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## Abstract

This study was conducted to supplement single and complex probiotics to investigate the effect on growing-finishing pigs and compost. In experiment 1, the 64 crossbred [(Landrace × Yorkshire) × Duroc] pigs with an initial body weight of  $18.75 \pm 0.33$  kg and a birth of 63 days were assigned to a completely randomized four treatment groups based on the initial body weight (4 pigs in a pen with 4 replicate pens for each treatment). For 13 weeks, the dietary treatments were provided: 1) Control (CON; basal diet), 2) T1 (CON + 0.2% *Bacillus subtilis*), 3) T2 (CON + 0.2% *Saccharomyces cerevisiae*), 4) T3 (CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*). In experiment 2, the pig manure was obtained from Chungbuk National University (Cheongju, Korea) swine farm. For 12 weeks, the supplementary treatments were provided: 1) CON, non-additive compost; 2) T1, spray *Bacillus subtilis* 10g per 3.306 m<sup>2</sup>; 3) T2, spray *Bacillus subtilis* 40 g per 3.306 m<sup>2</sup>; 4) T3, spray *Saccharomyces cerevisiae* 10g per 3.306 m<sup>2</sup>; 5) T4: spray *Saccharomyces cerevisiae* 40 g per 3.306 m<sup>2</sup>; 6) T5, spray *Bacillus subtilis* 5 g + *Saccharomyces cerevisiae* 5 g per 3.306 m<sup>2</sup>; 7) T6, spray *Saccharomyces subtilis* 20 g + *S. cerevisiae* 20 g per 3.306 m<sup>2</sup> and there were 6 replicates each treatment. In experiment 1, During the overall experimental period, T3 showed significantly improved ( $p < 0.05$ ) feed conversion ratio and average daily gain compared to other groups. In average maturity score, T3 showed significantly higher ( $p < 0.05$ ) than other groups. Supplementing complex probiotics group improved ( $p < 0.05$ ) H<sub>2</sub>S emissions and fecal microflora compared to the non-supplementing group. In experiment 2, additive probiotics groups had no effect ( $p > 0.05$ ) on moisture content than the non-additive group at 9 and 12 weeks. T6 showed a significantly improved ( $p < 0.05$ ) average maturity score at all periods and ammonia emissions at 1 week and 4 weeks compared to other groups. In summary, supplementation complex probiotics induced positive effects on both pigs and compost.

**Keywords (3 to 6):** Compost Maturity, Finishing pigs, Growing pigs, Probiotics

## **Introduction**

Various issues have emerged associated with improving livestock manure management (LM) due to the increasing demand for animal-sourced foods [1]. LM generally contains heavy metals (arsenic, copper, and zinc) that can be hazardous to humans and the environment. It also generates various harmful compounds (e.g., volatile fatty acids, alcohols, amines, hydrogen sulfide (H<sub>2</sub>S), and ammonia (NH<sub>3</sub>)) that can cause an unpleasant odor [2]. In particular, NH<sub>3</sub> is produced at a higher concentration than other odorous gases. It is involved in air pollution as a precursor to secondary ultrafine dust [3].

Composting is the primary method of LM treatment applied on a farm [4]. However, the process of composting can have various adverse effects such as nitrogen loss and increase in greenhouse gas due to rapid degradation of nitrogenous organic matter and the presence of anaerobic space in feedstocks [5]. Previous studies have shown that nitrogen losses during composting can result in the production of NH<sub>3</sub>, nitrous oxide, and leachate, which can reduce the agricultural value of composted products, and contribute to increase in greenhouse gas emissions and unpleasant odor [6].

Probiotics have been shown to improve the environment of digestive organ microorganisms by reducing harmful microorganisms in the intestine, resulting in improved nitrogen utilization and reduced nitrogen excretion in pigs [7]. Li and Kim [8] have reported that supplementation of *Saccharomyces cerevisiae* (*S. cerevisiae*) can improve nitrogen digestibility in growing pigs. Other studies have suggested that supplementation including *Bacillus subtilis* (*B. subtilis*) can attenuate NH<sub>3</sub> release by suppressing the urease-producing microorganisms in the gastrointestinal lumen by producing the protein-digesting enzyme such as subtilisin in pigs [8]. Gong et al. [9], have reported that *S. cerevisiae* can reduce the *Methanobrevibacter* spp. known to produce methanogen and methane. According to results of previous studies, supplementation containing *Bacillus* spp. might reduce NH<sub>3</sub> emissions in pigs [8,10].

However, there are few studies on adding complex probiotics to swine diets and manure. Therefore, the objective of this study was to determine effects of single and complex probiotics in growing-finishing pigs and compost on growth performance, odorous gas emissions, blood profiles, and compost maturity.

## **Materials and Methods**

### **Ethics approval and consent to participate**

All experimental procedures received prior approval from the Animal Ethics Committee of Chungbuk National University (CBNUA-1740-22-02).

### **Experiment 1**

#### **Experimental Design, Animals, and Housing**

A total of 64 crossbred growing pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight (BW) of  $18.75 \pm 0.33$  kg and a birth of 63 days were used for 13 weeks in this study. All pigs were assigned to a completely randomized four treatment groups based on the initial BW. There were 4 pigs in a pen with 4 replicate pens for each treatment. Dietary treatments were as follows: 1) Control (CON; basal diet), 2) T1 (CON + 0.2% *B. subtilis*), 3) T2 (CON + 0.2% *S. cerevisiae*), 4) T3 (CON + 0.2% *B. subtilis* + 0.2% *S. cerevisiae*). The probiotic used in this study such as *B. subtilis* and *S. cerevisiae* contains  $2.0 \times 10^{10}$  CFU kg<sup>-1</sup> and  $3.0 \times 10^{10}$  CFU kg<sup>-1</sup>, respectively. All diets were formulated to meet or exceed the NRC [11] requirement (Table 1). The diet was divided into two phases: the grower phase (0-6 weeks) and the finisher phase (7-13 weeks). Each of the pigs had *ad libitum* access to water. A nipple drinker and single-sided stainless steel automated feeder were placed with each pen.

## Measurements and sampling

### Growth performance

BW was recorded on initial, 6, 9, and 13 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). The ADG was calculated by subtracting the BW of the previous time point from the BW of the current time point and dividing it by the period. ADFI was calculated by subtracting the remaining feed amount from the initial feed amount and dividing it by the period, and FCR was calculated by dividing FI by ADG.

