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# Odor Reduction Monitoring in Swine Farms Following the Application of Domestic Commercial Microbial Products

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**Abstract:** This study evaluated the effects of commercially available microbial preparations (used as feed additives and environmental treatments) on swine farms, with a focus on odor reduction, gut microbiota, and immune responses. The results showed a significant reduction in major livestock odors, such as ammonia, volatile fatty acids, and phenolic compounds, although reductions in sulfur-based odors like hydrogen sulfide were limited. Five swine farms were selected for a ten-month study during which microbial feed additives and environmental improvement products were administered to assess their effectiveness in reducing odor emissions and altering the intestinal microbiota. Monitoring involved measurements of odorant concentrations, quantitative analysis of fecal microbiota, and microbial community profiling. A reduction in ammonia was observed, accompanied by an increase in hydrogen sulfide and other odorants. The change of metagenomics revealed a decrease in the abundance of *Firmicutes* from 77% to 56% and an increase in *Bacteroidetes* from 17% to 35% in the pig feces from the swine firms. Additionally, all farms maintained normal levels of the intestinal inflammation index. Microbial treatments led to a reduction in certain beneficial bacteria, such as *Lactobacillus*, but increased the abundance of short-chain fatty acid (SCFA), producing bacteria, including *Ruminococcaceae*, *Porphyromonadaceae*, and *Faecalibacterium prausnitzii*, suggesting improved energy metabolism and fiber degradation. Calprotectin levels (an inflammation marker) decreased in some farms, indicating potential reductions in intestinal inflammation. Despite no significant changes in total bacterial counts, species richness and diversity improved, and inflammation markers remained within normal physiological ranges. Overall, the study suggests that consistent use of microbial products, in combination with modern farm infrastructure, can improve both odor control and gut health, potentially enhancing the sustainability of swine farms and reducing community complaints.

**Keywords:** Gut microbiota Calprotectin levels, Metagenomics, Ordor reduction, Swine farms

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## Introduction

The livestock industry in Korea has shown continuous growth in response to increasing consumer demand for meat and the overall rise in national income.

According to Statistics Korea, livestock production has increased by an annual average of 12.2% over the past 54 year period from 1965 to 2018), accounting for 39.4% (19.7 trillion KRW) of the total agricultural output in 2018. During the same period, the supply of meat (beef, pork, chicken) increased at an average annual rate of 5%, while per capita meat consumption rose by 4.2% annually.

However, this rapid development in livestock farming has led to environmental and societal concerns, particularly due to the increased generation of livestock manure. As public complaints regarding livestock-related odors escalated, the Ministry of Environment enacted the Odor Prevention Act in 2004. Local governments subsequently allocated substantial budgets to odor management initiatives. Despite these efforts, such as the annual investment exceeding 150 billion KRW as of 2018, effective odor mitigation outcomes remain uncertain.

Many advanced livestock-producing countries have implemented minimum separation distances between animal facilities and residential areas to address odor conflicts [1]. For example, European nations have long established zoning regulations based on factors such as herd size, housing systems, manure storage types, feed composition, and proximity to residential areas [2]. In contrast, Korea's guidelines, such as the uniform 700-meter separation distance recommended for pig farms housing between 1,000 and 3,000 pigs, are applied uniformly, without consideration for local variables. Consequently, odor-related complaints persist, while farmers face increasing regulatory burdens without adequately tailored mitigation strategies.

In this context, over 60% of Korean livestock farms reportedly use microbial products aimed at improving odor conditions [3]. Nonetheless, concerns about their effectiveness persist, partly due to the proliferation of unverified products on the market, which has contributed to skepticism among farmers [4,5]. Despite these concerns, microbial additives continue to be widely used, particularly in pig farming [6]. When administered via feed, microbial products have been shown to improve digestive efficiency by promoting beneficial gut flora and suppressing harmful microorganisms. This, in turn, enhances manure fermentation and reduces the emission of odor-causing compounds such as ammonia and amines [7-10]. In pigs, improved digestion through probiotic supplementation has been associated with decreased emissions of ammonia, hydrogen sulfide, and volatile fatty acids, while also supporting gut microbial stability, weight gain, feed conversion efficiency, and immune function [11-14]. Additionally, environmental microbial treatments applied to manure have demonstrated the potential in reducing odor emissions and residual pollutants during the liquid composting process [15,16].

Given these considerations, this study aimed to evaluate the effects of six commercial microbial products, three feed additives and three environmental treatments, administered over a ten-month period across five pig farms experiencing severe odor issue. The evaluation included on-site odorant monitoring and fecal sample analysis to assess both odor reduction efficacy and improvements in intestinal microbial composition.

## Materials and Methods

### Selection of swine farms

Five swine farms were selected through a preliminary survey to conduct a field-based demonstration. The experimental livestock field sites included Koryeo farm (KR), Daeho farm (DH), Donbeot farm (DB), Daeseong farm (DS), and Kilim farm (KL). Selection criteria included the urgency of resolving odor complaints, willingness to participate in on-site testing, and availability of operational and productivity data. The characteristics of the selected farms, including scale, barn type, and manure management system, are summarized in Table 1. The herd size ranged from 1,000 to 3,500 pigs. Barns were classified as either open or enclosed slurry systems, and all farms utilized liquid fertilizer storage systems classified as either open or enclosed slurry systems, and all farms utilized liquid fertilizer storage systems.


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**Table 1. Summary of general conditions across the five swine farms**

| Farms | Location | Number of swine | Swine farm type |
|---|---|---|---|
| Koryeo (KR) | 35.07°N, 126.49°E | 1,000 | Wean-to-finish farm |
| Daeho (DH) | 35.09°N, 126.47°E | 2,500 | Slurry pit/Windowless |
| Donbeot (DB) | 35.10°N, 126.49°E | 2,600 | Slurry pit/Windowless |
| Daeseong (DS) | 35.17°N, 126.46°E | 3,500 | Slurry pit/Windowless |
| Kilim (KL) | 35.07°N, 126.48°E | 2,800 | Slurry pit/Windowless |

* Facility environment: Liquid fertilizer storage

## Selection and application of microbial products

The microbial products used in this study were commercially available and registered under the Feed Management Act (Article 8, Clause 1). Products with a microbial count of at least $10^7$ CFU/g were evaluated for efficacy, and six high-performing products were selected, three microbial-based formulation as feed additives (Spoazim, Cucumax 9 and EcomPower) and three microbial products as environmental treatment agents (Zero Base Plus, Odor escape-bacteria and Saccharo-20). The characteristics of the microbial products used to monitor livestock odor reduction in this study are presented in Table 2.

