a significant impact on the average concentrations of NH₃, CO₂, and CH₄ in the rabbit house. Cleaning the feces in the rabbit breeding house two to three times a day significantly reduced the NH₃ concentration, and, on the contrary, cleaning the feces four times a day increased the NH₃ concentration in rabbit house; increasing the manure removal frequency significantly reduced the concentrations of CO₂ and CH₄ in the rabbit house. Considering the average concentrations of NH₃, CO₂, N₂O, and CH₄ in the rabbit house and economic cost, it was better to remove feces twice a day. The average NH₃ and CO₂ concentration declined significantly within 3 days in the summer and winter; the N₂O concentration declined within 3 days in the summer but did not decline in the winter; and there was no effect on the CH₄ concentration in the summer and in the winter after spraying the rabbit house with microbial deodorant. Therefore, it was better to spray microbial deodorant twice a week on Monday and Thursday to reduce the NH₃, CO₂, and N₂O concentrations in rabbit houses. The NH₃, CO₂, N₂O, and CH₄ concentrations first showed a decreasing trend and then an increasing trend over 5 days in the summer and 7 days in the winter after VenaZn deodorant was sprayed in the rabbit house, and the NH₃, CO₂, N₂O, and CH₄ concentrations on day 3 and day 4 were significantly lower than they were on the other days.

Keywords: ammonia; greenhouse gas; manure remove frequency; microbial deodorant; VenaZn deodorant



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1. Introduction

CO₂ (carbon dioxide), CH₄ (methane), and N₂O (nitrous oxide) are three major anthropogenic greenhouse gases (GHGs). Worldwide, 52.4 Gt CO₂-eq GHGs was emitted in 2019 [1], 11.39 Gt (23%) of which was from agriculture [2]. Livestock is a main source of the GHG emissions from agricultural sources. Research has shown that, globally, the CH₄ and N₂O produced from livestock represent 80% of the non-CO₂ greenhouse gases produced from agriculture and 12% of global anthropogenic greenhouse gases [3,4]. In 2015, the emissions of non-CO₂ greenhouse gases from non-ruminant livestock accounted for 33% of the GHG emissions from livestock in China, with the CO₂ emission equivalent of greenhouse gas in manure accounting for 37.29% of the total greenhouse gas emissions from livestock [5].

The large amounts of manure accumulated in high-density and large-scale livestock houses produce a large amount of ammonia and greenhouse gases [6]. In China, about 90%

of the total ammonia emissions come from agricultural sources, 50–60% of which come from livestock production [7,8]. NH_3 emissions from livestock buildings accounted for about 32% of the NH_3 produced by livestock in China in 2017 [9]. The NH_3 and greenhouse gases (CH_4 , N_2O , and CO_2) in livestock houses come from the metabolism and decomposition of the organic matter containing carbon and nitrogen in manure. The improper disposal of manure in livestock houses causes a large amount of greenhouse gas emissions.

Ammonia (NH_3) accelerates the formation of fine particulate in the atmosphere and plays a very important role in the acidification and eutrophication processes in the ecosystem [10]. NH_3 enters the atmosphere in two forms, ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) and ammonium nitrate (NH_4NO_3), to form the precursor for fine particulate matter, $\text{PM}_{2.5}$ [11], which is then transported into rivers and oceans, where extra N_2O is produced [12]. At the same time, N_2O , CH_4 , and CO_2 further enhance the scattering of solar radiation by changing the physical properties of aerosols and clouds, thus affecting climate change [13]. In livestock houses, NH_3 is a notorious irritant gas that results in the reduced production, health, and welfare of livestock [14]. People who work in livestock houses for long hours and who are continuously exposed to airborne pollutants show an increased incidence of cough, itchy throat, burning eyes, chronic bronchitis, and reduced lung function compared with those who do not work in livestock houses [15–17]. NH_3 that has been attached to dust and that is transported via air currents might travel several miles or several feet, depending on specific conditions [18,19], affecting the lives of surrounding residents. Thus, to some extent, these harmful gases restrict the development of livestock production. How to reduce and control ammonia and greenhouse gases in livestock houses and improve animal welfare and productivity at the same time is a major problem for the animal husbandry industry.

Ammonia and greenhouse gas emissions from livestock houses depend on animal age and manure management; the nitrogen content in the feed and in the feces; and environmental conditions [20,21]. Previous studies have made numerous changes to diet ingredients to reduce the harmful gases in livestock houses [21,22]. Most livestock houses (such as those for pigs and rabbits) in China use shallow septic tanks for the short-term storage of manure. The residence time or cleaning frequency of manure in livestock houses affects the emission of harmful gases [23–25]. However, it is not clear how the cleaning frequency of manure using scraping systems affects the concentrations of harmful gases and their emission in livestock houses. There are many methods to manage the harmful gases generated in livestock houses, such as chemical methods, physical methods, and biological methods [26]. However, the most harmful gases are discharged outside due to the economic cost of harmful gas treatment. There are relatively few studies on directly spraying deodorants to remove harmful gases in livestock houses. China's rabbit production has become more large-scale and intensive, and about 319 million rabbits were sold in China in 2020. On large-scale rabbit farms, the weight of fresh feces produced by adult rabbits every day is about 100–150 g/rabbit, and urine output of adult rabbits is about 200–300 mL/rabbit. Therefore, large amounts of manure accumulate, and ammonia and greenhouse gases are emitted in rabbit houses every day. Therefore, under the existing conditions, there is increasing demand for practical technologies that can reduce the NH_3 and greenhouse gas emissions generated from livestock production. In particular, innovative manure management technologies are urgently needed to alleviate NH_3 and greenhouse gas emissions in livestock houses [27]. The focus of this study is the mitigation of NH_3 and GHGs using different manure removal frequencies, microbial deodorant, and VenaZn deodorant to provide technical support for ammonia and greenhouse gas control in rabbit breeding houses.

2. Materials and Methods

The experiments were conducted in the experimental rabbit farm at the Luhe animal science base of the Jiangsu Academy of Agricultural Science in Nanjing, China.

2.1. Experimental Room and Ventilation Management

The experimental rabbit house was a rabbit breeding house. It was a closed building with a sandwich-colored steel plate structure with one door on both the east and west walls, respectively. There were four windows that were 1.3 m in length and 0.6 m in height along the north wall and south wall, respectively. The experimental rabbit house was 50 m long, 12 m wide, and 3.5 m high. There were four rows of rabbit cages and five corridors. Vents that were 7 cm in width were reserved for each window for ventilation during the experiment in the winter. Nine unpowered hoods were set on the middle of the roof of rabbit house and were placed 5 m apart from each other, in a north–south sequence. Scraper-type tools for cleanings were equipped on the bottom of the feces tank, which was 30 cm away from the ground.