### Nutrient digestibility

In experiment periods, fresh fecal samples are collected at 6 and 13 weeks using rectal massage after each treatment. Fresh fecal and feed samples were stored in a freezer at -20 °C after collection immediately. The stored fecal samples were dried at 70 °C for 3 days and then crushed on a 1 mm screen at the end of the experiment. Chromic oxide was analyzed immediately after supplementation of 0.2 % as an indigestible marker that was added to the pig's diet the apparent total tract digestibility (ATTD) of crude protein (CP), dry matter (DM), and gross energy (GE). Chromium levels were analyzed with ultraviolet absorption spectrometry (UV-1201, Shimadzu, Kyoto, Japan) using a method used by Williams et al. [12]. The procedures utilized for the determination of DM (method 930.15), and CP (method 999.03) were conducted with the methods of AOAC [13], and GE using a bomb calorimeter (Parr 6400 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA).

Calculating the ATTD used the following formula:

$$\text{“Digestibility (\%)} = [1 - \{(Nf \times Cd)/(Nd \times Cf)\}] \times 100\text{”}$$

Nf =nutrient concentration in feces (DM %), Nd = nutrient concentration in diet (DM %), Cd =chromium concentration in diet (DM %), and Cf = chromium concentration in feces (DM %).

### **Blood profiles**

At 6 and 13 weeks, blood samples from the anterior vena cava of 4 pigs per treatment. Blood samples were collected into vacuum tubes containing K<sub>3</sub>EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) for complete blood count analysis, and nonheparinized tubes for serum analysis, respectively. After collection, serum samples were centrifuged (3,000 g) for 15 min at 4 °C. The white blood cell (WBC), and red blood cell (RBC) levels were determined using an automatic blood analyzer (ADVIA 1200<sup>®</sup>, Bayer Lab, NY, USA). The blood urea nitrogen (BUN), creatinine, and total protein levels were measured using a chemistry analyzer (Cobas C702, Roche, Munich, Germany).

### **Maturity score of compost**

Compost was used by mixing sawdust with manure obtained from pigs. The manure was collected at the 6 and 13 weeks. Compost was prepared by adding sawdust to swine manure at a ratio of 4:1 (swine manure: sawdust) for adjusting moisture content (MC) at the beginning of the experiment. Each compost was stored in a plastic box with air holes. Each compost was mixed weekly to supply oxygen. Compost maturity was evaluated using a maturity analyzer (CoMMe-100, E&A TECH Corp., Dangjin, Korea), according to maturity analyses specified in the fertilizer quality inspection and sampling standards in Korea. MC was adjusted to be around 50% for all samples before they were analyzed for maturity score according to methods described by Song et al. [14]: score 1, immature (barely progressing in compost maturity); score of 2, initial maturity (an initial state in which maturity progressed); score of 3, the middle of maturity (compost maturity in which a longer stay was required); score of 4, the latter part of maturity (compost was almost mature); score of 5, maturity completion (compost was mature). The samples collected at 6 weeks were ripened for 14 weeks, and the samples collected at 13 weeks were ripened for 13 weeks.

### **Odorous gas emissions**

The feces (150 g) that collected for 2 pigs each treated by a rectal massage at 6 and 13 weeks. The samples were mixed with 150 g of collected feces 100 g of sawdust, and 50 g of urine to analyze gas emissions. About samples were stored in a 4.2 L plastic box at room temperature 26 °C and fermented for 72 h. The plastic boxes with small holes sealed with plaster were used for analyzing fecal NH<sub>3</sub>, H<sub>2</sub>S, and acetic acid (CH<sub>3</sub>COOH) emissions of samples. The samples with plastic boxes are shaken 20 s to break down any crust formation before the measurement. NH<sub>3</sub> concentrations were determined within the scope of 5.0 - 100.0 ppm (No.3La, detection tube, Gastec Corp.,

Kanagawa, Japan), H<sub>2</sub>S concentrations were determined within scope of 2.0 - 20.0 ppm (No.4LK, detection tube, Gastec Corp., Kanagawa, Japan), and CH<sub>3</sub>COOH concentrations were determined within the scope of 2.5 - 10.0 ppm (No.81L, detection tube, Gastec Corp., Kanagawa, Japan).

### **Fecal microflora**

The samples of fresh fecal were collected by rectal massage at 6 and 13 weeks from 4 pigs in each treatment by rectal massage. The samples were immediately packaged in plastic bags and transferred to the laboratory freezer (-20 °C) for the duration of the experiment. To count the number of *Lactobacillus* and *Escherichia coli* (*E. coli*), 1 g of samples from each treatment were diluted with 9 mL of 1 % peptone broth (Becton, Dickinson and Co, Franklin Lakes, NJ, USA) and homogenized. In 6-fold to 4-fold dilution (1 % peptone solution) samples were used to analyze the viability of *E. coli* on MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and *Lactobacillus* on de Man, Rogosa, and Sharpe agar plates (Difco Laboratories) respectively. *E. coli* were incubated at 37 °C for 24 h and *Lactobacillus* were incubated for 48 h.

### **Experiment 2**

#### **Experimental Design, Animals, and Housing**

This study used swine manure and sawdust as raw materials for composting. Swine manure from CON of experiment 1 was obtained. The manure was collected from all CON replicates and mixed. The compost was prepared with the same method as experiment 1. The compost was matured in a 12 L plastic box and mixed weekly to provide oxygen and ripening at ambient temperature for 12 weeks. There were 6 replicates for each treatment. Experimental treatments were as follows: 1) CON, normal compost without probiotics; 2) T1, spray *B. subtilis* 10 g per 3.306 m<sup>2</sup>; 3) T2, spray *B. subtilis* 40 g per 3.306 m<sup>2</sup>; 4) T3, spray *S. cerevisiae* 10g per 3.306 m<sup>2</sup>; 5) T4, spray *S. cerevisiae* 40 g per 3.306 m<sup>2</sup>; 6) T5, spray *B. subtilis* 5 g + *S. cerevisiae* 5 g per 3.306 m<sup>2</sup>; 7) T6, spray *B. subtilis* 20 g + *S. cerevisiae* 20 g per 3.306 m<sup>2</sup>. In this study,  $2.0 \times 10^{10}$  CFU kg<sup>-1</sup> of *B. subtilis* and  $3.0 \times 10^{10}$  CFU kg<sup>-1</sup> of *S. cerevisiae* were used.

#### **Measurements and sampling**

##### **Moisture content of compost**

The collected compost samples were determined before and after drying at 105 °C for 24 h to analyze for MC according to methods and calculating formula described by Singh et al. [15].

Calculating the MC used the following formula:

$$\text{“MC (\%)} = \{(W_w - W_d)/W_w\} \times 100\text{”}$$

$W_w$  = weight of the sample before drying,  $W_d$  = weight of the sample after drying.

