Pakistan J. Zool., vol. 51(6), pp 2265-2273, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2265.2273

# **Trends in Nutrient Status and Gas Production** during the Decomposition of Poultry Manure

Qiuxia Lei<sup>1,2</sup>, Haixia Han<sup>1,2</sup>, Jinbo Gao<sup>1,2</sup>, Yan Zhou<sup>1,2</sup>, Wei Liu<sup>1,2</sup>, Chunlei Wang<sup>3</sup>, Jie Liu<sup>1,2</sup>, Dingguo Cao<sup>1,2</sup>, Huimin Li<sup>1</sup>, Baohua Huang<sup>1,\*</sup> and Fuwei Li<sup>1,2,\*</sup>

<sup>1</sup>Poultry Institute, Shandong Academy of Agricultural Sciences, Jinan 250023, Shandong, China <sup>2</sup>Poultry Breeding Engineering Technology Center of Shandong Province, Jinan250023, Shandong, China <sup>3</sup>Central Lab of Shandong Topsunshine Biological Technology Co., Ltd., Weifang 261000, Shandong, China

Qiuxia Lei and Haixia Han have contributed equally to the article.

# ABSTRACT

To develop the effective methods for the innocuous treatment of the poultry manure, this study explored the efficiency of bacteria in decomposing the poultry manure through testing the variation of gas production and nitrogen(N), phosphorus (P), potassium(K)in the fermentation process. Poultry litter, sawdust and rice husk were mixed in equal quantities by volume. One was treated with a single strain of Bacillus; another was treated with a consortium of bacteria comprising several species; and the third kept as the control without adding any bacteria. The results showed that decomposition started earlier in the treated batches than in the control group. During the fermentation period, there was no influence between the internal temperature of the plies and the addition of bacteria. After adding strains, the time to reach the fermentation temperature was advanced by 1 to 2 days. The content of ammonia and hydrogen sulfide were increased at the first weekend, reaching up to 10ppm and 3.5 ppm respectively. Three weeks later, the content of the ammonia (NH<sub>3</sub>) and hydrogen sulfide (H<sub>2</sub>S) were decreased to 0.3 ppm and 0.6 ppm respectively. Total N, P and K were increased gradually with time, whereas the content of ammonium nitrogen was decreased. SAS software analysis showed that there was no significant difference in H<sub>a</sub>S,  $CO_{2}$  and  $NH_{2}$  between the three groups (p>0.05), the same results showed that N, P, K, organic matter and ammonium nitrogen had no significant difference (p>0.05). These results suggested that the inoculation with Bacillus genus bacteria improved the microbial proliferation and shortened the time that the substrate reached to the fermentation temperature, resulting in the retention of N, P, K and the reduction of the NH<sub>4</sub>, H<sub>2</sub>S from the fermentation litter.

# **INTRODUCTION**

dding selected microorganisms to a substrate of manure, particularly animal manure, can hasten its decomposition. The manure is usually mixed with inert matter such as sawdust and rice husk to make the manure easier to handle, to regulate its moisture content, and to facilitate microbial activity. Using this technology, namely adding sawdust and rice husk and deploying select microorganisms, has other benefits as well. For example, by absorbing undesirable gases, the substrate improves air quality, thereby contributing to animal welfare and making farm animals more productive. Also, by improving the

Corresponding author: lifuwei1224@163.com; jqsyzs@163.com

0030-9923/2019/0006-2265 \$ 9.00/0

Copyright 2019 Zoological Society of Pakistan



Article Information Received 03 March 2019 Revised 30 April 2019 Accepted 25 May 2019 Available online 28 August 2019

#### Authors' Contribution

OX. FW and BH conceived and designed the experiments. QX, FW and CL performed the experiments. FW, HX and LW analyzed the data while YZ, DG and JB contributed reagents/materials/analysis tools. OX. FW, ZY, and BH wrote the paper.