2.2. Experimental Design

2.2.1. Experiment 1: Effects of Feces-Cleaning Frequency on Ammonia and Greenhouse Gas Concentrations

The first experiment was carried out from 26 February 2021 to 10 March 2021. The experimental treatments included treatment A (feces cleaned once a day at 6:00), treatment B (feces cleaned twice a day at 6:00 and 19:00), treatment C (feces cleaned three times a day at 6:00, 19:00, and 23:00) and treatment D (feces cleaned four times a day at 6:00, 19:00, 23:00, and 3:00). The doors of the experimental rabbit room were opened for ventilation every morning from 8:00 to 9:00 and every afternoon from 15:00 to 16:00.

There were 540 rabbits in the experimental house, and the rabbits were fed twice a day at 8:30 and 16:00 and were able to drink freely during the experiment.

2.2.2. Experiment 2: Effects of Microbial Deodorant and ZnMNP Deodorant on Ammonia and Greenhouse Gas Concentrations

The second experiment was carried out from 20 August 2021 to 2 October 2021, during the summer, and from 8 December 2021 to 24 January 2022, during the winter. First, the microbial deodorant was sprayed into the air and the bottom of the feces tank once every 7 days at the same time. Then, the VenaZn deodorant (the main component of which was ZnMNPs) was sprayed into the air and the bottom of the feces tank bottom once every 7 days at the same time. The ammonia and greenhouse gases in the experimental rabbit house were monitored for 5–7 days after the microbial deodorant and VenaZn deodorant had been sprayed. The microbial deodorant was mainly composed of *Lactobacillus plantarum*, *Bacillus subtilis*, and *Bacillus licheniformis* and was invented by the Institute of Animal Science, Jiangsu Academy of Agricultural Sciences. The ZnMNP deodorant was provided by Nanjing NanoKC Technology Co., Ltd. Nanjing, Jiangsu, China.

There were 400 rabbits in the experimental house, and the rabbits were fed twice a day at 8:30 and 16:00 and were allowed to drink freely during the experiment. The manure was cleaned once a day. The doors of the experimental rabbit room were opened for ventilation every morning from 8:00 to 9:00 and every afternoon from 15:00 to 16:00 in the winter, and the doors and the windows were opened for natural ventilation in the summer.

2.2.3. Ammonia, Greenhouse Gases, Temperature, and Relative Humidity Determination Methods

The ammonia and greenhouse gases (CO_2 , CH_4 , N_2O) were monitored by an Innova 1512 photoacoustic gas monitor. There were six monitor points in the rabbit house (Figure 1), and the monitoring points were located 30 cm from the ground. The instrument was set to monitor the area continuously and monitored one piece of data per minute. The instrument had six channels to monitor gases at the six monitor points, and each channel monitored one piece of data every 6–7 min.

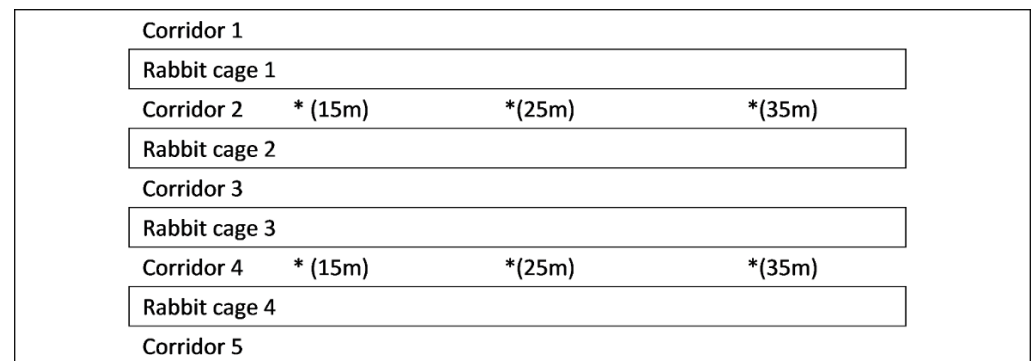


Figure 1. Monitoring points (*) in the rabbit house.

The temperature and relative humidity were monitored by a pocket weather tracker Kestrel 4500, and the data was recorded every 20 min. The temperature and relative humidity in the rabbit house during the experiment are shown in Table 1.

Table 1. The temperature and relative humidity in the rabbit house during the experiment.

	Season	Temperature (°C)	Relative Humidity (%)
Experiment 1	Winter	14.93	73.77
Experiment 2	Summer	26.04	81.04
	Winter	12.39	77.98

2.3. Statistical Analysis

Experimental data were analyzed in Excel and plotted in Sigmaplot 12.5, and the differences among the means for the NH_3 , CO_2 , N_2O , and CH_4 concentrations according to the days after spraying the microbial deodorant and ZnMNP deodorant were analyzed by two-way ANOVA. All of the statistical tests were performed at the significance level of 0.05 using SPSS 17.0 for Windows.

3. Results

3.1. Effects of Feces-Cleaning Frequency on Ammonia and GHG Concentration in Rabbit Houses

3.1.1. Effects of Feces-Cleaning Frequency on NH_3 Concentration

The overall trend in the NH_3 concentration in the rabbit house first increased and then decreased from 12:00 to 12:00 the next day (Figure 2). The NH_3 concentration was at a low level from 12:00 to 16:00 and began to increase gradually after the doors of the rabbit room closed at 16:00 and remained high from 19:00 to 8:00 the next day and then declined rapidly after opening the doors for ventilation at 8:00 the next day. The average NH_3 concentrations for treatments A, B, C, and D were 16.17 mg/m^3 , 14.31 mg/m^3 , 14.71 mg/m^3 , and 16.95 mg/m^3 , respectively; those of treatment A and D were significantly higher than those of treatment B and C, over the course of 24 h (Table 2).

Table 2. Effect of feces-cleaning frequency on the average concentrations of NH_3 and GHGs over 24 h in the rabbit house (different letters indicate significant differences at $p < 0.05$).