**Table 2. Characteristics of microbial products used to monitor livestock odor reduction**

| Microbial samples | | Registered ingredients | Manufactures |
|---|---|---|---|
| Feed additives | Spoazim | *Bacillus subtilis* >1×$10^7$ | Woo Jin B&G |
| | Cucumax 9 | *Lactobacillus plantarum* >1×$10^8$ | Jeon Nong Bio |
| | EcomPower | *Lactobacillus* sp. >1×$10^9$ | Hampyeong livestock Co. Asso. |
| Environmental treatment agents | Zero Base Plus | Soil microorganisms | Geon Nong Co., Ltd. |
| | Odor escape-bacteria | *Bacillus subtilis* >1×$10^6$ | Institute of livestock odor control |
| | Saccharo-20 | *Saccharomyces cerevisiae* >1×$10^6$ | Urim Bio incorporated |

Feed additives were evaluated based on viable cell count, microbial dominance, acid and bile tolerance, antimicrobial activity, and odor-reducing potential, specifically, the reduction of ammonia and amines. Environmental products were assessed for viable cell count, microbial dominance, and odor reduction effectiveness. Three different combinations were made by mixing one type of the feed additive with one type of the livestock odor reducer, and these were administered to five swine farms.

Recent research provided a large dataset that led to revisions in the feeding standards [17]. New additions include estimates of feed intake, standardized total tract digestible phosphorus needs, and nutrient emission data. In this study, the daily feed intake for fattening pigs was set at 2.3 kg, and feed additives were administered at approximately twice the manufacturer's recommended rate to ensure a minimum concentration of $10^7$ CFU/g. Environmental treatments were diluted according to the product instructions and applied around the barns at least twice per week. The specific products and application rates used on each farm are presented in Table 3.

## Odor monitoring in the swine farms

Field monitoring was conducted four times between May and December 2022 (May, July, September and November). The May sampling served as the baseline (pre-treatment). During sampling, barn ventilation fans were turned off for 5 minutes, and air was collected from 1 meter above the barn floor using polyester aluminum bags (Top Trading Engineering, Korea) placed inside an indirect suction box. A total of 10 liters of air was collected for each sample.


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**Table 3. Rate and amount of microbial product usage in the five swine farms**

| | Feed additive farms | | | Environmental improvement |
|---|---|---|---|---|
| | Product name | Product labeling application rate (%) | Actual application rate (%) | Product name |
| KR | F | 0.20 | 0.38 | I |
| DH | H | 0.20 | 0.40 | K |
| DB | H | 0.20 | 0.40 | J |
| DS | G | 0.10 | 0.17 | J |
| KL | G | 0.10 | 0.17 | K |

Odor compounds were analyzed using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS; VOICE 200 ultra, Syft Technologies, New Zealand), targeting 23 specific odorants. Each sampling point was measured three times (at the beginning, middle, and end), and the average value was used.

The limit of detection (LOD) was defined as three times the standard deviation of blank values using high-purity nitrogen (Rigas 99.999%, Daejeon, Korea). Air was drawn into the SIFT-MS at 25 sc cm, with measurements taken every 4 seconds over 5-minute period. Average values from minutes 4 to 5, when concentrations stabilized, were used. The Odor contribution was calculated by dividing the concentration of each odorant by its odor detection threshold [18].

The livestock odor monitoring data before the microbial-based products was applied in the five of five swine farms (KR, DH, DB, DS, KL) were subjected to one-way analysis of variance (ANOVA) using statistical analysis system (SAS) software (9.4). The mean comparisons were conducted using the least significant difference (LSD) test at a significant level of $p<0.005$.

## Quantitative analysis of intestinal microorganisms and NGS analysis

Fecal samples were collected from 10 randomly selected fattening pigs per farm, both before and after microbial treatment, using the anal massage method. In order to prepare standard DNA for quantitative PCR (qPCR), plasmid DNA was obtained via TA cloning and transformation [19]. DNA concentration was measured at 260 and 280 nm using a spectrophotometer, and copy numbers were calculated through serial 10-fold dilutions from $10^{10}$ to $10^{2}$ copies [20].

Total DNA was extracted using the Qiamp Genomic DNA Purification Kit (Qiagen, USA). Each qPCR reaction mixture (20 μL) contained 50 ng template DNA, 10 μL 2 × Ampigene qPCR Green Mix Lo-ROX, 0.8 μL forward primer, and 0.8 μL reverse primer (Table 4). Reactions were performed using a CFX96 Real-Time PCR Detection System (Bio-Rad) [21]. Sequencing was carried out with the Illumina iSeq 100 system (Illumina, San Diego, CA, USA) in accordance with the manufacturer's guidelines.

**Table 4. Overview of primers employed in qPCR assays**

| Target | Primer information | Primer sequence 5′-3′ | References |
|---|---|---|---|
| *Bacteria** | Eub 338 F | ACT CCT ACG GGA GGC AGC AG | [22] |
| | Eub 518 R | ATT ACC GCG GCT GCT GG | |
| *Lactobacillus* | LBLMA1 F | CTC AAA ACT AAA CAA AGT TTC | [23] |
| | 16-1 R | CTT GTA CAC CGC CCG TCA | |
| *Bifidobacterium* | xfp F | ATC TTC GGA CCB GAY GAG AC | [24] |
| | xfp R | CGA TVA CGT GVA CGA AGG AC | |
| *Faecalibacterium prausnitzil* | FPR-2 F | GGA GGA AGA AGG TCT TCG G | [25] |
| | Fprau645 R | AAT TCC GCC TAC CTC TGC ACT | [24] |
| Enterobacteriaceae | ent-F | ATG GCT GTC GTC AGC TCG T | [26] |
| | ent-R | CCT ACT TCT TTT GCA ACC CAC TC | |

* The bacterial taxonomy follows that described in the latest edition of Bergey's manual of systematic bacteriology


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The resulting raw reads were processed using the Mothur standard operating procedure outlined in the MiSeq SOP [27].

### Intestinal microbial community analysis

Total microbial DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, USA) following the manufacturer's instructions. For bacterial community profiling, the V3–V4 hypervariable regions of the 16S rRNA gene were amplified using primers Bakt_341F and Bakt_805R, which included Illumina overhang adapters [28].

Each 25 μL PCR reaction contained 2.5 μL microbial DNA, 1 μM of each primer, and 12.5 μL 2 × KAPA HiFi HotStart Ready Mix. PCR cycling conditions were as follows; initial denaturation at 95°C for 3 minutes; 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes. PCR products were purified with Agencourt AM Pure XP beads (Beckman Coulter, USA) and stored at -20°C until sequencing [29].

### Calprotectin analysis

Three commercial calprotectin ELISA kits were used to quantify intestinal inflammation: MBS033848 (Mybiosource, USA), DAEF-012 (Creative Diagnostics, USA), and Calprest (Eurospital, Italy). Stool samples (~56 mg) were homogenized using the Easy Cal device and immersed in 2.8 mL of extraction buffer. The samples were vortexed for 60 seconds, mixed on a roller shaker for 20 minutes, and then centrifuged at 5,000 rpm for 10 minutes.

Phosphate-buffered saline (PBS) without calcium or magnesium was used for extraction, and all samples were diluted 1:250. Absorbance measurements were performed using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, Finland), following each kit's manufacturer protocol [30].