Key words Poultry manure, Inoculant preparation, Microflora, Bacillus, Fermentation

texture of the substrate and lowering its moisture content, the technology facilitates the disposal of livestock manure and is, therefore, a promising adjunct to livestock farming. However, manure piles, as they grow layer by layer with continual addition of fresh manure, have seldom been a subject of detailed study. Wang et al. (2018) Study on the pollution status and control measures for the livestock and poultry breeding to completely utilize the manure and straw obtaining higher economic value. Zhao et al. (2014) studied the effects of turning frequency (the frequency with which a pile of manure is turned over) on the emissions of GHG and ammonia from such piles. The effects were significant: frequent turning not only increased the emissions of both GHG and ammonia however also increased the percentage of total nitrogen lost as ammonia (approximately 42% and 70% when the piles were turned over once a week or twice a week, respectively). The volatile organic compounds from poultry manure are released mainly during its aerobic fermentation. Dahunsi *et al.* (2017) studied explored biogas produciton from the co-digestion of Arachis hypogaea hull and poultry droppings. Sultan *et al.* (2019) reported that phytase is effective in improving the utilization of sorghum by broilers at day-21 and also reduces the losses of nutrients into the litter.

Zhang et al. (2015) studied the emissions of volatile gases from different types of animal manure during aerobic fermentation and found that the emissions occurred mainly during the earlier stages of fermentation and that ammonia production continued for the longest duration. Poultry manure produced eight different volatile organic compounds. Three composting systems, which consisted of different ratios of chicken manure, sawdust, and poultry carcasses, were used to investigate the effect of substrate on the identification of microbial communities and microorganisms associated with poultry carcass decomposition by characterizing the microbial communities and physicochemical parameters (Wang et al., 2014). Jiang et al. (2011) studied the effects of turning over manure piles and of covering them, on emissions of GHG and ammonia during winter. The heat during decomposition maintained the compost piles warm enough for more than 3 weeks to ensure that most of the pathogens were killed. However, as the stock of degradable carbon was exhausted from the piles, the continuing cold weather would freeze the piles, causing the maturation of the piles (decomposition or fermentation) to cease. Approximately, 0.14%-0.76% of the initial carbon was lost as methane, and ammonia and nitrous oxide emissions accounted for 10.3%-29.5% and 0.81%-3.93% of the initial nitrogen, respectively. Statistical analysis showed that turning over the manure decreased the emissions of nitrous oxide and methane but increased the loss of nitrogen as ammonia significantly. Li et al. (2013) studied nitrogen transformations during the composting of poultry manure mixed with rice husk and sawdust, and found that the conversion of ammonium nitrogen to nitrate nitrogen was inhibited. Thus, the transformation of organic nitrogen and ammonium nitrogen to ammonia accounted for most of the nitrogen transformation, the latter resulting in the loss of nitrogen.

Whereas, some questions related to the decomposition of such manure piles remain unanswered, such as the following: What species of microorganisms should be deployed for more efficient decomposition? Should we limit the choice to a single species or should we opt for a consortium of species? How should the production of undesirable gases be limited? How to introduce any changes during the prolonged period of decomposition without affecting the process adversely? How long does the process last? How to manipulate the process so that it

# Q. Lei et al.

yields richer manure?

The present research seeks to contribute to the solution by focusing on sawdust and rice husk as the raw materials to be mixed with poultry manure and on the process of decomposition as fresh manure is continually added to the manure pile. In particular, we studied how the production of ammonia and other gases-and, in turn, the quality of manure-changes over time and studied the effects of different microbial strains (as single species as well as consortia of strains) on the efficiency of decomposition and on the contents of nitrogen, phosphorus, potassium, and organic matter. These results will provide some useful data on the bedding material to be used within the cages of layer hens and the eventual decomposition of that bedding material mixed with poultry manure.

# **MATERIALS AND METHODS**

#### Experimental material

Rice husk (moisture content 12.17%) came from Qihe county, Dezhou City, Shandong province. Sawdust (moisture content 14.18%) came from a wood-processing plant (mainly pine wood) in the neighbouring Jiyang County, Ji'nan City, Shandong province. *Bacillus* was cultured from a single spore and some cultures were purchased from Shandong Tianyuan Agriculture and Animal Husbandry Limited Company. The consortium included lactic acid bacteria, yeast, *Bacillus, Actinomycetes*, and other useful microorganisms and their metabolites (digestive enzymes, protease, amylase, cellulose, amino acids, vitamins, and so on).