Treatment	$\text{NH}_3 \text{ (mg/m}^3\text{)}$	$\text{CO}_2 \text{ (mg/m}^3\text{)}$	$\text{N}_2\text{O (mg/m}^3\text{)}$	$\text{CH}_4 \text{ (mg/m}^3\text{)}$
A	$16.17 \pm 4.87\text{a}$	$1803 \pm 341\text{a}$	$0.796 \pm 0.037\text{b}$	$4.13 \pm 0.79\text{a}$
B	$14.31 \pm 3.82\text{b}$	$1681 \pm 183\text{b}$	$0.817 \pm 0.047\text{a}$	$2.80 \pm 0.82\text{b}$
C	$14.71 \pm 4.88\text{b}$	$1590 \pm 225\text{b}$	$0.803 \pm 0.026\text{ab}$	$2.29 \pm 0.78\text{c}$
D	$16.95 \pm 6.13\text{a}$	$1623 \pm 191\text{b}$	$0.799 \pm 0.029\text{b}$	$2.32 \pm 0.71\text{c}$

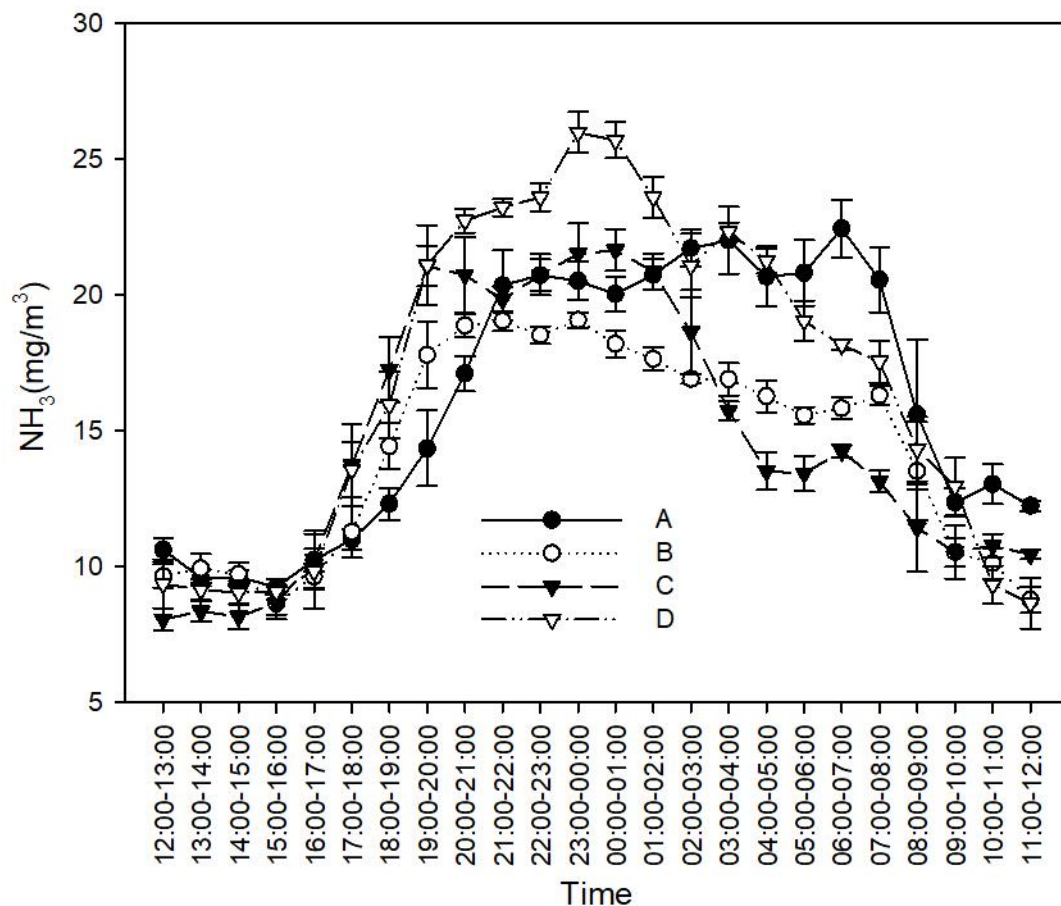


Figure 2. The effect of the feces-cleaning frequency on the NH_3 concentration in the rabbit house.

The NH_3 concentration of treatment A was more than 20 mg/m^3 after 11 h, from 21:00 to 8:00 the next day, and the average NH_3 concentration was 20.98 mg/m^3 . The highest value was 22.46 mg/m^3 at 6:00–7:00 after the feces had been cleaned, and it fell rapidly to 12.37 mg/m^3 at 9:00–10:00 after opening the doors at 8:00.

The NH_3 concentration of treatment B was between 15.58 – 19.09 mg/m^3 , and the average of concentration was 17.47 mg/m^3 from 19:00 to 8:00 the next day. Between 23:00 and 6:00 the next day, the NH_3 concentration demonstrated a slow downward trend and rose slightly after 6:00 the next day, at which point the feces were cleaned, and it decreased rapidly after opening the doors at 8:00 the next day.

The NH_3 concentration of treatment C in the rabbit house was between 19.84 and 21.66 mg/m^3 , with an average concentration of 20.93 mg/m^3 from 19:00 to 2:00 the next day, and it then decreased in waves from 18.64 mg/m^3 at 2:00–3:00 to 13.16 mg/m^3 at 7:00–8:00. The NH_3 concentration increased within one hour after feces cleaning started at 6:00, 19:00, and 23:00.

The NH_3 concentration of treatment D in the rabbit house was between 21.09 and 25.99 mg/m^3 , with an average concentration of 23.07 mg/m^3 from 19:00 to 5:00 the next day, and it then declined linearly from 19.06 mg/m^3 at 5:00–6:00 to 9.38 mg/m^3 at 10:00–11:00. The NH_3 concentration rose within one hour of the start of each fecal-cleaning period at 19:00, 23:00, and 3:00 and continued to fall at 6:00.

3.1.2. Effects of the Feces-Cleaning Frequency on the CO_2 Concentration in the Rabbit House

Figure 3 shows that the overall trend of the CO_2 concentration in the rabbit house first increased and then decreased from 12:00 to 12:00 the next day. The average concentration of CO_2 within 24 h of treatments A, B, C, and D were 1803 mg/m^3 , 1681 mg/m^3 , 1590 mg/m^3 ,

and 1623 mg/m³, respectively; that of treatment A was significantly higher than those of treatment B, C, and D (Table 2). At its highest value, treatment A had a concentration higher than 2000 mg/m³.