### Statistical analysis

The information was processed using Statistical Analysis System (SAS) version 9.4 from SAS Institute in 2022. Analysis of variance (ANOVA) was employed to assess the livestock odor monitoring and calprotectin concentrations data before and after the microbial-based products that had been applied in the five of five swine farms (KR, DH, DB, DS, KL). Mean comparisons were conducted through the least significant difference (LSD) test at a significance level of $p<0.05$.

## Result and Discussion

### Livestock odor monitoring in swine farms

In order to establish a baseline, the concentrations of odor-causing substances were measured at five pig farms prior to the administration of six commercially available microbial products (three feed additives and three environmental treatments) (Table 5). Post-treatment samples were collected every two months (at 2, 4, and 6 months) from the same locations and at consistent time intervals within the barn (start, middle, and end) (Table 6). According to the analysis by farm, it was commonly observed that after applying the microbial product, odor-causing substances decreased at two months, but showed an increase at four and six months. This is considered to be due to seasonal effects.

As shown in the Table 7, an analysis of the average values from five pig farms showed that, compared to the control group, ammonia was reduced by 19.99%, and dimethyl disulfide of the sulfur compound by 28.99%. Propionaldehyde among aldehydes, was reduced by 3.42%, butyraldehyde by 13.21%, and valeraldehyde by 8.71%. Volatile organic compounds such as methyl isobutyl ketone were reduced by 15.45%, and butyl acetate by 43.21%. Volatile fatty acids including propionic acid were reduced by 25.25%, butyric acid by 23.45%, and i-Valeric acid by 26.74%. Indole was reduced by 10.62%, and phenol by 20.32%. The average complex


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**Table 5. The odor concentration emitted form five swine housing facilities prior to application of the microbial products**

| Odorous compounds | | Odor concentration (mg/kg)±SD* | | | | | LSD | F-value | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | KR | DH | DB | DS | KL | | | |
| Nitrogen compound | Ammonia | 4.363±0.450 b | 8.273±0.620 a | 3.707±0.380 b | 9.953±0.710 a | 1.843±0.210 b | 3.177 | 0.011 | 0.0010 |
| | Trimethylamine | 0.031±0.003 c | 0.054±0.004 b | 0.028±0.002 c | 0.070±0.005 a | 0.012±0.001 d | 0.015 | 0.021 | 0.0001 |
| Sulfur compound | Hydrogen sulfide | 0.662±0.080 c | 2.600±0.230 b | 0.890±0.090 c | 3.830±0.310 a | 0.229±0.020 c | 1.159 | 0.017 | 0.0002 |
| | Methyl mercaptan | 0.077±0.007 a | 0.050±0.004 ab | 0.015±0.001 bc | 0.035±0.003 bc | 0.010±0.001 c | 0.039 | 0.005 | 0.0172 |
| | Dimethyl sulfide | 0.097±0.009 a | 0.016±0.002 b | 0.011±0.001 b | 0.014±0.002 b | 0.005±0.001 b | 0.015 | 0.062 | 0.0001 |
| | Dimethyl disulfide | 0.496±0.050 a | 0.201±0.020 b | 0.038±0.004 b | 0.214±0.030 b | 0.247±0.020 b | 0.243 | 0.005 | 0.0237 |
| Aldehydes compound | Acetaldehyde | 0.159±0.015 a | 0.082±0.010 b | 0.095±0.009 b | 0.094±0.009 b | 0.052±0.005 c | 0.027 | 0.020 | 0.0001 |
| | Propionaldehyde | 0.030±0.003 a | 0.021±0.002 b | 0.015±0.001 bc | 0.020±0.002 b | 0.013±0.001 c | 0.006 | 0.010 | 0.0014 |
| | Butyraldehyde | 0.042±0.004 a | 0.036±0.003 a | 0.024±0.002 bc | 0.036±0.003 ab | 0.021±0.002 c | 0.012 | 0.006 | 0.0129 |
| | Valeraldehyde | 0.022±0.002 ab | 0.023±0.002 a | 0.011±0.001 c | 0.016±0.001 bc | 0.013±0.001 c | 0.007 | 0.005 | 0.0136 |
| Volatile organic compound | Styrene | 0.006±0.001 ab | 0.006±0.001ab | 0.004±0.001 bc | 0.007±0.001 a | 0.003±0.001 c | 0.002 | 0.006 | 0.0122 |
| | Toluene | 0.010±0.001 a | 0.006±0.0001 ab | 0.008±0.001 ab | 0.008±0.001 ab | 0.005±0.001 b | 0.005 | 0.002 | 0.2726 |
| | Xylene | 0.005±0.001 a | 0.004±0.001 a | 0.003±0.001 a | 0.004±0.001 a | 0.003±0.001 a | 0.002 | 0.002 | 0.2586 |
| | Methyl ethyl ketone | 0.018±0.002 a | 0.018±0.002 a | 0.010±0.001 b | 0.014±0.001 ab | 0.010±0.001 b | 0.005 | 0.006 | 0.0122 |
| | Methyl isobutyl ketone | 0.005±0.001 ab | 0.004±0.001 ab | 0.003±0.001 c | 0.004±0.001 bc | 0.005±0.001 a | 0.001 | 0.005 | 0.0144 |
| | Butyl acetate | 0.014±0.001 a | 0.008±0.001 b | 0.005±0.001 b | 0.005±0.001 b | 0.006±0.001 b | 0.005 | 0.006 | 0.0089 |
| | Butyl alcohol | 0.060±0.005 a | 0.019±0.002 b | 0.020±0.002 b | 0.020±0.002 b | 0.017±0.002 b | 0.012 | 0.021 | 0.0010 |
| Volatile fatty acid | Propionic acid | 0.261±0.02 a | 0.163±0.015 b | 0.103±0.012 bc | 0.154±0.014 b | 0.074±0.007 c | 0.078 | 0.008 | 0.0030 |
| | Butyric acid | 0.115±0.010 a | 0.066±0.006 b | 0.040±0.004 bc | 0.066±0.006 b | 0.023±0.002 c | 0.031 | 0.013 | 0.0006 |
| | i-Valeric acid | 0.038±0.003 a | 0.021±0.002 b | 0.010±0.001 b | 0.014±0.001 b | 0.012±0.001 b | 0.013 | 0.008 | 0.0042 |
| Livestock odor | Indole | 0.002±0.001 ab | 0.002±0.001 ab | 0.001±0.001 bc | 0.002±0.001 a | 0.001±0.001 c | 0.0004 | 0.005 | 0.0217 |
| | p-Cresol | 0.003±0.001 b | 0.004±0.001 a | 0.001±0.001 b | 0.003±0.001 a | 0.001±0.001 b | 0.0003 | 0.013 | 0.0001 |
| | Phenol | 0.003±0.001 a | 0.003±0.001 a | 0.001±0.001 b | 0.003±0.001 a | 0.001±0.001 b | 0.0013 | 0.007 | 0.0051 |
| Particulate matter | $PM_{10}$ | 0.795±0.009 b | 1.171±0.028 a | 0..859±0.170 b | 0.321±0.037 c | 0.490±0.133 c | 0.220 | 0.023 | 0.0001 |
| | $PM_{2.5}$ | 5.107±0.009 a | 3.359±0.183 abc | 4.912±0.748 ab | 1.163±0.161 c | 3.189±0.686 bc | 0.189 | 0.006 | 0.0120 |
| Complex odor | Sensor-based complex odor | 0.045±0.004 c | 0.101±0.003 a | 0.029±0.011 c | 0.081±0.005 b | 0.028±0.011 c | 0.017 | 0.036 | 0.0001 |

Means followed by the same letter within a column are not significantly different according to the Least significant difference (LSD) test at a significance level of $p<0.05$
* SD: Standard deviation (n=3)

odor measured by sensors was reduced by 34.39%, particulate matter ($PM_{10}$) by 77.31%, and fine particulate matter ($PM_{2.5}$) by 69.40% (Table 7).