Fresh chicken manure was collected daily from the poultry farm of the Institute of Poultry Research of the Academy of Agricultural Sciences in Shandong province. Yeast extract peptone (LB) medium, Martin medium, and a commercial medium (High no. 1) containing xylose lysine deoxycholic salt agar (XLD) were purchased from Qingdao Hope Bio-Technology Co., Ltd.

## Laboratory equipment

Temperature and humidity were checked with a Kestrel 4000 Weather Meter (KestrelMeters, Birmingham, Michigan, USA); NH<sub>3</sub>, with ZDL-800; H<sub>2</sub>S, with ZDL-900 (Environmental Sensors Co., Boca Raton, Florida, USA); and CO<sub>2</sub>, with testo 535 (testo AG, Lenzkirch, Germany). The constant temperature incubator was SLI-700 (Eyela, Tokyo); the table model of high-speed centrifuge was Sigmal-14; for PCR, the Eppendorf Mastecycler pro system was used; the electrophoresis apparatus was DYY-6C; for gel imaging, the AlphaImager EP System was used (ProteinSimple, San Jose, California, USA) and pH was measured with PHS-3C.

## Experimental method

Poultry litter was mixed, in equal quantities by volume, with a mixture containing equal quantities, also by volume, of sawdust and rice husk. The nutrient status of the manure and of the sawdust and rice husk is given in Table I. This composite mixture was inoculated with two inoculants, namely *Bacillus subtilis* alone and a consortium of bacteria comprising bacilli, lactobacilli, yeast, etc. The control consisted of an uninoculated batch of the composite mixture. The experiments were carried out in fermentation tanks, each measuring  $1 \text{ m} \times 1 \text{ m} \times 0.7 \text{ m}$ .

Table I.- Nutrient and moisture status of raw materials(% by wet weight).

Raw	Moisture	Total	Total	Total	Organic
material		Ν	Р	K	matter
Sawdust	14.18	0.12	0.07	0.07	83.40
Rice husk	12.17	0.67	1.52	1.32	65.81
Poultry	73.61	5.80	3.62	2.10	83.62
manure	-78.62				

## Packing and stacking

The requisite quantities of poultry manure, sawdust, and rice husk were thoroughly mixed and divided into three piles; 4 kg of fresh chicken manure was added to each pile. Sterile water (60 kg, being half the quantity of total water required) was added to the first pile, taking care to wet the mix evenly. This pile served as the control. The second pile was inoculated with Bacillus subtilis mixed in 60 kg of water to ensure uniform distribution, and the third pile was similarly inoculated with the consortium of bacteria. Heat insulation material, retained in place by nylon bags, was added around and at the top of each pile. The piles were sealed from the sides to retain heatso that the decomposition would be faster-and the top was covered with a porous nylon bag to ensure normal ventilation. The pile temperature was monitored regularly; when it decreased to 30°C, the first step, namely packing and stacking, was considered complete.

The temperature of the piles was recorded daily at 08:00 am and 08:00 pm as the average of five readings at different points and the pH-at a depth of 20-30 cm-was determined every time the pile temperature was 35°C.

# Application of fresh litter

Fresh poultry manure at the rate of 4 kg for each pile was added daily between 08:00 and 09:00 h by raking it into each pile and turning the pile over once.

Ambient temperature and humidity were recorded daily at 08:00 am and 5:00 pm. The concentration of three gases-namely NH<sub>3</sub>, H<sub>2</sub>S and CO<sub>2</sub> was recorded

approximately 4 min before adding the daily supplement of fresh chicken manure. For this purpose, a box (0.6 m  $\times$ 0.5 m  $\times$  0.5 m) was placed atop each pile at its centre and the probe was suspended within the box. The pH of the pile was measured once a week and the moisture content of the pile was determined by drying a sample from it at 105 °C for 2 h in an oven (NY525-2002, the Standard of organic fertilizer (2002).

The nutrient status of the decomposing litter was monitored weekly by determining the contents of ammonium nitrogen, total N, total P, total K, and organic matter before turning over the piles. Ammonium nitrogen was determined by the distillation and titration method (AOAC 973.49, EPA350.2); total N, by the Kjeldahl method (using  $H_2SO_4$ - $H_2O_2$  for the digestion); total P, by vanadium molybdenum yellow colorimetry (using  $H_2SO_4$ - $H_2O_2$  for the digestion); total K, by flame photometry (using  $H_2SO_4$ - $H_2O_2$  for the digestion) and organic matter, by the potassium dichromate volumetric method and heating over a water bath.