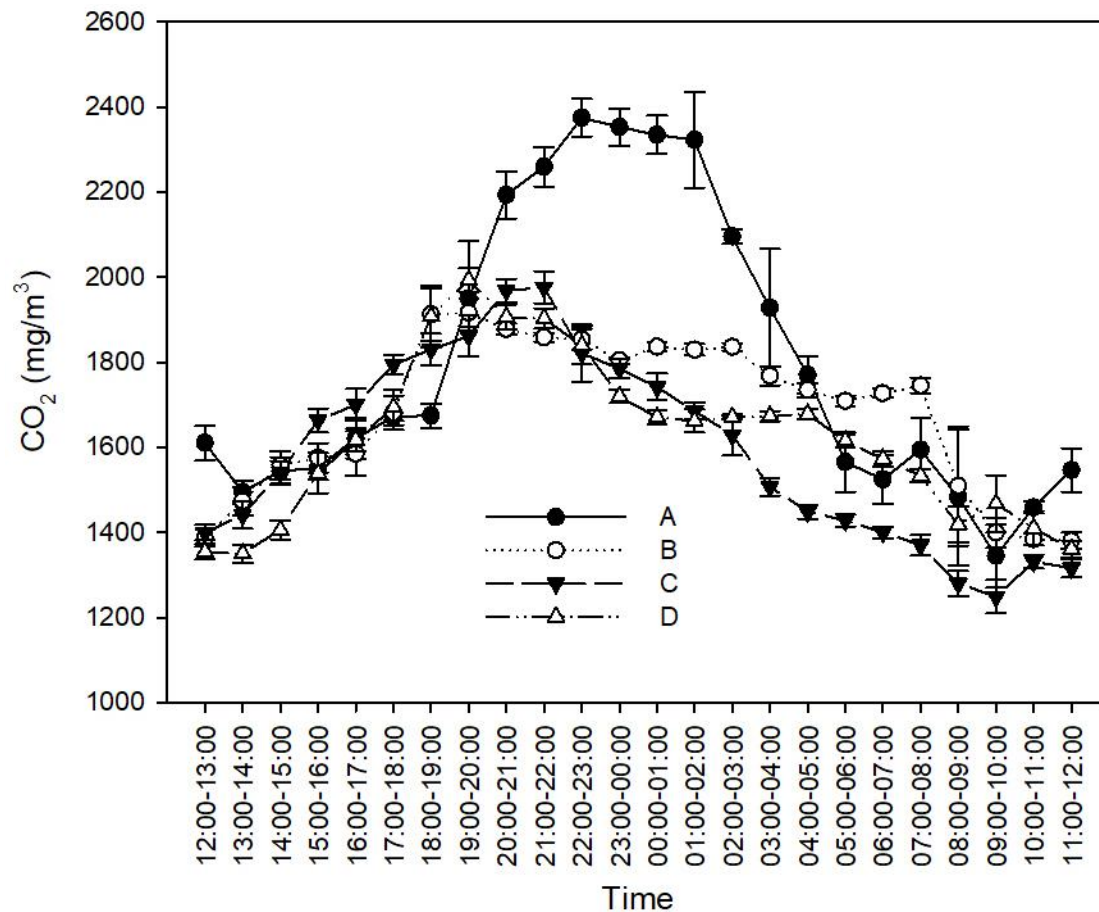


Figure 3. Effects of feces-cleaning frequency on the CO₂ concentration in the rabbit house.

The CO₂ concentration of treatment A was at its peak between 2096–2375 mg/m³, the average concentration was 2276 mg/m³ from 20:00 to 3:00 the next day, and it dropped rapidly to 1594 mg/m³ at 7:00–8:00. The CO₂ concentration of treatment B reached its highest value of 1917 mg/m³ at 20:00–21:00, and it then slowly decreased to 1746 mg/m³ at 7:00–8:00. The CO₂ concentration of treatment C rose to its highest value of 1976 mg/m³ at 22:00–23:00 and then declined linearly to 1370 mg/m³ at 7:00–8:00. The CO₂ concentration of treatment D reached its highest value of 1993 mg/m³ at 19:00–20:00, and it then slowly descended to 1533 mg/m³ at 7:00–8:00.

3.1.3. Effects of Feces-Cleaning Frequency on the N₂O Concentration in the Rabbit House

The N₂O concentration in the experimental rabbit house first showed an increasing trend and then a reducing trend from 12:00 to 12:00 the next day (Figure 4). The average N₂O concentrations in the experimental rabbit house within 24 h of treatments A, B, C, and D were 0.796 mg/m³, 0.817 mg/m³, 0.803 mg/m³, and 0.799 mg/m³, respectively (Table 2).

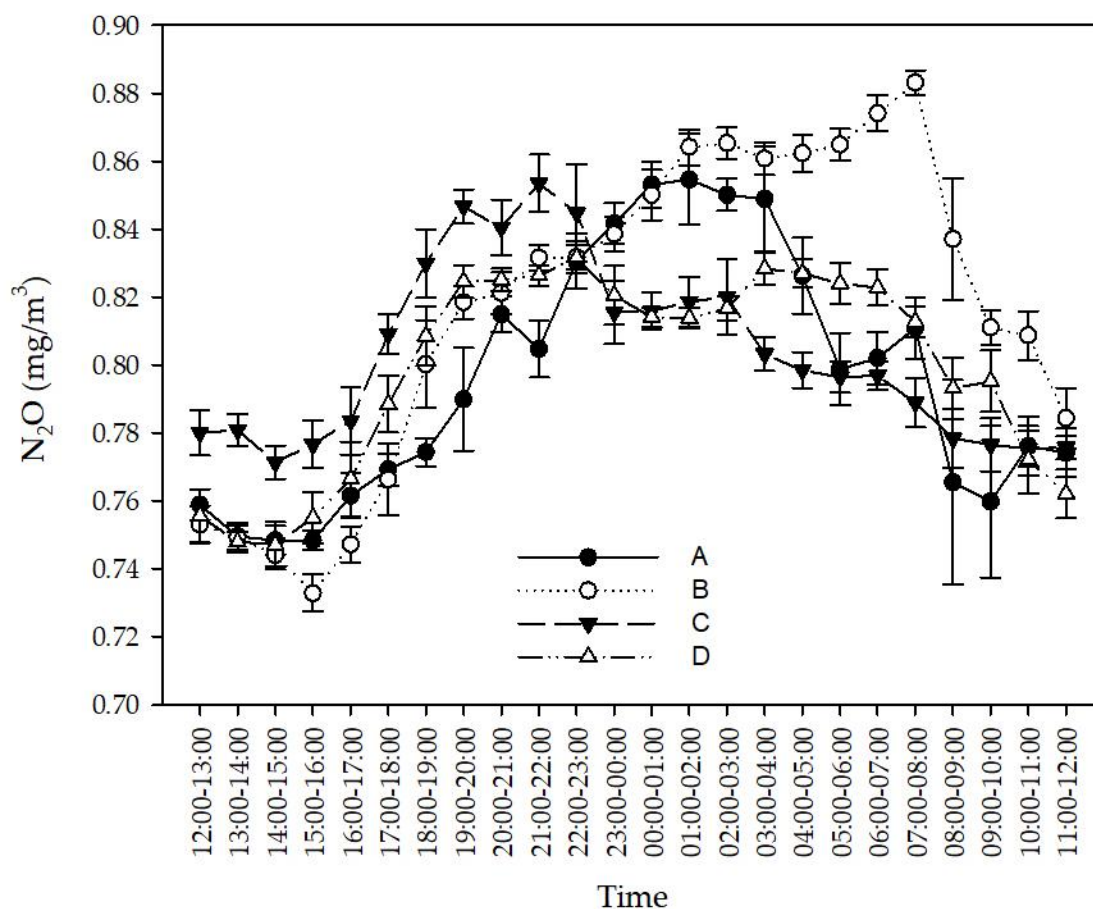


Figure 4. The effect of feces-cleaning frequency on the N₂O concentration in the rabbit house.