These results suggest that the combined use of microbial feed additives and environmental products led to a reduction in ammonia and a diversification of odor compounds to include hydrogen sulfide and others. While major livestock odorants such as ammonia, volatile fatty acids, indole and phenol were significantly reduced, sulfur-containing compounds showed relatively limited mitigation.

## Changes in the microbiome of pig feces following microbial application on swine farms

The intestinal microbiome of pigs was analyzed using fecal samples collected before and after the administration of microbial products across five swine farms. Results from the alpha-diversity analysis confirmed improvements in both species richness and diversity at all five farms. Alpha-diversity is a comprehensive metric that reflects both species richness and evenness within a specific environment or sample. The Chao1 index estimates species richness based on the number of observed species and the


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**Table 6. The odor concentration emitted from five swine housing facilities following the application of microbial products**

| Odorous compounds | | Change in odor reduction rate of all farms (μg/g)±SD* | | | |
|---|---|---|---|---|---|
| | | Free-microbial treatment** | Two months post-microbial treatment | Four months post-microbial treatment | Six months post-microbial treatment |
| Nitrogen compound | Ammonia | 5,628±110 | 4,042±80 | 5,215±100 | 4,252±85 |
| | Trimethyamine | 39.1±1.90 | 26.5±1.30 | 70.8±3.50 | 93.6±4.70 |
| Sulfur compound | Hydrogen sulfide | 1642±50 | 1561±45 | 3130±95 | 3825±115 |
| | Methyl mercaptan | 37.6±1.80 | 28.9±1.40 | 107.4±5.40 | 198±10 |
| | Dimethyl sulfide | 28.5±1.20 | 11.4±0.70 | 89.5±4.50 | 58.2±2.80 |
| | Dimethyl disulfide | 239.3±8.50 | 320±12 | 150.4±5.70 | 39.3±1.50 |
| Aldehydes compound | Acetaldehyde | 96.4±4.00 | 59.7±2.50 | 151.4±6.00 | 203.1±8.00 |
| | Propionaldehyde | 19.69±0.98 | 14.66±0.73 | 25.90±1.30 | 16.50±0.83 |
| | Butyraldehyde | 31.89±1.59 | 18.65±0.93 | 40.26±2.01 | 24.13±1.21 |
| | Valeraldehyde | 16.79±0.84 | 9.38±0.47 | 21.21±1.06 | 15.41±0.77 |
| Volatile organic compound | Styrene | 4.81±0.24 | 4.03±0.20 | 6.79±0.34 | 4.12±0.21 |
| | Toluene | 7.44±0.37 | 21.03±1.05 | 16.33±0.82 | 44.79±2.24 |
| | Xylene | 3.75±0.19 | 3.41±0.17 | 5.94±0.30 | 13.05±0.65 |
| | Methyl ethyl ketone | 13.67±0.68 | 10.70±0.54 | 16.80±0.84 | 21.64±1.08 |
| | Methyl isobutyl ketone | 4.43±0.22 | 3.25±0.16 | 3.83±0.19 | 4.17±0.21 |
| | Butyl acetate | 7.71±0.39 | 3.15±0.16 | 4.93±0.25 | 5.06±0.25 |
| | Butyl alcohol | 27.09±1.35 | 13.33±0.67 | 35.50±0.80 | 71.38±3.57 |
| Volatile fatty acid | Propionic acid | 151.05±7.55 | 80.81±4.04 | 178.83±8.94 | 79.08±3.95 |
| | Butyric acid | 61.81±3.09 | 27.76±1.39 | 74.77±3.74 | 39.43±1.97 |
| | i-Valeric acid | 18.78±0.94 | 10.73±0.54 | 20.39±1.02 | 10.16±0.51 |
| Livestock odor | Indole | 1.44±0.07 | 0.91±0.05 | 1.73±0.09 | 1.23±0.06 |
| | Phenol | 2.18±0.11 | 2.08±0.10 | 1.99±0.10 | 1.14±0.06 |
| | p-Cresol | 0.79±0.04 | 0.46±0.02 | 0.96±0.05 | 1.05±0.05 |

* SD: Standard deviation (n=3)
** Free-microbial treatment: No microbial application

presence of rare species, while the Shannon index accounts for both species abundance and relative evenness to evaluate overall diversity (Table 8).

As shown in Table 8, on average, the Chao1 index increased from 463.31 to 643.09, representing a 38.81% increase, while the Shannon-Weaver index rose from 6.51 to 7.22, indicating a 10.91% improvement. These results suggest that both the richness and diversity of microbial species increased following microbial supplementation. Additionally, the Good's coverage value was 99.84%, indicating that most species present in the samples were successfully analyzed.

These findings suggest that supplementation with microbial products as feed additives contributed to a more diverse and abundant gut microbial community in pigs, thereby helping to maintain microbial balance and potentially enhancing digestive function.

The relative abundance of microbial taxa was compared based on microbiological taxonomic levels to analyze the intestinal microbiota. As shown in the Table 9, at the phylum level, noticeable shifts were observed in the gut microbial community structure following microbial supplementation, particularly in the dominant phyla *Firmicutes* and *Bacteroidetes*. Prior to microbial treatment, *Firmicutes* accounted for the highest proportion at 77%, while *Bacteroidetes* represented 17% (Fig. 1). After microbial supplementation, the proportion of *Firmicutes* decreased to 56%, whereas *Bacteroidetes* increased to 35% (Fig. 1). This shift indicates a reduction in the *Firmicutes*/*Bacteroidetes* (F/B) ratio, suggesting an improved microbial balance in the pig gut.