### **Maturity score of compost**

The measurement methods are the same as experiment 1.

### **Odorous gas emissions of compost**

Initially, the samples were agitated and collected 300 g on 1, 4, 8, and 12 weeks, respectively. About 300 g samples were stored in a 4.2 L plastic box at room temperature 26 °C and fermented for 72 h. The plastic boxes with small holes sealed with plaster were used for analyzing H<sub>2</sub>S, NH<sub>3</sub>, and methyl mercaptan (CH<sub>3</sub>SH) emissions of samples. The samples with plastic boxes are shaken for 20 s to break down any crust formation before the measurement. H<sub>2</sub>S concentrations were determined within scope of 2.0 - 20.0 ppm (No.4LK, detection tube, Gastec Corp., Kanagawa, Japan), NH<sub>3</sub> concentrations were determined within scope of 5.0 - 100.0 ppm (No.3La, detection tube, Gastec Corp., Kanagawa, Japan), and CH<sub>3</sub>SH concentrations were determined within scope of 2.5 - 70.0 ppm (No.71, detection tube, Gastec Corp., Kanagawa, Japan).

### **Statistical analysis**

All data excluding compost maturity were analyzed with the PROC General Linear Models procedure of SAS 9.4 software (version 9.4, SAS Institute Inc., Cary, NC, USA). The maturity score was analyzed with a Chi-square test using the FREQ procedure of SAS. The GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA) was used to visualize the maturity score and MC. Tukey's multiple range test was used as post-hoc test was used to analyze the differences between means and a  $p < 0.05$  was considered statistically significant for analysis.

## **Results**

### ***Experiment 1***

#### **Growth performance**

The effects of supplemental probiotics on growth performance are presented in Table 4. There was no significant difference ( $p > 0.05$ ) in ADG, ADFI and FCR in 0-6 weeks and 6-13 weeks among treatments. In BW, there was no significant difference ( $p > 0.05$ ) at initial, 6 weeks and 9 weeks, respectively. However, T3 showed significantly higher ( $p < 0.05$ ) BW than CON in 13 weeks, significantly higher ( $p < 0.05$ ) ADG than CON and T2 in 9-13 weeks, and significantly lower ( $p < 0.05$ ) FCR than the other groups for 9-13 weeks and the overall experimental period (0-



13 weeks), respectively. Additionally, T3 showed significantly higher ( $p < 0.001$ ) ADG than other groups in the overall experimental period.

### **Nutrient digestibility**

The effects of supplemental probiotics on nutrient digestibility are presented in Table 5. There was no significant difference ( $p > 0.05$ ) in DM, GE, and CP at 6 weeks among treatments. Also, there was no significant difference ( $p < 0.05$ ) in GE, and CP at 13 weeks among treatments. However, T3 showed significantly higher ( $p < 0.05$ ) DM than CON at 13 weeks.

### **Blood profiles**

The effects of supplemental probiotics on blood profile are presented in Table 6. There was no significant difference ( $p > 0.05$ ) in total protein, creatinine, WBC, and RBC at 6 weeks among treatments. Probiotics did not affect the total protein, BUN, creatinine, WBC, and RBC at 13 weeks. However, supplementation of probiotic groups was significantly lower ( $p < 0.05$ ) BUN than non-supplementation of the probiotic group at 6 weeks.

### **Compost maturity**

The effects of supplemental probiotics on the maturity score of pigs (Exp 1) are presented in Figure 3. The chi-square test showed no difference ( $p > 0.05$ ) in the maturity score among treatments. However, supplementation of probiotic groups was significantly higher ( $p < 0.001$ ) average maturity score than non-supplementation of the probiotic group at 6 weeks. Moreover, T3 showed a significantly higher ( $p < 0.001$ ) average maturity score than other groups at 13 weeks.

### **Odorous gas emissions**

The effects of supplemental probiotics on gas emissions are presented in Table 8. There was no significant difference ( $p > 0.05$ ) in  $H_2S$ ,  $NH_3$ , and  $CH_3COOH$  at 6 weeks among treatments. Also, there was no significant difference ( $p > 0.05$ ) in  $NH_3$  and  $CH_3COOH$  at 13 weeks among treatments. However, T3 showed significantly lower ( $p < 0.05$ )  $H_2S$  than CON at 13 weeks.

### **Fecal microflora**

The effects of supplemental probiotics on fecal microflora are presented in Table 9. There was no significant difference ( $p > 0.05$ ) in *Lactobacillus* at 6 weeks among treatments. Also, there was no significant difference ( $p > 0.05$ ) in *E. coli* at 13 weeks among treatments. However, T1 and T3 showed significantly lower ( $p < 0.001$ ) *E. coli* than CON and T2 at 6 weeks. Moreover, T3 showed significantly higher ( $p < 0.05$ ) *Lactobacillus* than CON and T2 at 13 weeks.

## **Experiment 2**

### **Moisture content of compost**

The effects of supplemental probiotics on MC of compost are presented in Figure 1. Supplementation of probiotic groups was only numerically decreasing ( $p > 0.05$ ) MC compared to the non-supplementation group for 6 to 12 weeks.

### **Compost maturity**

The effects of supplemental probiotics on MC of compost are presented in Figure 1. Supplementation of probiotic groups was only numerically decreasing ( $p > 0.05$ ) MC compared to the non-supplementation group for 6 to 12 weeks.

### **Odorous Gas emissions of Compost**

The effects of supplemental probiotics on gas emissions of compost are presented in Table 3. There was no significant difference ( $p > 0.05$ ) in H<sub>2</sub>S and CH<sub>3</sub>SH emissions of compost during the overall measurement period among treatments. Although there was no significant difference in NH<sub>3</sub> emission at 8 and 12 weeks among treatments, T6 showed significantly lower ( $p < 0.05$ ) NH<sub>3</sub> emissions than other groups at 1 week and 4 weeks, respectively.

## **Discussion**

### **Experiment 1**

In addition to promoting the growth of beneficial bacteria, probiotics may also produce microbicidal substances that have effects against harmful microbes and gastrointestinal pathogens [16,17]. Furthermore, probiotics can improve growth performance by improving digestion, absorption, and uptake of nutrients in pigs [18]. Especially, *Bacillus* spp. can produce various digestive enzymes to degrade complex carbohydrates in feed and improve feed utilization [19]. Previous studies have indicated that supplementation of *B. subtilis* and *B. licheniformis* can increase ADFI and ADG [20] and decrease FCR [21] in pigs. The addition of *Lactobacillus acidophilus*, *S. cerevisiae*, and *B. subtilis* can also increase ADG [22] in pigs. Likewise, the results of this study revealed that the inclusion of complex probiotics such as *B. subtilis* and *S. cerevisiae* in the diets of growing-finishing pigs increased ADG and ADFI, while decreasing FCR. These study results agree with previous studies showing that complex probiotics have enhanced benefits in the gastrointestinal tract by integrating effects of different strains compared with a single probiotic [23].