The N₂O concentration of treatment A began to increase from its lowest value of 0.748 mg/m³ at 15:00–6:00, increasing to values between 0.849 and 0.853 mg/m³ from 0:00 to 4:00 the next day before declining in waves to 0.799 mg/m³ at 5:00–6:00 after 4:00 and then increasing slightly before 8:00. The N₂O concentration of treatment B increased continuously from 0.733 mg/m³ at 15:00–16:00 to its peak value of 0.883 mg/m³ at 7:00–8:00 for 12 h, and it then dropped rapidly after opening the doors for ventilation at 8:00. The N₂O concentration of treatment C increased from 0.771 mg/m³ at 14:00–15:00 to its highest value of 0.853 mg/m³ at 21:00–22:00. It remained at a high concentration of 0.840–0.853 mg/m³ for 4 h from 19:00 to 23:00 before slowly and continuously declining to 0.776 mg/m³ in waves at 11:0–12:00. The N₂O concentration of treatment D increased from 0.747 mg/m³ at 14:00–15:00 to 0.825 mg/m³ at 19:00–20:00, and it was between 0.813–0.832 mg/m³ from 19:00 to 8:00 for 13 h before dropping rapidly after opening the doors.

3.1.4. Effect of Feces-Cleaning Frequency on CH₄ Concentration in the Rabbit House

The CH₄ concentration first increased and then decreased from 12:00 to 12:00 the next day (Figure 5). The average CH₄ concentrations of treatments A, B, C, and D were 4.13 mg/m³, 2.80 mg/m³, 2.29 mg/m³, and 2.32 mg/m³, respectively, and that of treatment A was significantly higher than those of treatments B, C, and D (Table 2). The CH₄ concentration of treatment A rose in a zigzag pattern from 3.11 mg/m³ at 13:00–14:00 to its highest value of 5.55 mg/m³ at 0:00–1:00 and then decreased in waves to 3.97 mg/m³ at 5:00–6:00 before rising slightly at 8:00 before the doors were opened. The average CH₄ concentration of treatment B was slightly higher than that of treatments C and D. The CH₄ concentration rose rapidly from 1.57 mg/m³ at 12:00–13:00 to 3.81 mg/m³ at 20:00–21:00 and then decreased slowly to 2.88 mg/m³ at 5:00–6:00 and rose slightly before the doors were opened at 8:00. The CH₄ concentration of treatment C rose rapidly from 1.31 mg/m³

at 13:00–14:00 to its highest value of 3.47 mg/m^3 at 21:00–22:00 and then decreased linearly to 1.81 mg/m^3 at 7:00–8:00. The CH_4 concentration of treatment D increased rapidly from 1.21 mg/m^3 at 12:00–13:00 to its peak concentration of 3.51 mg/m^3 at 19:00–20:00 before continuously declining in a zigzag pattern to 2.14 mg/m^3 at 7:00–8:00.

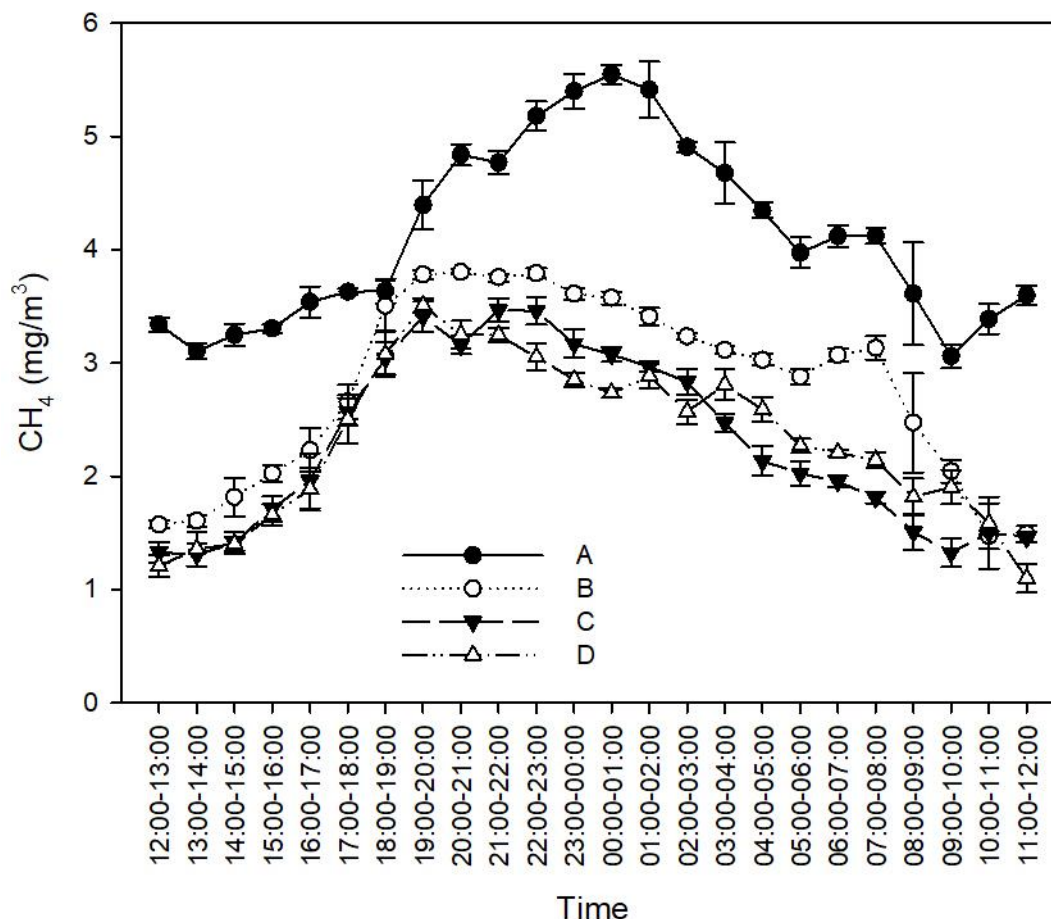


Figure 5. The effect of feces-cleaning frequency on CH_4 concentration in the rabbit house.

3.2. Effects of Microbial Deodorant on NH_3 and Greenhouse Gases in the Rabbit House

Overall, after spraying the microbial deodorant, the NH_3 , CO_2 , and N_2O concentrations in the rabbit house were higher in the winter than they were in the summer, and the CH_4 concentration in the rabbit house was significantly higher in the summer than it was in the winter (Table 3).