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**Table 7. The extent of odor reduction at five swine housing facilities subsequent to microbial product application**

| Odorous compounds | | Change in odor reduction rate across the swine farms (%)* | | | |
|---|---|---|---|---|---|
| | | Two months after microbial treatment | Four months after microbial treatment | Six months after microbial treatment | Overall average |
| Nitrogen compound | Ammonia | 28.18 | 7.33 | 24.45 | 19.99 |
| | Trimethyamine | 32.41 | -80.87** | -139.21 | -62.56 |
| Sulfur compound | Hydrogen sulfide | 4.94 | -90.59 | -132.95 | -72.87 |
| | Methyl mercaptan | 22.75 | -187.55 | -430.05 | -198.29 |
| | Dimethyl sulfide | 59.95 | -213.79 | -104.12 | -85.99 |
| | Dimethyl disulfide | -33.74 | 37.13 | 83.59 | 28.99 |
| Aldehydes compound | Acetaldehyde | 38.04 | -56.99 | -110.62 | -43.19 |
| | Propionaldehyde | 25.56 | -31.52 | 16.22 | 3.42 |
| | Butyraldehyde | 41.52 | -26.23 | 24.33 | 13.21 |
| | Valeraldehyde | 44.16 | -26.28 | 8.26 | 8.71 |
| Volatile organic compound | Styrene | 16.23 | -41.00 | 14.32 | -3.48 |
| | Toluene | -182.77 | -119.56 | -502.28 | -268.20 |
| | Xylene | 9.23 | -58.23 | -247.58 | -98.86 |
| | Methyl ethyl ketone | 21.76 | -22.90 | -58.30 | -19.81 |
| | Methyl isobutyl ketone | 26.77 | 13.67 | 5.91 | 15.45 |
| | Butyl acetate | 59.18 | 36.01 | 34.43 | 43.21 |
| | Butyl alcohol | 50.80 | -31.06 | -163.52 | -47.93 |
| Volatile fatty acid | Propionic acid | 46.50 | -18.39 | 47.65 | 25.25 |
| | Butyric acid | 55.09 | -20.96 | 36.21 | 23.45 |
| | i-Valeric acid | 42.85 | -8.53 | 45.90 | 26.74 |
| Indole | Indole | 37.01 | -20.15 | 14.99 | 10.62 |
| Phenols | Phenol | 4.40 | 8.77 | 47.79 | 20.32 |
| | p-Cresol | 41.39 | -22.04 | -33.17 | -4.61 |

* Percentage change in odor reduction rate across all swine farms
** This (-) means that a specific odor concentration in a pig house increased after the use of the microbial product compared to before its use

**Table 8. Alpha-diversity indices of the gut microbiota in the pig feces from five swine farms**

| Farms | Application of microbial products[a)] | Chao index[b)] | Shannon-weaver index[c)] | Simpson's index[d)] | Good's coverage[e)] |
|---|---|---|---|---|---|
| KR | Free-microbial treatment | 515.74 | 6.98 | 0.9660 | 0.9991 |
| | Post-microbial treatment | 565.64 | 7.50 | 0.9840 | 0.9990 |
| DH | Free-microbial treatment | 438.90 | 6.36 | 0.9502 | 0.9992 |
| | Post-microbial treatment | 699.67 | 7.27 | 0.9790 | 0.9980 |
| DB | Free-microbial treatment | 485.07 | 6.94 | 0.9762 | 0.9992 |
| | Post-microbial treatment | 482.69 | 7.36 | 0.9852 | 0.9991 |
| DS | Free-microbial treatment | 492.21 | 6.70 | 0.9493 | 0.9990 |
| | Post-microbial treatment | 670.70 | 7.02 | 0.9687 | 0.9982 |
| KL | Free-microbial treatment | 384.64 | 5.57 | 0.8938 | 0.9993 |
| | Post-microbial treatment | 796.76 | 6.96 | 0.9677 | 0.9976 |
| Total average | Free-microbial treatment | 463.31 | 6.51 | 0.9471 | 0.9992 |
| | Post-microbial treatment | 643.09 | 7.22 | 0.9769 | 0.9984 |

[a)] Three microbial-based formulation as feed additives and three microbial products as environmental treatments, [b)] Species richness index, [c)] Species diversity index, [d)] Species diversity index, [e)] Sequencing index


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**Table 9. Changes in metagenomic profiles at the phylum level in pig feces before and after microbial application across five swine farms**

| Kingdom | Phylum | Application of microbial products[a] | | p-Value | SEM[b] |
|---|---|---|---|---|---|
| | | Free-treatment | Post-treatment | | |
| Bacteria | Firmicutes | 77.24 | 56.44 | 0.0054 | 3.7468 |
| Bacteria | Bacteroidetes | 17.23 | 34.51 | 0.0296 | 4.2514 |
| Bacteria | Spirochaetes | 1.46 | 4.70 | 0.1309 | 1.0913 |
| Bacteria | Planctomycetes | 0.89 | 0.67 | 0.6156 | 0.2875 |
| Bacteria | Kiritimatiellaeota | 0.57 | 0.77 | 0.4107 | 0.1654 |
| Bacteria | Patescibacteria | 0.43 | 0.22 | 0.1110 | 0.0823 |
| Bacteria | Cyanobacteria | 0.26 | 0.45 | 0.2365 | 0.0946 |
| Archaea | Euryarchaeota | 0.35 | 0.24 | 0.4715 | 0.0996 |
| Bacteria | Proteobacteria | 0.42 | 0.60 | 0.4049 | 0.1377 |
| Bacteria | Actinobacteria | 0.44 | 0.34 | 0.6891 | 0.1554 |
| Bacteria | Tenericutes | 0.60 | 0.89 | 0.3488 | 0.1788 |

[a] Three microbial-based formulation as feed additives and three microbial products as environmental treatments
[b] Standard error of mean (n=3)

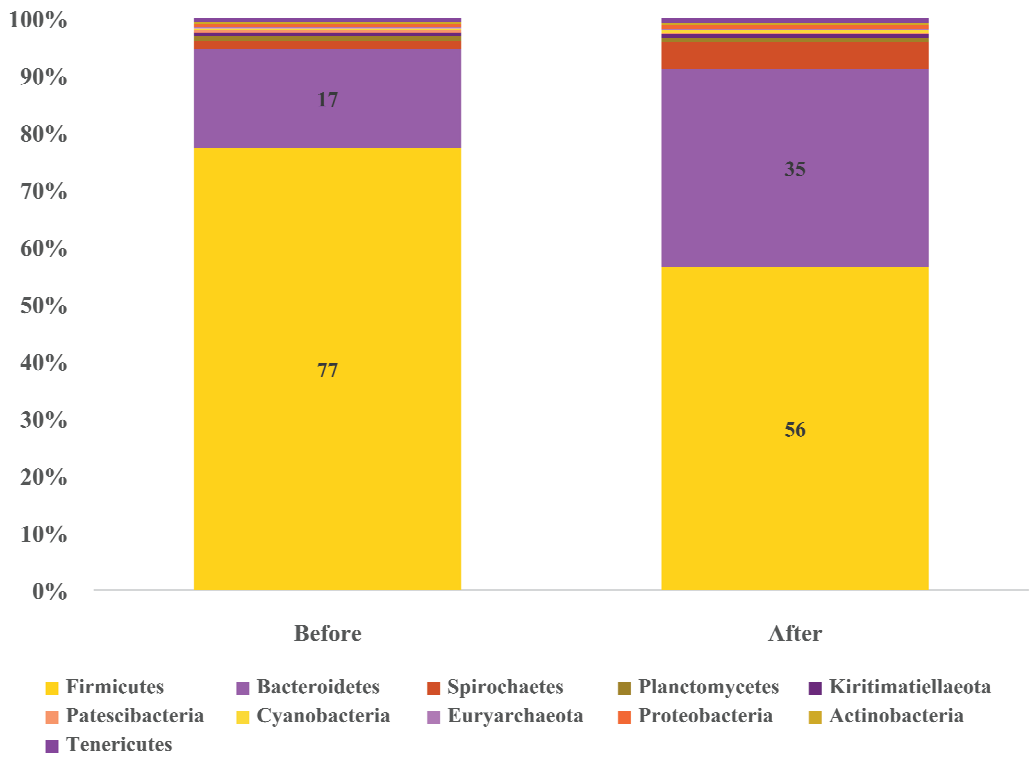


**Fig. 1 Bar plot showing differences identified through cluster analysis of gut microbiota at the phylum level in pig feces from five major swine farms (cut-off < 0.1%).**