The improved growth performance after adding probiotics might be related to enhanced nutrient digestibility by improving the gastrointestinal tract [24]. The mechanism of probiotics involves production of antimicrobials that can affect the composition and function of microbial communities, thus promoting overall gut health [25]. Previous studies have indicated that supplementation of complex probiotics (*B. subtilis*, *Clostridium butyricum*, *B. licheniformis*, and *B. coagulans*) can improve DM and nitrogen digestibility in growing-finishing pigs that addition of complex probiotics (*B. subtilis* and *S. cerevisiae*) can improve DM and GE digestibility in growing pigs [26,27]. Similarly, in this study, dietary addition of probiotics to pigs improved DM digestibility in the grower phase. However, another study has suggested that supplement containing complex probiotics (*B. subtilis*, *B. licheniformis*, and *S. cerevisiae*) has no effect on nutrient digestibility in growing pigs [28]. Such inconsistent results on nutrient digestibility might be due to different probiotic species and dose levels.

In the current study, there was no significant difference in blood profile including WBC, RBC, creatinine, or total protein after supplementing probiotics to diets. However, there was a significant decrease in BUN concentration in groups supplemented with probiotics. BUN concentration might be used as a method for quantifying nitrogen utilization in livestock [29]. In addition, Otsuka et al. [30] have reported that increased BUN concentration is associated with an increase in feed intake. However, another study has revealed that high concentrations of BUN-impaired kidneys are harmful to pigs [31]. Probiotics can increase the efficiency of nitrogen utilization, improve nitrogen utilization, and increase BUN concentrations in pigs [32,33]. On the other hand, other studies have demonstrated that supplementation of probiotics has no effect on blood profiles of growing pigs [24,34]. These results were probably due to feed intake time or amount and gender differences.

Immature manure can generate odorous gas and cause civil complaints in nearby livestock facilities [35]. Its solutions include reducing nitrogen excretion in urine and feces and supplying feed additives to improve gastrointestinal microbial manipulation [18]. Scheuermann [36] has reported that supplementation of *Lactobacillus* in growing pigs can increase nitrogen retention and reduce nitrogen content in manure. In addition, Ramons et al. [37] have revealed that a reduction of nitrogen content can accelerate maturity period. Similarly, the present study showed that manure composting of dietary supplementation probiotics pigs accelerated the maturity period. These results might reduce BUN to enhance pig intestinal N retention. However, there have been few studies that have measured the maturity of manure excreted after feeding probiotics to pigs, so more research is needed.

High levels of noxious gases such as NH<sub>3</sub>, volatile sulfur, and volatile organic compounds can negatively affect animal health and performance. They, not only affect the health of workers but also cause environmental pollution

[18]. Volatile sulfur-degrading properties of *Bacillus* spp. and increased absorption of nutrients in the gut by *S. cerevisiae* can reduce the substrate for microbial fermentation and decrease emissions of these gases [38,39]. Prior studies have reported that addition of *Bacillus*-based can reduce H<sub>2</sub>S emissions in growing pigs [24] and sows [40]. At the end of the experiment, H<sub>2</sub>S emissions were reduced in groups supplemented with probiotics. Supplement of complex probiotics significantly decreased H<sub>2</sub>S from 4 weeks. This indicates that *Bacillus*-based complex probiotics might have potential to reduce gas emissions in pigs and improve air quality of swine farms efficiently with positive effects on pigs.

*E. coli* and *Lactobacillus* are representative intestinal pathogens and beneficial bacteria, respectively. Moreover, these bacteria are associated with gastrointestinal conditions, health status, and immune system [41]. Prior studies have shown that *S. cerevisiae* can decrease the level of potential pathogens in the intestinal lumen and generate antibacterial substances and that *Bacillus* can generate some effective enzymes (such as  $\alpha$ -amylase,  $\alpha$ -galactosidase,  $\beta$ -glucanase,  $\beta$ -mannanase, cellulase) to improve the intestinal condition [42,43]. In this study, adding complex probiotics increased *Lactobacillus* but decreased *E. coli*. Similarly, previous studies have reported that supplementation of probiotics can increase the counts of gastrointestinal *lactobacillus* but decrease the counts of *E. coli* [26,44]. The present result was consistent with Balasubramanian et al. [26] showing that continuous feeding of probiotics could maintain beneficial intestine microbiota by generating organic acids and hydrogen peroxide, thereby preventing pathogenic bacteria activation into the intestine and excreting antagonistic activity.

## **Experiment 2**

In compost, there are beneficial microorganisms that take charge of regular composting process and potentially harmful microorganisms for humans and the environment. These deactivations of harmful microorganisms and beneficial microbiome development are important goals of composting [45]. In addition, previous studies have reported that providing sufficient quantity of probiotics as beneficial microorganisms could enhance microbial enzyme activity and offset effects of pathogenic microorganisms [46,47]

In addition to microorganisms, factors that affect composting include porosity, aeration, moisture, and temperature [48]. Especially, low values of MC, an important environmental parameter during composting, can cause premature dehydration known to arrest biological processes, resulting in biologically unstable compost, while high values of MC will halt composting activity due to creation of anaerobic conditions caused by water logging [49]. Besides, moisture is related to heat capacity in compost [50]. It can influence metabolic activities of probiotics [51]. Lee et al. [52] have reported that microorganisms produce heat during enzymatic catabolism of substrates and

synthesis of cell material. Therefore, we hypothesize that reducing MC during composting due to microorganisms could generate heat as they decompose organic material. However, in this study, single and complex probiotics (*B. subtilis*, *S. cerevisiae*) supplementation only numerically decreased MC compared to the non-supplemented group. Since no study has reported the relationship between probiotics and MC of compost, further research is needed.

Normal composting involving aerobic decomposition proceeds with the following phases: (i) fermentation, (ii) acid formation, (iii) thermophilic activity, and (iv) temperature decline [53]. Shortening the thermophilic activity phase during the degradation phase can delay the maturity period [54]. Prior studies have suggested that adding *B. subtilis* can prolong the thermophilic phase by increasing high-temperature-resistant bacteria [55]. Xu and Li. [56] have shown that inoculating *B. subtilis*, *B. licheniformis*, *Phanerochaete chrysosporium*, *Trichoderma koningii*, and *S. cerevisiae* into compost can promote compost maturity. Results of this study agree with those of previous studies showing that supplementation of *B. subtilis* and *S. cerevisiae* can reduce the maturation time of composting [57]. For this reason, complex probiotics such as *B. subtilis* can be used to accelerate the maturation.