In the summer, the average NH_3 concentration in the rabbit house on days 1, 2, and 3 after spraying the microbial deodorant was 3.50 mg/m^3 , 3.44 mg/m^3 , and 3.64 mg/m^3 , respectively. The concentration increased from day 4 and reached its maximum of 4.67 mg/m^3 on day 5. As such, the NH_3 concentration in the rabbit house in the first three days after spraying the microbial deodorant was significantly lower than it was on days 4 and 5.

The CO_2 concentration in the rabbit house in the summer was lower on days 1 and 2 after the microbial deodorant had been sprayed and was 944 mg/m^3 and 929 mg/m^3 , respectively. The concentration increased to 1090 mg/m^3 from days 3 to 5. That is, the CO_2 concentration was significantly lower in the first two days after the microbial deodorant was sprayed than it was on days 3, 4, and 5.

Table 3. Effects of microbial deodorant on NH_3 and greenhouse gas concentrations in the rabbit house (different letters indicate significant differences at $p < 0.05$).

	NH_3 (mg/m ³)	CO_2 (mg/m ³)	N_2O (mg/m ³)	CH_4 (mg/m ³)
Summer				
1d	3.50 ± 0.47c	944 ± 33c	0.89 ± 0.04c	7.20 ± 0.40b
2d	3.44 ± 0.55c	929 ± 29c	0.91 ± 0.04bc	8.07 ± 0.74a
3d	3.64 ± 0.86c	1014 ± 124b	0.91 ± 0.03bc	7.04 ± 0.75b
4d	4.25 ± 1.25b	1056 ± 100a	0.93 ± 0.05b	5.42 ± 1.74d
5d	4.67 ± 1.03a	1090 ± 86a	0.99 ± 0.06a	6.13 ± 1.78c
6d	3.91 ± 1.12b	1006 ± 60b	0.97 ± 0.06a	5.99 ± 1.85cd
Mean value	3.94 ± 0.49	1007 ± 63	0.93 ± 0.04	6.61 ± 1.00
Winter				
1d	5.86 ± 0.49c	1350 ± 84c	1.02 ± 0.04a	0.46 ± 0.24a
2d	6.20 ± 0.59c	1348 ± 140c	1.02 ± 0.03a	0.40 ± 0.34ab
3d	5.89 ± 1.42c	1326 ± 176c	1.00 ± 0.05b	0.34 ± 0.25bc
4d	6.68 ± 0.50b	1368 ± 77bc	1.00 ± 0.04b	0.41 ± 0.24ab
5d	7.92 ± 0.78a	1406 ± 126ab	0.99 ± 0.02c	0.27 ± 0.28c
6d	7.59 ± 0.65a	1399 ± 102ab	1.01 ± 0.04ab	0.40 ± 0.32ab
7d	7.61 ± 0.80a	1430 ± 137a	1.02 ± 0.03a	0.47 ± 0.32a
Mean value	6.82 ± 0.88	1375 ± 37	1.01 ± 0.01	0.39 ± 0.07

During the summer, the N_2O concentration in the rabbit house increased from 0.89 mg/m³ on day 1 to 0.99 mg/m³ on day 5 after the microbial deodorant had been sprayed. The N_2O concentration on day 1 was significantly lower than it was on days 4 and 5, before returning to its normal value on day 5.

After spraying the microbial deodorant, the CH_4 concentration in the rabbit house was higher on the first of the three days in the summer and was significantly lower on days 4, 5, and 6.

In the winter, the average NH_3 concentration in the rabbit house was low on days 1, 2, and 3 after spraying the microbial deodorant, achieving concentrations of 5.86 mg/m³, 6.20 mg/m³, and 5.89 mg/m³, respectively, before increasing on day 4 and reaching the peak concentration of 7.92 mg/m³ on day 5. As such, the NH_3 concentration within the first 3 days after spraying the microbial deodorant was significantly lower than it was during the last 4 days in the winter study period (Table 3).

The CO_2 concentration in the rabbit house in the winter was low on days 1, 2, and 3 after spraying the microbial deodorant, achieving values of 1350 mg/m³, 1348 mg/m³, and 1326 mg/m³, respectively, before increasing to its highest concentration of 1430 mg/m³ on day 7. As such, the CO_2 concentration within the first 3 days after spraying the microbial deodorant was significantly lower than it was during the last 3 days of the winter study period.

After spraying the microbial deodorant in the winter, the N_2O concentration in the rabbit house first decreased and then increased, varying significantly between 0.99 mg/m³ and 1.02 mg/m³, and on days 3, 4, and 5, it was lower than it was on the other days.

The CH_4 concentration in the rabbit house first decreased and then increased, and the lowest value was 0.27 mg/m³ on day 5 after spraying the microbial deodorant.

3.3. Effect of VenaZn Deodorant on NH_3 and Greenhouse Gases in the Rabbit House

Generally, after spraying the VenaZn deodorant, the NH_3 , CO_2 , and N_2O concentrations in the rabbit house in the winter were higher than they were in the summer, and the CH_4 concentration in the summer was significantly higher than it was in the winter (Table 4).

Table 4. Effects of the VenaZn deodorant on the NH₃ and greenhouse gas concentrations in the rabbit house (different letters indicate significant differences at $p < 0.05$).

	NH ₃ (mg/m ³)	CO ₂ (mg/m ³)	N ₂ O (mg/m ³)	CH ₄ (mg/m ³)
Summer				
1d	4.73 ± 1.06a	1038 ± 153a	0.89 ± 0.04a	6.21 ± 2.54b
2d	3.91 ± 1.29b	994 ± 107b	0.87 ± 0.04b	5.77 ± 2.51bc
3d	2.37 ± 0.46c	898 ± 43c	0.82 ± 0.02c	4.98 ± 2.42cd
4d	2.67 ± 0.70c	913 ± 57c	0.82 ± 0.03c	4.88 ± 1.67d
5d	4.23 ± 1.11b	1010 ± 96ab	0.91 ± 0.03a	7.24 ± 2.01a
Mean value	3.58 ± 1.02	809 ± 62	0.86 ± 0.04	5.81 ± 0.97
Winter				
1d	7.93 ± 1.05a	1403 ± 77a	1.00 ± 0.03b	0.23 ± 0.47a
2d	6.62 ± 1.23bc	1261 ± 141b	0.98 ± 0.02c	0.02 ± 0.05b
3d	5.53 ± 0.39d	1202 ± 66c	0.97 ± 0.03c	0.01 ± 0.03b
4d	5.81 ± 0.60d	1173 ± 61c	0.95 ± 0.01d	0.00 ± 0.00b
5d	6.36 ± 0.72c	1184 ± 102c	0.95 ± 0.01d	0.00 ± 0.00b
6d	6.99 ± 0.55b	1278 ± 69b	1.03 ± 0.02a	0.04 ± 0.07b
7d	7.70 ± 0.70a	1384 ± 73a	1.00 ± 0.01b	0.01 ± 0.01b
Mean value	6.70 ± 0.90	1269 ± 93	0.98 ± 0.03	0.04 ± 0.01

After spraying the VenaZn deodorant in the summer, the average NH₃ concentration in the rabbit house decreased from 4.73 mg/m³ on day 1 to 2.37 mg/m³ on day 3 and then increased to 4.23 mg/m³ on day 5. The ammonia concentration on days 3 and 4 were significantly lower than they were on days 1, 2, and 5.