# Statistical analyses

The test data were sorted out by Excel software, and one-way ANOVA was performed by SAS 10.0 statistical software. Duncan's method was used for multiple comparisons. The results were expressed as "average value ( $\pm$ SD)".

# **RESULTS AND DISCUSSION**

#### Packing and stacking

## Temperature

The overall trend of the changes in temperature (Fig. 1A) was the same for the two treatments as well as for the control: an initial increase followed by a decrease. The temperatures inside the pile rose to 50 °C, remained fairly high for 3-5 days, and then decreased gradually to 33°C in all the piles. The average temperature of the group one was  $33.53\pm11.07$ °C, which was higher than that of group two of  $32.78\pm11.28$ °C and groups three of  $32.40\pm10.89$ °C, however there was no significant difference in temperature between the three groups(*p*>0.05).

## pН

The pH increased only slightly in the control pile and in the pile inoculated with *Bacillus* (the increase being less in the control pile), whereas in the pile inoculated with the consortium, changes in the pH were more marked (Table II).

#### Application of fresh litter

Ambient temperature, humidity and litter temperature Ambient temperature and humidity fluctuated a great deal (Fig. 1B); their values within the piles, irrespective of the treatment, were fairly constant (Fig. 1C) however reflected the changes in ambient conditions.

Table II.- Changes in pH during packing and stacking.

Phase	Control	Bacillus	Consortium
Initial rise in tem-	$\begin{array}{c} 6.62 \pm \\ 0.12^{\scriptscriptstyle B} \end{array}$	6.26±	7.52±
perature (30 °C)		0.21 <sup>B</sup>	0.18 <sup>A</sup>
Steadily high tem- perature (49 °C)	$\begin{array}{c} 6.45 \pm \\ 0.26^{\scriptscriptstyle B} \end{array}$	6.63± 0.34 <sup>B</sup>	$8.45 \pm 0.52^{\text{A}}$
Low temperature (33 °C)	6.95±	6.82±	6.74±
	0.25	0.27	0.42

Different letters on the same line show significant differences (p < 0.01), unmarked letters or identical letters show no significant difference (p>0.01).

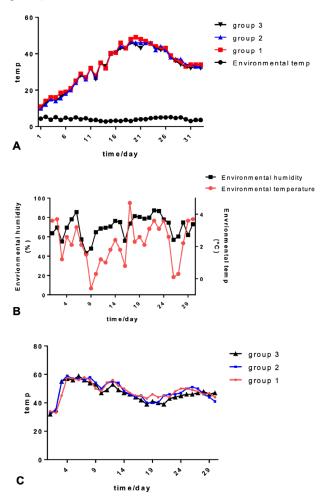


Fig. 1. Ambient and internal temperatures during packing and stacking (A), ambient temperature and humidity (B) and changes in temperature within the piles (C) (at 30 cm depth).

# Gas production

The production of ammonia increased initially and then decreased to remain fairly constant in all the treatments (Fig. 2A). The highest value (10 ppm) was recorded in the *Bacillus* treatment on the 8th day, and the lowest (7.8 ppm) in the consortium treatment on the same day. Before fresh litter was added, all the three treatments produced large quantities of H<sub>2</sub>S and CO<sub>2</sub> although the production of H<sub>2</sub>S (Fig. 2B) fluctuated more than that of CO<sub>2</sub> (Fig. 6). The average ammonia concentration in group two was 0.959±1.462 ppm higher than that in group one of 0.833±1.208ppm and group 3 0.89±1.17, however the three groups was not significant(p>0.05).

Although the control pile produced more  $H_2S$  than the other two piles, it produced less in the first week (the production peaked at the end of the second week). The production of  $H_2S$  was the least in the consortium group, as can be seen in Figure 2B. The pattern of ammonia production in the initial stages was the exact opposite of that of  $H_2S$  however similar to that of  $CO_2$  (Fig. 2C). After the last addition of fresh litter, production of all the three gases decreased slightly. Overall, the treatments showed significant differences in the production of  $NH_3$  and  $H_2S$  in the first 10 days, however none in the production of  $CO_2$ . There was no significant difference in  $H_2S$ ,  $CO_2$  and  $NH_3$ between the three groups (p>0.05).