Numerous studies have demonstrated a strong association between gut microbiota and immune function, intestinal development, and productivity in pigs. The gut microbiota also plays a critical role in host nutrient metabolism, including the metabolism of carbohydrates, amino acids, and lipids [31]. *Firmicutes* and *Bacteroidetes* are the two predominant phyla, comprising approximately 90% of the mammalian gut microbial community [32,33]. *Firmicutes* are involved in energy absorption and fat storage, whereas *Bacteroidetes* are known to promote gut health by degrading complex carbohydrates and producing short-chain fatty


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acids (SCFAs) [34]. Therefore, the observed compositional changes may reflect an enhancement in digestive function resulting from microbial supplementation.

As shown in the Fig. 2, *Lactobacillus*, a major genus within *Firmicutes* known for its beneficial effects such as lactic acid production, pH regulation, pathogen inhibition, and immune modulation, represented the highest initial relative abundance at the genus level [35]. Likely, *Lactobacillus* as a probiotic, it is known to reside in the human gut and play various roles such as improving the gut microbiota, regulating the immune system, lowering cholesterol, inhibiting the growth of pathogenic bacteria, and alleviating lactose intolerance [36]. However, its proportion decreased from 21% to 12% following treatment, which may be indicative of a restructuring of the microbial community, possibly due to environmental shifts in the gut or competitive interactions among microbial species.

The genus Terrisporobacter, belonging to the Peptostreptococcaceae family and associated with hyperammonemia production, showed an increase post-treatment, but this change was not statistically significant. On the other hand, a number of fiber-degrading

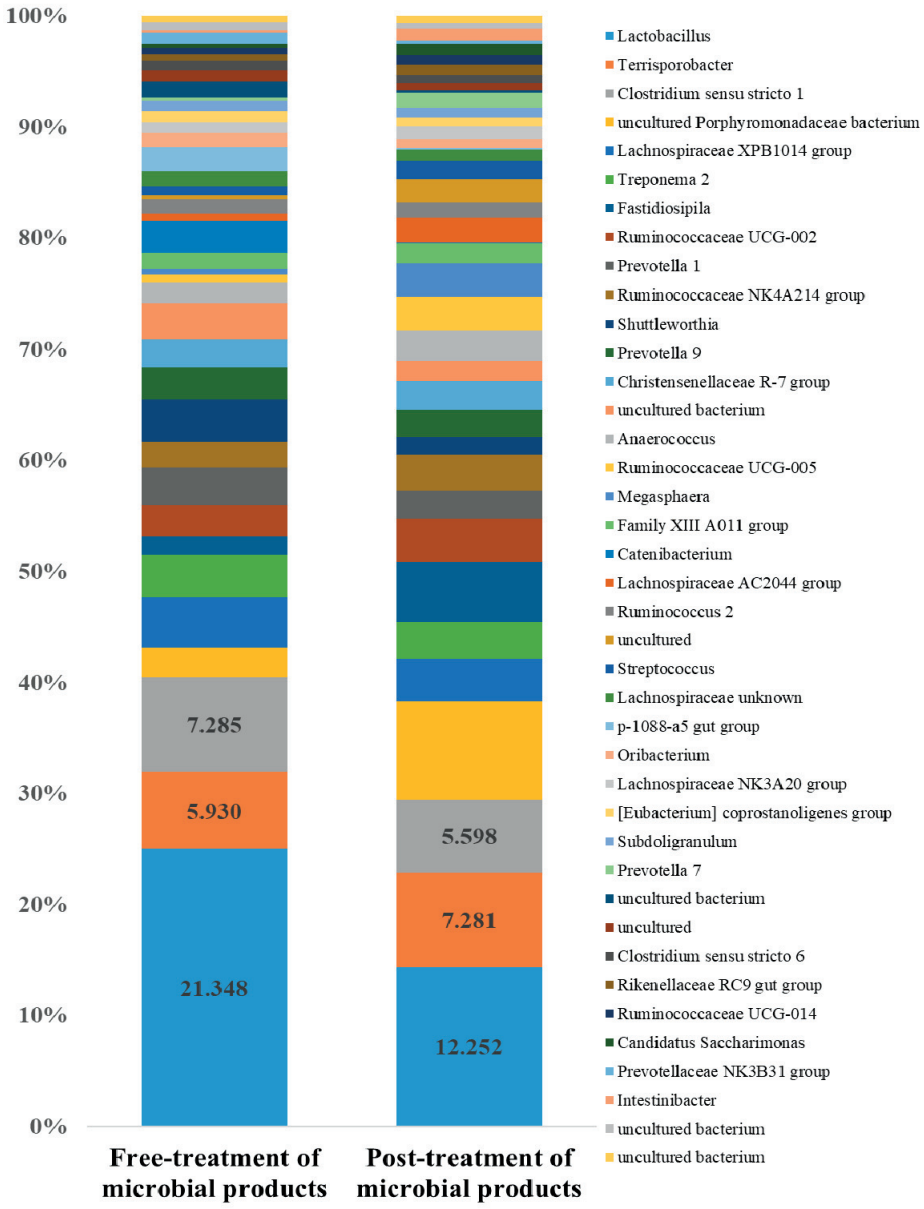


**Fig. 2. Bar plot showing cluster analysis of genus-level gut microbiota differences in pig feces from five major swine farms (cut-off < 0.5%).**


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and SCFA-producing genera exhibited notable growth, with the uncultured *Porphyromonadaceae* bacterium rising from 2.263% to 7.541%, Shuttleworthia from 1.222% to 3.780%, *Ruminococcaceae* UCG-005 from 0.650% to 2.957%, and Megasphaera from 0.400% to 2.559%. In addition, a relative increase in rare taxa such as *Anaerococcus*, *Ruminococcus* 2, and *Catenibacterium* suggests a reorganization of the overall community structure, contributing to enhanced diversity and balance (Fig. 2).