Accelerating compost mature period can improve several problems such as greenhouse gas emissions, including loss of nitrogen via NH<sub>3</sub> volatilization, and leaking of inorganic/organic pollutants from compost substrates [58]. In general, the genus *Bacillus* grows by assimilating ammonium nitrogen during composting, which causes NH<sub>3</sub> emissions reduction [59]. A prior study has suggested that supplementation of *Bacillus*-based probiotics can reduce emissions of gases such as NH<sub>3</sub>, H<sub>2</sub>S, CH<sub>3</sub>COOH, CO<sub>2</sub>, and CH<sub>3</sub>SH [60]. In addition, supplementation of *S. cerevisiae* can reduce NH<sub>3</sub> by 10.2% and amine gas by 45.5% in swine manure [61]. In this study, supplementation of probiotics decreased NH<sub>3</sub> emissions compared with non-supplementation. However, other odorous gas emissions (CH<sub>3</sub>SH and H<sub>2</sub>S) were not affected by supplementation of probiotics. These inconsistent results are attributed to temperature and humidity.

## **Conclusion**

This study indicates that supplementation of probiotics at the complex probiotics are more improved on growth performance, nutrient digestibility, blood profile, compost maturity, gas emissions, and fecal microflora in pigs and on MC, compost maturity, and gas emissions in compost than single and non-supplementation. Therefore, these results revealed that complex probiotics (*S. cerevisiae* and *B. subtilis*) had positive effects in pigs and compost, respectively. However, supplementation of complex probiotics in compost rarely investigates so more studies are needed.

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## Tables and Figures

**Table 1. Ingredients and chemical composition of the basal experimental diets (as fed basis).**

Items	Grower Phase (0-6w)	Finisher Phase (7-13w)
<b>Ingredients (%)</b>		
Corn	53.479	55.776
Soybean meal	15.660	13.100
Wheat (11%)	3.750	3.750
Rice bran	6.500	6.500
DDGS	11.500	11.500
Limestone	1.270	0.910
Vegetable oil	1.320	1.720
Sugar	4.590	4.870
Poultry oil	0.200	0.200
Salt	0.358	0.376
Choline chloride	0.040	0.040
Lysine sulphate	0.724	0.711
L-Methionine (99%)	0.083	0.077
Tryptophan (98%)	0.049	0.038
Threonine	0.146	0.162
MDCP	0.061	0.000
Emulsifier	0.050	0.050
Vitamin and mineral premix *	0.220	0.220
Total	100	100
<b>Calculated values</b>		
Dry matter (%)	86.69	86.45
Protein (%)	15.90	14.89
Fat (%)	5.51	5.75
Fiber (%)	3.83	3.79
Ash (%)	5.19	4.41
Calcium (%)	0.72	0.46
Phosphorus (%)	0.49	0.44
Na (%)	0.20	0.20
Cl (%)	0.35	0.35
NE (kcal/kg)	2408.70	2446.50
<b>Analyzed values, g/kg</b>		
AID Arg	7.40	6.70
AID Ile	4.63	4.26
AID Leu	12.07	11.48
AID Lys	8.90	8.20
AID Met+Cys	5.25	5.00
AID Met	3.17	3.01
AID Thr	5.43	5.25
AID Trp	1.60	1.39
AID Val	5.71	5.28

\* Provided per kilogram of complete diet: vitamin A, 11,025 U; vitamin D<sub>3</sub>, 1103 U; vitamin E, 44 U; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg; Cu (as CuSO<sub>4</sub> · 5H<sub>2</sub>O), 12 mg; Zn (as ZnSO<sub>4</sub>), 85 mg; Mn (as MnO<sub>2</sub>), 8 mg; I (as KI), 0.28 mg; and selenium (as Na<sub>2</sub>SeO<sub>3</sub> · 5H<sub>2</sub>O), 0.15 mg.

Abbreviation: DDGS, Distiller's dried grains with solubles; MDCP, monocalcium phosphate; NE, Net energy; AA, amino acids; Arg, arginine; Ile, Isoleucine; Leu, Leucine; Lys, lysine; Met, methionine; Cys, cystine; Thr, tryptophan; Val, valine; AID, apparent ileal digestibility.

**Table 2.** Effects of single and complex probiotics supplementation on growth performance in growing-finishing pigs (Exp 1)

Items	CON	T1	T2	T3	SE	<i>p</i> -value
<b>BW, kg</b>						
0 week	18.52	18.81	18.84	18.83	0.974	0.995
6 weeks	51.01	51.73	51.77	52.17	1.237	0.931
9 weeks	70.38	71.44	71.25	72.32	1.387	0.811
13 weeks	95.98 <sup>b</sup>	98.83 <sup>ab</sup>	97.97 <sup>ab</sup>	101.29 <sup>a</sup>	1.279	0.047
<b>0-6 weeks</b>						
ADG, kg	0.77	0.78	0.79	0.79	0.010	0.561
ADFI, kg	1.70	1.72	1.69	1.70	0.033	0.923
FCR, kg/kg	2.20	2.20	2.16	2.14	0.036	0.569
<b>6-9 weeks</b>						
ADG, kg	0.92	0.94	0.93	0.96	0.018	0.455
ADFI, kg	2.41	2.46	2.42	2.46	0.048	0.815
FCR, kg/kg	2.62	2.63	2.62	2.56	0.047	0.724
<b>9-13 weeks</b>						
ADG, kg	0.91 <sup>b</sup>	0.98 <sup>ab</sup>	0.95 <sup>b</sup>	1.03 <sup>a</sup>	0.026	0.021
ADFI, kg	2.87	2.84	2.86	2.87	0.054	0.978
FCR, kg/kg	3.15 <sup>a</sup>	2.91 <sup>b</sup>	3.00 <sup>b</sup>	2.79 <sup>c</sup>	0.040	<.001
<b>0-13 weeks</b>						
ADG, kg	0.85 <sup>c</sup>	0.88 <sup>b</sup>	0.87 <sup>bc</sup>	0.91 <sup>a</sup>	0.007	<.001
ADFI, kg	2.22	2.24	2.22	2.23	0.021	0.896
FCR, kg/kg	2.61 <sup>a</sup>	2.55 <sup>a</sup>	2.55 <sup>a</sup>	2.47 <sup>b</sup>	0.024	0.001

Abbreviation: CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SE, standard error

<sup>a-c</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )

**Table 3.** Effects of single and complex probiotics supplementation on nutrient digestibility in growing-finishing pigs (Exp 1)

Items	CON	T1	T2	T3	SE	<i>p</i> -value
<b>6 weeks</b>						
DM	84.01	84.46	84.77	84.88	0.287	0.193
GE	74.95	75.38	75.77	75.96	0.347	0.225
CP	70.72	71.29	71.15	72.79	0.592	0.130
<b>13 weeks</b>						
DM	83.32 <sup>b</sup>	83.73 <sup>ab</sup>	83.74 <sup>ab</sup>	84.03 <sup>a</sup>	0.144	0.032
GE	70.55	71.51	71.70	72.64	0.545	0.113
CP	70.56	70.65	70.82	71.63	0.805	0.780

Abbreviation: CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error

<sup>a-b</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )

ACCEPTED

**Table 4.** Effects of single and complex probiotics supplementation on blood characteristics in growing-finishing pigs (Exp 1)

Items	CON	T1	T2	T3	SE	<i>p</i> -value
<b>6 weeks</b>						
Total Protein, g/dL	5.78	6.00	6.03	6.03	0.187	0.742
BUN, mg/dL	8.25 <sup>a</sup>	6.75 <sup>b</sup>	7.25 <sup>b</sup>	6.50 <sup>b</sup>	0.260	0.002
Creatinine, mg/dL	1.18	1.19	1.40	1.39	0.080	0.121
WBC, 10 <sup>3</sup> /uL	23.03	23.47	23.18	23.21	0.794	0.983
RBC, 10 <sup>6</sup> /uL	7.80	7.69	7.99	7.86	0.168	0.657
<b>13 weeks</b>						
Total Protein, g/dL	6.28	6.70	6.58	6.70	0.168	0.284
BUN, mg/dL	14.00	12.25	13.00	13.25	1.365	0.839
Creatinine, mg/dL	1.20	1.21	1.43	1.41	0.084	0.141
WBC, 10 <sup>3</sup> /uL	17.00	19.02	18.04	17.43	1.180	0.658
RBC, 10 <sup>6</sup> /uL	6.75	6.94	7.14	6.77	0.117	0.121

Abbreviation: CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; BUN, blood urea nitrogen; WBC, white blood cell, RBC, red blood cell; SE, standard error

<sup>a-b</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )



**Table 5.** Effects of single and complex probiotics supplementation on odorous gas emissions in growing-finishing pigs (Exp 1)

Items, ppm	CON	T1	T2	T3	SE	<i>p</i> -value
<b>6 weeks</b>						
H <sub>2</sub> S	5.47	5.32	5.42	5.23	0.180	0.788
NH <sub>3</sub>	8.10	7.99	8.00	7.95	0.113	0.815
CH <sub>3</sub> COOH	3.11	3.09	3.10	3.06	0.049	0.888
<b>13 weeks</b>						
H <sub>2</sub> S	6.42 <sup>a</sup>	6.23 <sup>ab</sup>	6.31 <sup>ab</sup>	6.11 <sup>b</sup>	0.066	0.036
NH <sub>3</sub>	9.35	9.24	9.27	9.21	0.083	0.685
CH <sub>3</sub> COOH	3.14	3.06	3.10	3.04	0.041	0.384

Abbreviation: CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; H<sub>2</sub>S, hydrogen sulfide; NH<sub>3</sub>, ammonia; CH<sub>3</sub>COOH, acetic acid; SE, standard error

<sup>a-b</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )

ACCEPTED

**Table 6.** Effects of single and complex probiotics supplementation on fecal bacteria counts in growing-finishing pigs (Exp 1)

Items, log CFU/g	CON	T1	T2	T3	SE	<i>p</i> -value
<b>6 weeks</b>						
<i>Lactobacillus</i>	8.89	9.01	8.98	9.07	0.158	0.864
<i>E. coli</i>	6.43 <sup>a</sup>	6.21 <sup>b</sup>	6.33 <sup>a</sup>	6.15 <sup>b</sup>	0.038	<0.001
<b>13 weeks</b>						
<i>Lactobacillus</i>	9.02 <sup>c</sup>	9.09 <sup>ab</sup>	9.08 <sup>bc</sup>	9.14 <sup>a</sup>	0.020	0.003
<i>E. coli</i>	6.45	6.33	6.36	6.30	0.798	0.976

Abbreviation: CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; *E. coli*, *Escherichia coli*; SE, standard error

<sup>a-c</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )

ACCEPTED

**Table 7.** Effects of probiotics supplementation on gas emissions in growing-finishing pigs manure compost (Exp 2)

Items, ppm	CON	T1	T2	T3	T4	T5	T6	SE	<i>p</i> -value
<b>1 week</b>									
H <sub>2</sub> S	6.00	3.00	2.47	3.18	2.49	2.52	1.85	0.980	0.124
NH <sub>3</sub>	16.64 <sup>a</sup>	8.83 <sup>b</sup>	6.24 <sup>bc</sup>	8.98 <sup>b</sup>	5.29 <sup>c</sup>	5.34 <sup>c</sup>	2.43 <sup>d</sup>	0.922	<0.001
CH <sub>3</sub> SH	2.86	1.45	1.24	1.53	1.30	1.31	0.99	0.504	0.232
<b>4 weeks</b>									
H <sub>2</sub> S	1.09	0.41	0.34	0.42	0.36	0.36	0.19	0.205	0.111
NH <sub>3</sub>	5.05 <sup>a</sup>	2.17 <sup>b</sup>	1.67 <sup>b</sup>	2.34 <sup>b</sup>	1.92 <sup>b</sup>	1.99 <sup>b</sup>	0.80 <sup>c</sup>	0.212	<0.001
CH <sub>3</sub> SH	0.95	0.81	0.69	0.82	0.71	0.72	0.52	0.097	0.127
<b>8 weeks</b>									
H <sub>2</sub> S	0.30	0.15	0.12	0.16	0.13	0.12	0.09	0.059	0.289
NH <sub>3</sub>	0.70	0.31	0.25	0.32	0.26	0.26	0.16	0.126	0.129
CH <sub>3</sub> SH	0.11	0.07	0.05	0.08	0.07	0.07	0.03	0.198	0.243
<b>12 weeks</b>									
H <sub>2</sub> S	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.003	0.123
NH <sub>3</sub>	0.02	0.01	0.00	0.13	0.01	0.01	0.00	0.004	0.156
CH <sub>3</sub> SH	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.005	0.605