In all the three treatments, the pH of the piles fluctuated with time however the pattern of the fluctuations was similar: an initial increase followed by a decrease, only to be followed by another increase and decrease (Fig. 3A). In the initial 5 weeks, the changes were small (0.06-0.33) however increased markedly (0.65-1.23) over the next 3 weeks. The increase was minimum in the consortium treatment. The lowest value in the 5th week was probably due to the organic acids produced as a result of decomposition. SAS analysis software was used to analyze the three groups. There was no significant difference between the three groups (P>0.05).

#### Nutrient status

#### Ammonium nitrogen

The content of ammonium nitrogen (Fig. 3B) followed the same trend as that of pH, the two being closely related. The lowest value (0.701 g/kg) was recorded at the end of the 5th week and the highest 2 weeks later (in the *Bacillus* treatment in both cases). The average concentration of ammonium nitrogen in the third groups was  $1.371\pm0.187$ g/kg higher than of the first group  $1.265\pm0.287$  g/kg and second group of  $1.285\pm0.405$  g/kg, however there was no significant difference between the three groups (P>0.05).

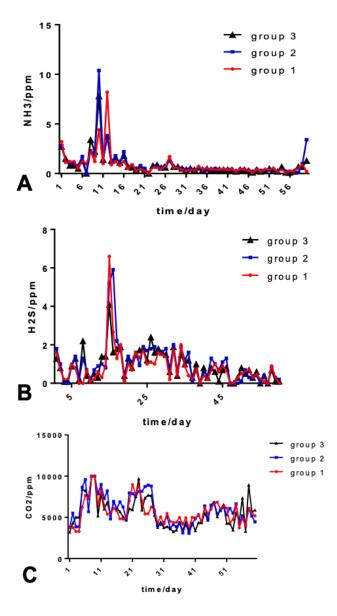


Fig. 2. Changes in ammonia production (A), hydrogen sulfide production (B) and carbon dioxide production (C).

#### Total nitrogen

The content of total N (Fig. 3C) rose steadily in all the three piles, although it was the lowest in the control pile, as was the extent of increase (0.418%). The average total N content of the second groups was  $1.103\pm0.196\%$ higher than that of the first group  $1.013\pm0.57\%$  and third group of  $1.086\pm0.197\%$ , however there was no significant difference between the three groups (*P*>0.05).

# Total phosphorus

The content of total P (Fig. 4A) also rose steadily in all the three piles although the extent of change was greater than that for total N. The inoculated piles recorded higher total P than the control pile did, especially between the 3rd and the 4th week and also between the 6th and the 8th week, probably because the bacteria utilized P more efficiently. The average total P content of the second groups was 1.648±0.737% higher than that of first group 1.488±0.658% and third group of 1.584±0.712%, however there was no significant difference between the three groups (P>0.05).

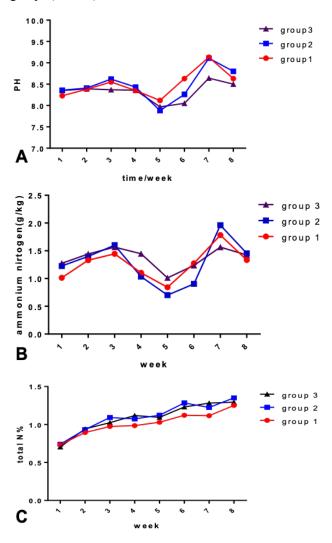


Fig. 3. Changes in pH (A), ammonium nitrogen content changes (B) and total N (C) over the first 8 weeks.

## Total potassium

The content of total K (Fig. 4B) showed the same trend as that shown by total N and total P. Over the first six weeks, all the three groups followed a closely similar trend but the consortium group diverged from the other two over the last two weeks. As with N and P, both the inoculated

2269

piles contained more K than the control pile. The average total K content of the second groups was  $0.962\pm0.357\%$  higher than that of the first group  $0.908\pm0.355\%$  and third group of  $0.946\pm0.376\%$ , however there was no significant difference between the three groups (*P*>0.05).