**Table 10. Composition of gut microbiota in pig feces collected from five swine farms (KR, DH, DB, DS, KL)**

| | Genus Family | Free-microbial treatment | Post-microbial treatment |
|---|---|---|---|
| Lactobacillaceae | Lactobacillus | 21.348 | 12.252 |
| Peptostreptococcaceae | Terrisporobacter | 5.930 | 7.281 |
| Clostridiaceae 1 | Clostridium sensu stricto 1 | 7.285 | 5.598 |
| Muribaculaceae | Uncultured porphyromonadaceae bacterium | 2.263 | 7.541 |
| Lachnospiraceae | Lachnospiraceae XPB1014 group | 3.919 | 3.307 |
| Spirochaetaceae | Treponema 2 | 3.265 | 2.831 |
| Ruminococcaceae | Fastidiosipila | 1.427 | 4.624 |
| Ruminococcaceae | Ruminococcaceae UCG-002 | 2.378 | 3.301 |
| Prevotellaceae | Prevotella 1 | 2.913 | 2.165 |
| Ruminococcaceae | Ruminococcaceae NK4A214 group | 1.983 | 2.783 |
| Lachnospiraceae | Shuttleworthia | 3.272 | 1.370 |
| Prevotellaceae | Prevotella 9 | 2.447 | 2.079 |
| Christensenellaceae | Christensenellaceae R-7 group | 2.188 | 2.237 |
| p-2534-18B5 gut group | Uncultured bacterium | 2.772 | 1.490 |
| Family XI | Anaerococcus | 1.500 | 2.335 |
| Ruminococcaceae | Ruminococcaceae UCG-005 | 0.650 | 2.597 |
| Veillonellaceae | Megasphaera | 0.400 | 2.559 |
| Family XIII | Family XIII A011 group | 1.254 | 1.555 |
| Erysipelotrichaceae | Catenibacterium | 2.454 | 0.054 |
| Lachnospiraceae | Lachnospiraceae AC2044 group | 0.595 | 1.901 |
| Ruminococcaceae | Ruminococcus 2 | 1.090 | 1.173 |
| Erysipelotrichaceae | Uncultured | 0.289 | 1.801 |
| Streptococcaceae | Streptococcus | 0.688 | 1.402 |
| Lachnospiraceae | Lachnospiraceae unknown | 1.155 | 0.877 |
| Pirellulaceae | p-1088-a5 gut group | 1.868 | 0.118 |
| Lachnospiraceae | Oribacterium | 1.106 | 0.683 |
| Lachnospiraceae | Lachnospiraceae NK3A20 group | 0.786 | 0.985 |
| Ruminococcaceae | Eubacterium coprostanoligenes group | 0.886 | 0.670 |
| Ruminococcaceae | Subdoligranulum | 0.780 | 0.715 |
| Prevotellaceae | Prevotella 7 | 0.283 | 1.156 |
| Uncultured bacterium | Uncultured bacterium | 1.224 | 0.209 |
| Prevotellaceae | Uncultured | 0.872 | 0.529 |
| Clostridiaceae 1 | Clostridium sensu stricto 6 | 0.715 | 0.620 |
| Rikenellaceae | Rikenellaceae RC9 gut group | 0.499 | 0.805 |
| Ruminococcaceae | Ruminococcaceae UCG-014 | 0.511 | 0.762 |
| Saccharimonadaceae | Candidatus saccharimonas | 0.281 | 0.886 |
| Prevotellaceae | Prevotellaceae NK3B31 group | 0.901 | 0.241 |
| Peptostreptococcaceae | Intestinibacter | 0.184 | 0.905 |
| Uncultured | Uncultured bacterium | 0.596 | 0.430 |
| Muribaculaceae | Uncultured bacterium | 0.480 | 0.532 |


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As shown in Table 10, while the notable decline in *Lactobacillus* may raise concerns about potential reductions in immunological stability or impaired pH regulation in the pig gut, the simultaneous increase in SCFA-producing bacteria—such as *Ruminococcaceae* and *Porphyromonadaceae*—suggests a shift toward a more metabolically efficient microbial community, enhancing fiber degradation and energy utilization. Furthermore, the increased abundance of *Bacteroidetes* and other fiber-degrading genera is expected to support energy metabolism and contribute to a healthier intestinal mucosal environment [34].

## Composition of gut microbiota in pig feces collected from five swine farms

The application of microbial products, used as feed additives and for environmental improvement, did not cause significant changes in the total bacterial count, nor were there significant changes in the levels of beneficial gut bacteria such as Bifidobacterium and Faecalibacterium (Table 11).

However, as shown in Table 11, *Lactobacillus* spp. showed a statistically significant decrease from 8.432 (before supplementation) to 8.163 (after supplementation) ($p<0.05$). This trend was consistent with the results of the boxplot analysis ($F=10.41$, $p=0.0121$). Although the reduction in *Lactobacillus* may be viewed as a potentially negative outcome, it is more appropriately interpreted as part of a complex regulatory shift within the overall gut microbiota balance.

SCFA-producing genera, including *Faecalibacterium prausnitzii*, *Ruminococcaceae*, and *Porphyromonadaceae*, showed significant increases as indicated in Table 11. In particular, *F. prausnitzii* exhibited a marked increasing trend in specific farms (KR, DS and KL), with farm KR showing a substantial rise from 5.638 to 6.765 following feed supplementation (Table 11).

These results suggest a functional shift in the gut microbial community from dominance by pH-regulating *Lactobacillus* spp. to a metabolically oriented community favoring fiber degradation and SCFA production. Such a transition is considered a favorable outcome, as it may enhance energy absorption efficiency and contribute to improved stability of the intestinal environment.

## Changes in intestinal inflammation markers in pig feces collected from swine farms

Calprotectin is a calcium- and zinc-binding protein primarily derived from neutrophils and is widely used as a non-invasive biomarker to assess the degree of intestinal mucosal inflammation by measuring its concentration in feces. In this study, calprotectin concentrations (μg/g) were measured before and after microbial supplementation in five swine farms to evaluate the immunological impact of microbial products on gut health.

Table 12 presents the calprotectin concentrations (μg/g) before and after microbial treatment across five swine farms. On

**Table 11. Quantitative assessment of intestinal microbiota across individual swine farms**

| Odorous compounds | | Total bacteria | Bifido bacteria | *Lactobacillus* spp. | *Faecalibacterium prausnitzii* | Entero bacteriaceae |
|---|---|---|---|---|---|---|
| KR | Free-microbial treatment | 9.178 | 3.580 | 8.374 | 5.638 | 4.307 |
| | Post-microbial treatment | 10.184 | 3.096 | 8.236 | 6.765 | 4.222 |
| DH | Free-microbial treatment | 8.406 | 3.154 | 8.241 | 5.818 | 3.638 |
| | Post-microbial treatment | 9.215 | 3.210 | 7.942 | 5.443 | 3.141 |
| DB | Free-treatment | 9.014 | 3.767 | 8.362 | 6.088 | 4.052 |
| | Post-microbial treatment | 8.334 | 2.729 | 8.221 | 4.916 | 3.717 |
| DS | Free-microbial treatment | 9.029 | 4.742 | 8.645 | 5.836 | 3.387 |
| | Post-microbial treatment | 8.367 | 4.362 | 8.420 | 5.196 | 3.371 |
| KL | Free-microbial treatment | 8.646 | 4.177 | 8.538 | 5.345 | 3.514 |
| | Post-microbial treatment | 8.598 | 3.892 | 7.997 | 5.122 | 4.194 |
| Total mean | Free-microbial treatment | 8.855 | 3.884 | 8.432 a | 5.745 | 3.780 |
| | Post-microbial treatment | 8.940 | 3.458 | 8.163 b | 5.489 | 3.729 |