Abbreviation: CON, normal compost without probiotics; T1, spray *Bacillus subtilis* 10g per 3.306 m<sup>2</sup>; T2, spray *Bacillus subtilis* 40g per 3.306 m<sup>2</sup>; T3, spray *Saccharomyces cerevisiae* 10g per 3.306 m<sup>2</sup>; T4, spray *Saccharomyces cerevisiae* 40g per 3.306 m<sup>2</sup>; T5, spray (*Bacillus subtilis* 5g + *Saccharomyces cerevisiae* 5g) per 3.306 m<sup>2</sup>; T6, spray (*Bacillus subtilis* 20g + *Saccharomyces cerevisiae* 20g) per 3.306 m<sup>2</sup>; H<sub>2</sub>S, hydrogen sulfide; NH<sub>3</sub>, ammonia; CH<sub>3</sub>SH, methyl mercaptan; SE, standard error

<sup>a-d</sup> Means in the same row with different letters indicate different significantly ( $p < 0.05$ )

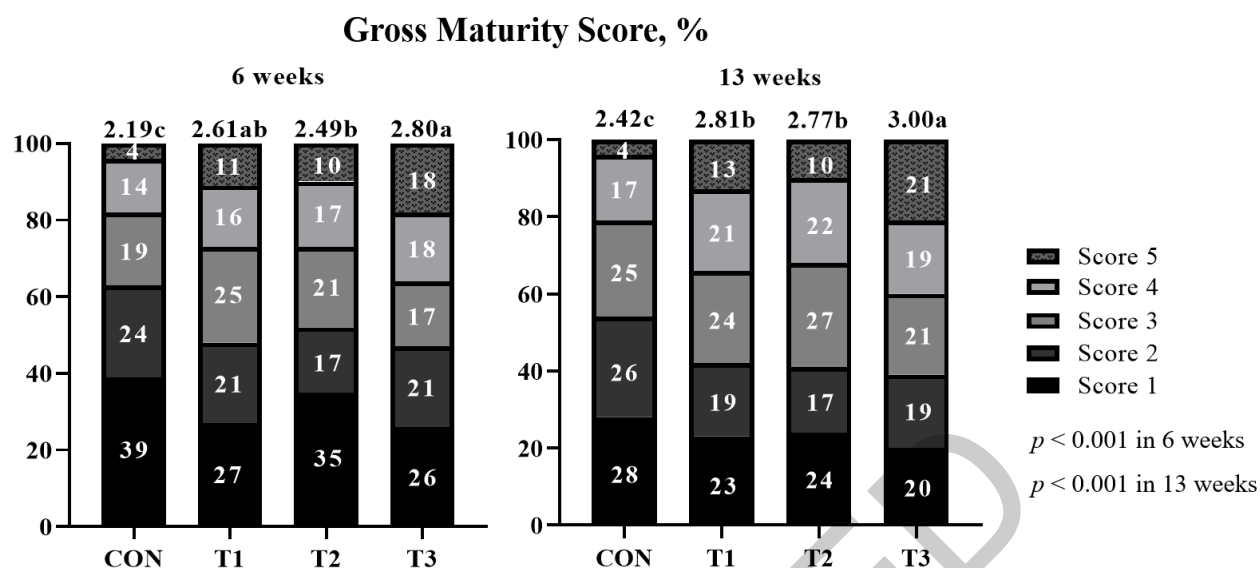


Figure 1. Effects of supplemental probiotics on maturity score from pigs (Exp 1). CON, basal diet; T1, CON + 0.2% *Bacillus subtilis*; T2, CON + 0.2% *Saccharomyces cerevisiae*; T3, CON + 0.2% *Bacillus subtilis* + 0.2% *Saccharomyces cerevisiae*; 6 weeks, collected manure 2 pigs per pen at 6 weeks and composting with sawdust; 13 weeks, collected manure 2 pigs per pen at 13 weeks and composting with sawdust. n = 6 pen/treatment.  $\chi^2 = 13.972$ ,  $p = 0.303$  in 6 weeks.  $\chi^2 = 13.387$ ,  $P = 0.342$  in 13 weeks. Numbers inside the bar indicates percentage of score out of total (100%) as shown in legend. a-c Means scores with different upward letters in the graph bar different significantly by the one-way ANOVA ( $p < 0.05$ )