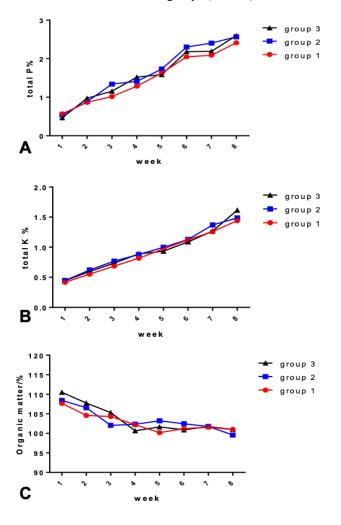


Fig. 4. Changes in total P (A), total K (B), organic matter content (C) over the first 8 weeks.

## Organic matter

Organic matter content (Fig. 4C) is probably the clearest indicator of the efficiency of decomposition. The value of the parameter decreased by 6.67%-8.86%, in all the three groups initially and then stabilized. The decrease was not only minimum in the control group but also the slowest. The average organic matter content of the third group was  $103.69\pm3.74\%$  higher than that of the first group  $102.84\pm2.49\%$  and the second group  $103.30\pm2.84\%$ .

# Changes in temperature and in production of gases

The temperature of the manure piles rose rapidly soon after fresh chicken manure was added, mainly because the organic matter in the manure was degraded rapidly, generating heat during the process. However, the organic matter was not fully utilized and was not easily degraded. The faster decrease in organic matter in the first four weeks is consistent with the rise in temperature. At the same time, the microorganisms, as they rapidly reproduce, utilize the nutrients in the manure and speed up its decomposition, a process aided by higher temperatures. Adding the microbial inoculum boosted the process, which is why the temperature in the inoculated piles began to rise 1-2 days earlier than that in the control pile. Later, the internal temperature tended to be stable as the addition of fresh litter continued. Thus, changes in the temperature of the manure piles were affected by the ambient temperature, humidity, addition of fresh manure, and the extent of microbial activity.

The production of gases, namely NH<sub>2</sub>, H<sub>2</sub>S, and CO<sub>2</sub>, was clearly affected by the properties of the substrate, which, in turn, were related to microbial activity Chen et al. (2008). Patterson et al. (2005) found moisture content to be an important factor affecting the volatilization of NH<sub>2</sub>. Production of NH<sub>3</sub> following microbial decomposition of manure requires ample moisture; therefore, the higher the moisture content of the litter, the greater the release of NH<sub>2</sub>. Bacillus subtilis and Bacillus cereus can decompose proteins and amino acids to produce NH<sub>2</sub> (Li *et al.*, 1993) Some strains of *Bacillus cereus* can produce H<sub>2</sub>S also, however the reaction is slow (Berkeley et al., 1981). Chen et al. (2011) found that aerobic bacteria can effectively reduce the volatilization of NH, in chicken manure and Jian et al. (2009) found that a mixture of bacteria removes NH, more efficiently.

The amount of ammonia produced on the 10th day following the addition of fresh manure from the inoculated piles was greater than that from the control pile because the substrate contained more water(Patterson, 2005): as the decomposition continued, the bacteria produced more NH<sub>3</sub> and H<sub>2</sub>S (Berkeley *et al.*, 1981).

Microbial decomposition at the early stages, when the moisture content of the substrate was high, led to  $NH_3$  and  $H_2S$  being released in larger quantities. Later, the efficient utilization of N led to a rapid decrease in the production of  $NH_3$  and  $H_2S$  and kept their production to the minimum.

The production of  $CO_2$  followed the same basic pattern, namely initial fluctuation followed by a steady state. A number of factors account for the decline and the steady state: changes in the substrate, greater share of anaerobic fermentation (which resulted in marked odour), lower population of the microorganisms, and the falling temperatures.