After spraying the VenaZn deodorant in the summer, the CO₂ concentration in the rabbit house first decreased from 1038 mg/m³ on day 1 to 898 mg/m³ on day 3 and then gradually rose to 1010 mg/m³ on day 5. The concentrations on days 3 and 4 were significantly lower than they were on the other days.

The N₂O concentration declined from 0.89 mg/m³ on day 1 to 0.82 mg/m³ on days 3 and 4 and then increased to 0.91 mg/m³ on day 5 after spraying the VenaZn deodorant in the summer. On days 3 and 4, the concentration was significantly lower than it was on the other days.

After spraying the VenaZn deodorant in the summer, the CH₄ concentration decreased from 6.21 mg/m³ on day 1 to 4.88 mg/m³ on day 4 and then increased to 7.24 mg/m³ on day 5. The concentrations on days 3 and 4 were significantly lower than they were on the other days (Table 4).

In the winter, after spraying the VenaZn deodorant, the average NH₃ concentration in the rabbit house dropped significantly, from 7.93 mg/m³ on day 1 to 5.53 mg/m³ on day 3 and 5.81 mg/m³ on day 4, and then increased significantly to 7.70 mg/m³ on day 7. The NH₃ concentration on days 3 and 4 was significantly lower than it was on the other days.

After spraying the VenaZn deodorant in the winter, the CO₂ concentration in the rabbit house first declined from 1403 mg/m³ on day 1 to 1173 mg/m³ on day 4 and then rose to 1384 mg/m³ on day 7. The CO₂ concentration on days 3, 4, and 5 was significantly lower than it was on the other days (Table 4).

After spraying the VenaZn deodorant in the winter, the N₂O concentration in the rabbit house first decreased from 1.00 mg/m³ on day 1 to 0.95 mg/m³ on days 4 and 5 and then increased to 1.03 mg/m³ on day 6. The N₂O concentration on days 4 and 5 was significantly lower than it was on the other days.

After spraying the VenaZn deodorant in the winter, the CH₄ concentration in the experimental rabbit house dropped significantly from 0.23 mg/m³ on day 1 to 0.00–0.04 mg/m³ from days 2 to 7.

4. Discussion

4.1. Effect of Feces-Cleaning Frequency on NH_3 and GHG Concentrations

The manure removal frequency in the livestock house played a major role in the mitigation of pollutant gas emission [28]. Under the given feeding conditions, if we want to control and reduce the NH_3 , CO_2 , N_2O , and CH_4 concentrations in livestock houses to the greatest extent, we need to minimize the contact time and area between the manure and the air in the house and clean up manure as soon as possible after manure is produced. According to Linyuan Cai's report, compared to manual manure cleaning, the average NH_3 , CH_4 , and CO_2 concentrations decreased by 56.8–70.6%, 14.7–33.7%, and 12.0–19.6%, respectively, when a rapid manure scraper system was implemented [29]. If the manure was not removed in time, the contact area between the manure and the air would continue to increase, resulting in more NH_3 and GHG emissions. Therefore, the manure-cleaning frequency greatly affected the air quality in the livestock houses. Research showed that, compared to deep slurry pits, cleaning manure from animal houses once a week could reduce NH_3 emissions by 35% [23]. Compared to cleaning the manure from animal houses once a week, removing the manure 2–3 times a week or every day significantly reduced ammonia emissions by 46% [23], reduced CO_2 emissions by 15%, and reduced N_2O emissions by 50% [24]. In hen laying houses, ammonia losses from weekly belt scraping, twice-weekly belt scraping, and daily belt scraping were 3.3, 1.6, and 1.3 g $[\text{NH}_3\text{-N}]\text{h}^{-1} 500 \text{ kg}^{-1} [\text{lw}]$, respectively [25]. In our results, the NH_3 concentration in a rabbit house when cleaning the manure two and three times a day was significantly lower than when it was only cleaned once a day, and the concentrations of CO_2 and CH_4 after cleaning the manure two, three, and four times a day were significantly lower than they were after cleaning the manure once a day, making these results similar to those obtained in previous research.

Using mechanical manure-cleaning facilities to remove feces from the house in a timely manner could shorten the amount of time in which harmful gases can be generated in livestock houses, but the manure-cleaning process would promote NH_3 emissions. In our study, the ammonia concentration in the rabbit house was highest at night when feces were cleaned four times a day, and frequent mechanical fecal cleaning accelerated the release of NH_3 . Using manure-scraping boards caused a large amount of NH_3 to be generated instantaneously, with NH_3 concentrations increasing by 70–75% and decreasing to 80–85% after one hour, in a beef cattle barn in the winter [30]. Our study also showed that there was an obvious increase in the NH_3 concentration in the rabbit house within 1 h after feces cleaning began. This could be because of crust formation on the manure surface, which could affect NH_3 emissions [31,32], and cleaning manure with a manure-scraping board destroyed the crusts on the surface of the feces and urine and broke the dung balls created by rabbits, resulting in more NH_3 gas being discharged in the livestock house. When manure was tumbled around, it was not easy for crusts to form on the surface of the feces. An excessive fecal-cleaning frequency accelerates the release of NH_3 , resulting in an excessive NH_3 concentration in rabbit houses.