[a-b] Means in each row with different superscripts are significantly different ($p<0.05$)


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**Table 12. Calprotectin concentrations in response to the application of microbial products**

| Swine farms | Calprotectin (μg/g) | | SEM* | p-value |
|---|---|---|---|---|
| | Free-microbial treatment | Post-microbial treatment | | |
| KR | 7.12b | 8.16a | 0.67 | 0.03 |
| DH | 7.53 | 7.48 | 1.07 | 0.93 |
| DB | 6.45 | 6.28 | 1.88 | 0.82 |
| DS | 7.91 | 8.14 | 1.31 | 0.71 |
| KL | 8.44a | 6.79b | 1.74 | 0.04 |
| Mean | 7.49 | 7.37 | 1.33 | 0.51 |

* Standard error of mean (tree replicates, n=3)
[a-b] Means in each row with different superscripts are significantly different ($p<0.05$)

average, fecal calprotectin levels slightly decreased from 7.49 μg/g before treatment to 7.37 μg/g after treatment. However, this difference was not statistically significant (p=0.51), indicating no substantial change at the population level. In contrast, farm-specific analysis revealed significant changes in farms KR and KL. As shown in Table 12, in the pig farms, calprotectin levels increased significantly from 7.12 μg/g to 8.16 μg/g after microbial application (p=0.0346), potentially indicating a transient elevation in immune response or gut mucosal stimulation. Conversely, Farm KL exhibited a significant decrease from 8.44 μg/g to 6.79 μg/g (p=0.0373), suggesting a reduction in intestinal inflammation and enhanced mucosal stability. These contrasting responses imply that the immunomodulatory effects of microbial supplementation may vary depending on the initial gut microbiota composition and baseline immune state of each swine farm.

Fecal calprotectin levels above 28 μg/g are strongly linked to hemorrhagic diarrhea, whereas levels near 26 μg/g are commonly found in cases of mucoid diarrhea. Values below 25 μg/g are regarded as normal [37]. As a noninvasive indicator of intestinal inflammation, fecal calprotectin is extensively applied in gastrointestinal diagnostics for humans [38,39]. The average intestinal inflammation index across all five swine farms remained within the normal range, further supporting the overall intestinal stability in the animals throughout the study.

This study comprehensively evaluated the effects of commercially available feed-additive and environmental microbial formulations on odor reduction, gut microbial community structure and immunological markers in swine farms.

Following microbial application, major odorants commonly associated with livestock operations-namely ammonia (19.99%), dimethyl disulfide (28.99%), volatile fatty acids (23~26%), and phenolic compounds (20.32%) were substantially reduced. Notably, complex odor levels decreased by 34.39%, and significant improvements were also observed in particulate matter levels, with $PM_{10}$ (particles with a diameter of 10 μm or less) reduced by 77.31% and $PM_{2.5}$ (particles with a diameter of 2.5 μm or less) by 69.40%. However, the effect on certain sulfur compounds, such as hydrogen sulfide, remained limited.

Although a significant reduction in beneficial bacteria such as *Lactobacillus* was observed, the relative abundance of SCFA-producing genera, including *Ruminococcaceae*, *Porphyromonadaceae*, and *F. prausnitzii*, increased. This indicates a favorable shift in the gut microbial community toward improved energy metabolism and fiber degradation capacity.

Calprotectin analysis, a biomarker of intestinal inflammation, revealed a significant decrease in some swine farms after microbial supplementation, suggesting a potential alleviation of intestinal inflammatory responses. The observed increase in SCFA-producing bacteria further supports the likelihood of positive physiological effects, particularly with regard to mucosal stability and immune modulation.

Collectively, these findings demonstrate that the combined application of microbial products can effectively contribute to both gut environment improvement and odor reduction in swine farms. Future efforts should focus on developing tailored microbial formulations that simultaneously maintain the stability of beneficial gut microbes and enhance the metabolic activity of functional strains, alongside farm-specific optimization strategies.


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## Conclusion

This study comprehensively evaluated the effects of applying commercially available feed additive-type and environment-improving microbial preparations to pig farms on odor reduction, gut microbial community analysis and immune response indicators. After microbial application, there was an overall reduction in major livestock odors such as ammonia (19.99%), dimethyl sulfide (28.99%), volatile fatty acids (23~26%), and phenolic compounds (20.32%). Notably, there were also significant improvements in complex odors (34.39%), particulate matter ($PM_{10}$, 77.31%), and fine particulate matter ($PM_{2.5}$, 69.40%). However, the reduction effects were limited for some sulfur compounds such as hydrogen sulfide. Although a significant decrease in beneficial bacteria such as *Lactobacillus* was observed, the relative abundance of strains related to short-chain fatty acid (SCFA) production, such as *Ruminococcaceae*, *Porphyromonadaceae*, and *F. prausnitzii*, increased. This suggests that the gut microbial community is shifting toward improved energy metabolism and fiber degradation efficiency. Analysis of calprotectin, an immune marker, showed a significant decrease in some farms following microbial application, indicating a potential alleviation of intestinal inflammation. In particular, the increase in SCFA-producing bacteria is expected to have positive physiological effects by enhancing gut mucosal stability and immune regulation.

These results demonstrate that the combined application of microbial preparations can effectively improve the intestinal environment and reduce odors in pig farms. Moving forward, it will be important to develop customized microbial formulations that not only maintain the stability of beneficial gut bacteria but also enhance the metabolic activity of functional strains, alongside implementing optimized strategies tailored to specific pig farm environments.

In conclusion, continuous application of a combination of microbial products (feed additives and environmental treatments) across five swine farms demonstrated a reduction in key livestock odorants including ammonia, volatile fatty acids, indole, and phenol. However, the reduction in sulfur-based compounds was limited. Microbial community analysis revealed no significant change in total bacterial counts but did indicate improvements in species richness and diversity. The intestinal inflammation index remained within the normal physiological range throughout the study.

The findings indicate that the regular application of microbial formulations, when paired with upgraded livestock facility infrastructure, can lead to significant improvements in odor control and the overall environmental conditions of pig farms. This integrated approach may help minimize community complaints and support the long-term sustainability of swine production systems.

**Data Availability:** All data are available in the main text or in the Supplementary Information.

**Author Contributions:** J.S.Y., C.Y.J., C.D. and C.I.K. conceived and designed the research; J.S.Y., C.Y.J., C.W.Y., H.S.E. and C.I.K. collected the data and performed the analysis; J.S.Y., C.Y.J., C.D. and C.I.K. wrote the first manuscript; C.W.Y., J.S.Y., C,D., H.S.E. and C.I.K. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Additional Information:**

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.5338/KJEA.2025.44.28

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