## Changes in pH

The optimal range of pH for microbial growth is 5-6 during the phase of aerobic fermentation (Berkeley et al., 1981). In the present experiment, changes in the pH were similar in all the three treatments, with least changes occurring in the consortium group. In microbial decomposition of organic matter, the increase in pH and production of NH<sub>2</sub> are related. In the 5th week, the nitrifying bacteria produced large amounts of H<sup>+</sup>, lowering the pH (Golueke et al., 1984), a decrease accentuated by the formation of organic acids as a result of decomposition of organic matter. By the 7th week, the pH began to rise gradually and even exceeded the upper bound of the range considered optimal, mainly because of low microbial activity and also because the larger quantities of NH, and H<sub>2</sub>S consumed more H<sup>+</sup>. These results are very similar to those reported by Li et al. (2004). That study also reported an initial increase in pH followed by a plateau and then a tendency to increase, resulting in weakly alkaline compost. Members of the consortium also affected the distribution of the bacteria in the litter to some extent, regulating the populations of the NH<sub>2</sub>-producing bacteria and the decomposing bacteria to keep them relatively stable, which explains why the extent of changes in the pH were minimal in that group. Thus, adding a consortium of bacteria can lower the magnitude of change in pH and thereby prevent the resulting compost from being alkaline.

#### Changes in nutrient status

#### Ammonium nitrogen

Changes in the content of ammonium nitrogen are directly related to the following factors: internal temperature, pH, the kind of bacteria, inorganic mineralization of organic N by nitrification and nitrification and denitrification (Ma *et al.*, 2009).

In the present experiment, changes in the contents of ammonium nitrogen in all the three groups followed a similar pattern, namely an initial increase followed by a decrease, which resulted in the lowest value (at the end of the 5th week), followed by yet another increase which resulted in the highest value (at the end of the 7th week).

In the first three weeks, the temperature was high, promoting the activity of ammonifying bacteria; in the 4th and the 5th weeks, the temperature decreased, inhibiting the activity of ammonifying bacteria and promoting the activity of nitrifying bacteria, which convert ammonium nitrogen into nitrate nitrogen-therefore, the content of ammonium nitrogen decreased whereas that of nitrate nitrogen increased (Sheng *et al.*, 1998). In the 7th week, the temperature increased again, and so did ammonium nitrogen; in the 8th week, the temperature fell, and

along with it the content of ammonium nitrogen. These fluctuations in ammonium nitrogen are due mainly to its content reaching a set concentration, at which ammonia is released following decomposition.

# Total nitrogen, total phosphorus, and total potassium

The content of all the three major nutrients, namely N, P. and K, increased in the present experiment, their levels in the inoculated piles being slightly higher than those in the control pile. Total N, total P, and total K contents reflect the composition of the bedding material (poultry litter, sawdust, and paddy husk) The total N content changed when fresh poultry manure was added, mainly because it was the only source of variable N in the litter. An earlier study (Yang *et al.*, 2005) showed N to be the nutrient that suffers maximum loss during aerobic fermentation. Martin *et al.* (1992) maintains that such loss can be as high as 77%.

In the present experiment, the amount of poultry manure added exceeded the carrying capacity of the litter: the amount of N lost from the manure being less than the N content of the manure, no decomposition occurred during the entire process, and total N content continued to increase. Total P in the litter, which takes the form of phytate phosphorus, is neither utilized nor lost easily. Therefore, with the addition of fresh manure, P continued to increase, and total P in the litter continued to accumulate. Potassium, being an element that is not lost easily, showed the same trend, and also continued to accumulate. The retention rates of N, P, and K were calculated from their fractions in the dry mass and the weight of the litter. As the litter dry weight increased linearly with time, so did N, P, and K. The retention rates of N, P, and K also reflect their levels in the bedding material; because the lost N, P, and K were not easy to use, their retention rates were significantly higher than those of N, P, and K.

The retention rates of N, P, and K were higher in the inoculated piles than in the control pile, whereas total N, total P, and total K were similar in all the three piles, which shows that the inoculation made the litter richer in the major nutrients. The *Bacillus* and Consortium groups was higher than of control group, which may proliferate microorganisms and promote the retention of N, P and K by microorganisms.

# Organic matter

Changes in organic matter were similar to those seen with the internal temperature and production of  $CO_2$ , namely wide fluctuations in the beginning followed by small fluctuations later. The explanation probably lies in the underlying physicochemical properties, microbial activity, and the interaction of other factors. At the beginning of the decomposition process, the more easily degradable components are broken down and used by the microorganisms to generate energy, rapidly producing  $CO_2$  and water vapour as by-products; later on, decomposition turns increasingly anaerobic, with corresponding changes in litter composition and microbial activity and population. Thus the content of organic matter decreased faster in the early stages, and  $CO_2$  production and temperature within the pile increased. The remaining ingredients were difficult to degrade; microbial activity slowed down; and changes in the content of organic matter,  $CO_2$  production, and temperature within the pile were both slower and smaller.