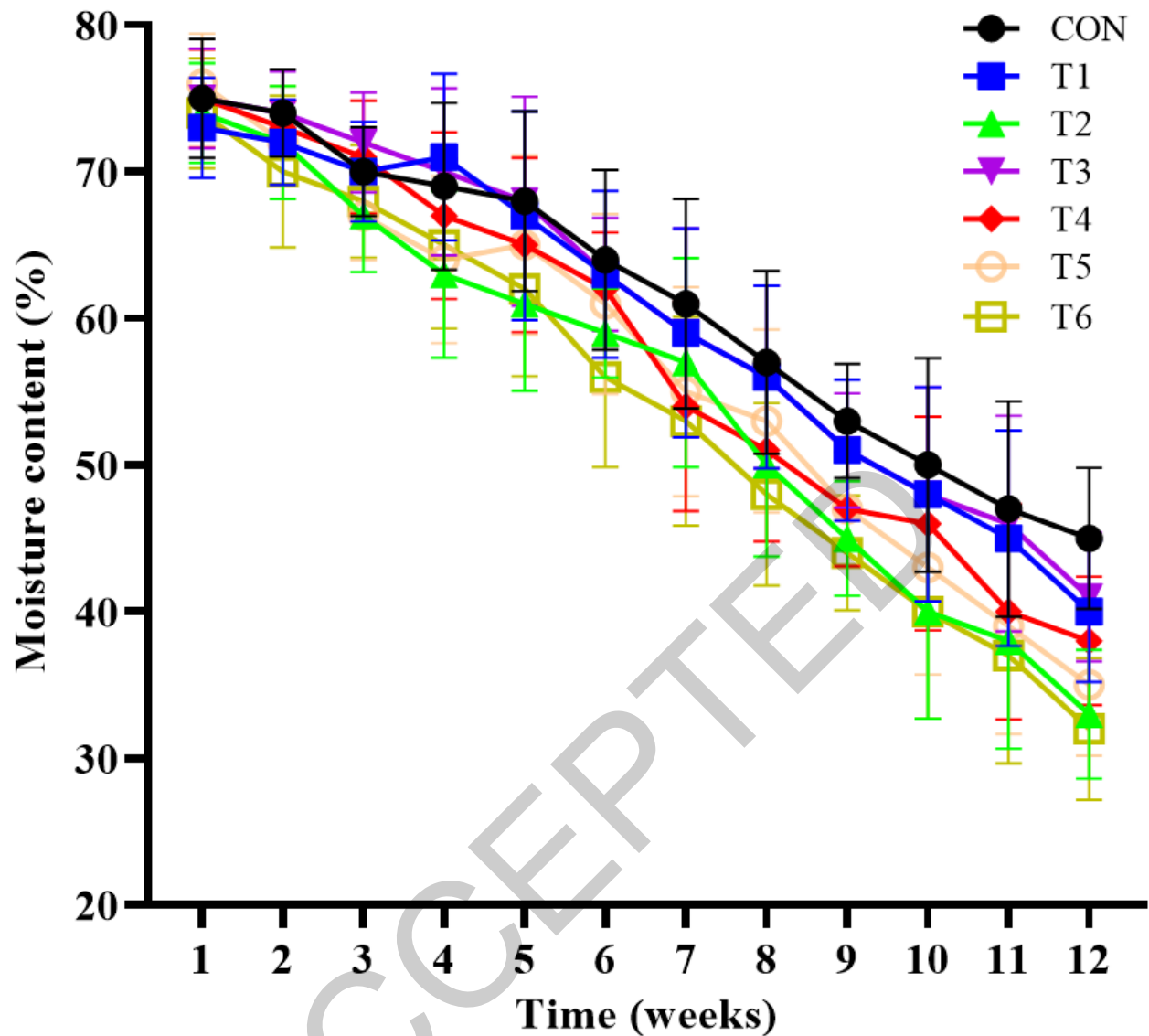


Figure 2. Effects of probiotics supplementation on moisture content change in growing-finishing pigs manure compost during composting (Exp 2). Bars denote standard errors. CON, normal compost without probiotics; T1, spray *Bacillus subtilis* 10g per 3.306 m<sup>2</sup>; T2, spray *Bacillus subtilis* 40g per 3.306 m<sup>2</sup>; T3, spray *Saccharomyces cerevisiae* 10g per 3.306 m<sup>2</sup>; T4, spray *Saccharomyces cerevisiae* 40g per 3.306 m<sup>2</sup>; T5, spray (*Bacillus subtilis* 5g + *Saccharomyces cerevisiae* 5g) per 3.306 m<sup>2</sup>; T6, spray (*Bacillus subtilis* 20g + *Saccharomyces cerevisiae* 20g) per 3.306 m<sup>2</sup>; T6, spray (*Bacillus subtilis* 20g + *Saccharomyces cerevisiae* 20g) per 3.306 m<sup>2</sup>

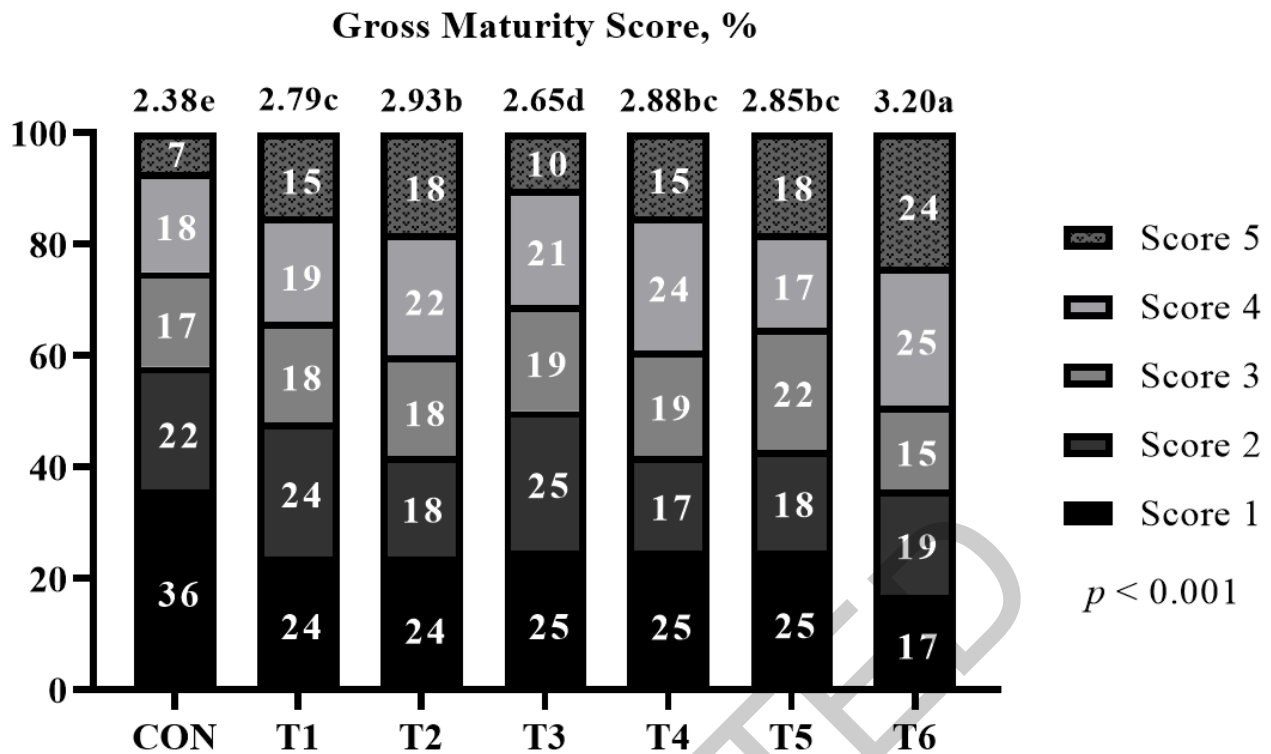


Figure 3. Effects of supplemental probiotics on maturity score (Exp 2). CON, normal compost without probiotics; T1, spray *Bacillus subtilis* 10g per 3.306 m<sup>2</sup>; T2, spray *Bacillus subtilis* 40g per 3.306 m<sup>2</sup>; T3, spray *Saccharomyces cerevisiae* 10g per 3.306 m<sup>2</sup>; T4, spray *Saccharomyces cerevisiae* 40g per 3.306 m<sup>2</sup>; T5, spray (*Bacillus subtilis* 5g + *Saccharomyces cerevisiae* 5g) per 3.306 m<sup>2</sup>; T6, spray (*Bacillus subtilis* 20g + *Saccharomyces cerevisiae* 20g) per 3.306 m<sup>2</sup>; T6, spray (*Bacillus subtilis* 20g + *Saccharomyces cerevisiae* 20g) per 3.306 m<sup>2</sup>. n = 6 pen/treatment.  $\chi^2 = 19.558$ ,  $p = 0.722$ . Numbers inside the bar indicates percentage of score out of total (100%) as shown in legend. a-e Means scores with different upward letters in the graph bar different significantly by the ANOVA ( $p < 0.05$ )