4.2. Effect of Temperature on NH_3 and GHGs Concentration

The main source of NH_3 in rabbit houses is the hydrolysis of the urea in urine and the decomposition of the organic nitrogen in the solid feces in shallow cement fecal tanks [21,33]. N_2O is an intermediate product of the complex biochemical processes of nitrification and denitrification that takes place in manure under conditions with low oxygen availability [34]. It was reported that the NH_3 emission flux of pigs increased as the temperature increased [35]. The NH_3 and N_2O concentrations in pig houses in the winter were higher than they were in the summer, and this was mainly caused by the reduced ventilation to increase the temperature in livestock houses in the winter [36–38]. Additionally, the results of our study were consistent with this. The CO_2 in the rabbit house mainly came from animal respiration and the decomposition of the organic matter in urine and feces. The results of our study indicated that the CO_2 concentration in the rabbit house was higher

than it was in the summer because of lower ventilation in the winter [36]. It had previously been reported that CH₄ emissions increased significantly with the air temperature in livestock houses [29,39,40]. Fecal fermentation was the main source of methane emissions in livestock houses, and methanogens were sensitive to temperature. Methane emissions increased significantly as house temperature increased [41–43]. The methane emissions in pig houses were significantly influenced by the season and were higher in the summer [44], which indicated that temperature had a significant impact on the methane concentration in pig houses. In our study, the methane concentration in the naturally ventilated rabbit house was significantly higher in the summer than it was in the winter with the 3–4 h ventilation created by opening the doors every day. This could mainly be because methanogens were greatly affected by temperature, and the lower temperature in the winter inhibited the activity of methanogens.

4.3. Deodorization Effect of Microbial Preparations

Microbial deodorant uses the physiological metabolic activities of one or more beneficial microorganisms to degrade odor substances and oxidize them into odorless and harmless end products, such as carbon dioxide, water, sulfates, and nitrates, for the purposes of deodorization. Spraying microbial deodorant to reduce odor generation in livestock houses is a widely used practice in livestock and poultry farms in China [45–47]. It has low energy consumption, low cost, and strong applicability and causes no secondary pollution [48,49]. Previous studies found that *Bacillus subtilis* and *Bacillus licheniformis* could assimilate ammonium nitrogen (NH₄⁺-N) under certain conditions and effectively inhibit the growth of major odor-producing bacteria such as *Escherichia coli* and *Salmonella* [50,51], reducing the ammonia concentration in livestock houses. Other studies showed that the use of microbial preparations of *Bacillus subtilis* [52], *Lactobacillus plantarum*, *Lactobacillus*, and *Bifidobacterium* [53] could reduce ammonia emissions from pig manure. According to Ye Fenxia's study, one complex microbial adsorbent made of *Candida tropicalis*, *Bacillus megaterium*, and *Streptomyces griseus* was effective in reducing ammonia and hydrogen sulfide by 78.4% and 66.7%, respectively, in pig manure [54]. The addition of a 0.2% probiotic composed of *Bacillus subtilis*, yeast, and *actinomyces* to pig slurry reduced the pH and carbon dioxide emissions [55]. The microbial deodorant used in our experiment was mainly composed of *Lactobacillus plantarum*, *Bacillus subtilis*, and *Bacillus licheniformis*. Our experimental results showed that the average concentrations of NH₃ and N₂O in the first three days after spraying the microbial deodorant in the summer were low. This was because the microbial agents, especially *Bacillus subtilis*, and *Bacillus licheniformis*, promoted the conversion of ammonia nitrogen to nitrate nitrogen [7,8]. However, spraying microbial agents could reduce the N₂O concentration in the rabbit house in the summer, but it did not reduce the N₂O concentration in the winter, and this could be due to the low temperature in the winter. The average concentrations of CO₂ in the first three days after spraying the microbial deodorant in the summer and winter were low. This could be because the microbial agents, especially *Lactobacillus plantarum*, inhibited the growth of decomposing bacteria, so as to inhibit the degradation of nitrogenous organics, reducing the concentrations of ammonia and carbon dioxide in the rabbit house. Regardless of whether it was summer or winter, the microbial agents had no effect on the concentration of CH₄ in the rabbit house.

4.4. Deodorization Effect of VenaZn Deodorant

Zn micro nanoparticles (ZnMNPs), the main component of the VenaZn deodorant, have a wide size range of 10 nm–600 nm in diameter, and their antibacterial activity is due to the combination of their small size and high surface-to-volume ratio, which enables them to interact closely with microbial membranes [56–59]. The VenaZn deodorant also contains other antibacterial active ingredients, such as tea polyphenols, lemon extract, pine extract, etc. Reports have indicated that lemon extract could change certain key structures of bacteria or inhibit bacteria by destroying the bacterial cell wall, and it had an obvious inhibitory effect on bacteria and fungi [60,61]. Tea polyphenols, healthy and

safe plant preparations [62,63], are broad-spectrum antibacterial agents that had inhibition and killing effects on *Staphylococcus aureus* and other pathogenic intestinal bacteria [64–66]. Pine bark and heart wood had antibacterial effects on *Staphylococcus aureus*, *Enterococcus faecium*, and *Bacillus subtilis* [67], and pine needle extract had antimicrobial effectiveness against foodborne illness-causing bacteria [68]. Therefore, the manufacturer believed that VenaZn deodorant could effectively inhibit the growth of the microorganisms that produced harmful gases and absorb, catalyze, oxidize and decompose the harmful gases in livestock houses. The results of our study confirmed this view. After spraying the VenaZn deodorant in the rabbit house, the concentrations of the harmful gases (NH_3 , CO_2 , N_2O , CH_4) in the rabbit house first decreased and then increased within one week, and the concentrations of harmful gases were significantly lower on the third and fourth days than they were on the other days.

5. Conclusions

- (1) The overall trends in the average concentrations of NH_3 , CO_2 , N_2O , and CH_4 in the experimental rabbit house first increased and then decreased from 12:00 to 12:00 the next day during the winter. The manure removal frequency had a significant impact on the average concentrations of NH_3 , CO_2 , and CH_4 in the rabbit house. Cleaning feces from animal houses two and three times a day significantly decreased the NH_3 concentration, and, in contrast, cleaning four times a day increased the NH_3 concentration in the rabbit house; increasing the manure removal frequency significantly reduced the CO_2 and CH_4 concentration in the rabbit house. Considering the average concentrations of NH_3 , CO_2 , N_2O , and CH_4 in the rabbit house and the economic cost, it is better to clean feces from animal houses twice a day.
- (2) The average NH_3 and CO_2 concentrations declined significantly within 3 days in the summer and winter, and N_2O concentration declined within 3 days in the summer but not in the winter. There was no effect on the CH_4 concentration in the summer or in the winter after the microbial deodorant was sprayed. Therefore, it was better to spray microbial deodorant twice a week on Monday and Thursday to reduce the concentrations of NH_3 , CO_2 , N_2O , and CH_4 in the rabbit house.
- (3) The average concentrations of NH_3 , CO_2 , N_2O , and CH_4 first showed a decreasing trend and then an increasing within 5 days to 7 days in the summer and winter after the VenaZn deodorant had been sprayed in the rabbit house, and the concentrations of NH_3 , CO_2 , N_2O , and CH_4 were significantly lower on the third and fourth days than they were on the other days.
- (4) The average NH_3 , CO_2 , and N_2O concentrations in the winter were higher than they were in the summer, and the average CH_4 concentration was higher in the summer than it was in the winter in the experimental rabbit house.

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