In the inoculated piles, organic matter reached its lowest level one or two weeks earlier, probably because the added bacteria began to degrade the organic matter in the substrate faster and in greater quantities. Thus, changes in the content of organic matter reflect to some extent the pattern of utilization of the substrate.

# CONCLUSION

Inoculation of *Bacillus genus* bacteria can guide microbial proliferation and start fermentation heating ahead of time during the stacking fermentation period and the initial application period of fermentation *Bacillus* genus bacteria of inoculation and that also have the good affection for the retention of nitrogen, phosphorus, potassium. The harmful gases NH<sub>3</sub> and H<sub>2</sub>S in fermented garbage were significantly reduced due to inoculation of fermented bacteria.

# ACKNOWLEDGEMENTS

This study was financially supported by China Agriculture Research system (CARS-40-S12), Genomewide Association analysis on body weight and carcass traits of Shandong Indigenous Chicken Breeds (2015YQN53) and Agricultural scientific and technological innovation project of Shandong Academy of Agricultural Sciences (CXGC2016A04).

# Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional file.

Statement of conflict of interest

Authors have declares that there is no conflict.

# REFERENCES

Anonymous, 2002. Organic matter content of NY 525-2002 [78] [S]. China Standard Press, Beijing.
Berkeley, R.C.W. and Goodfellow, M., 1981. The aerobic endospore-forming bacteria. *Classif. Identtif.*, **1998**: 315-331.

- Chen, L-Y., Wu, D. and Xia, L-Z., 2008. Livestock manure deodorizing isolation and screening of microorganisms. *Anim. Husb. Vet. Med.*, **12**: 59-61.
- Chen, G-Y., Zhan, K., Chen, L-Y., Wu, J-F., Zhu, Y-K., Li, J-Y. and Liu, W., 2011. Microbial screening for reducing ammonia emission from layers' manure. *China Poult.*, 33: 9-13.
- Dahunsi, S.O., Oranusi, S. and Efeovbokhan, V.E., 2017. Pretreatment optimization, process control, mass and energy balances and economics of anaerobic co-digestion of *Arachis hypogaea* (Peanut) hull and poultry manure. *Bioresour. Technol.*, 241: 454-464 https://doi.org/10.1016/j.biortech.2017.05.152.
- Feng, F., Fang, X. and Yu, M., 2009. Application of biological deodorization in the deodorant in the feces of livestock and poultry. *Mod. agric. Sci. Technol.*, 20: 286-287.
- Golueke, C.C., 1984. Overview of composting research. *Biocycle*, 25: 54-55
- Jiang, T., Suchuchardt, F. and Li, G-X., 2011. Effect of turning and covering on greenhouse gas and ammonia emissions during the winter composting. *Trans. Chinese Soc. Agric. Engineer.*, 27: 212-216.
- Li, Y., Liao, X-D. and Wu, Y-B., 2013. Nitrogen transformations during the layer-hen manure composting with rich bran and sawdust. *Acta Ecol. Anim. Domest.*, **34**: 32-36.
- Patterson, P.H. and Adrizal, B., 2005. Management strategies to reduce air emissions: Emphasis-dust and ammonia. *J. appl. Poult. Res.*, **14**: 638-650. https://doi.org/10.1093/japr/14.3.638
- Li, J-l., 1993. *Microbial physiology*. Peking Agricultural University Press.
- Li, J-J., 2004. Study on mechanism of high temperature composting of livestock manure and its application. PhD thesis, China Agriculture University, pp. 06.
- Ma, L-H., Huang, Y-M., Li, X-Z., Qi, J-H., Zhao, Z-Z. and Li, P., 2009. Changes of nitrogen forms and microbial physiological group diversity and their relations during composting of cow manure. J. Agro-Environ. Sci., 28: 2674-2679.
- Martins, O. and Dewes, T. 1992. Loss of nitrogenous compounds during composting of animal wastes. *Biotechnology*, **42**: 103-111. https://doi. org/10.1016/0960-8524(92)90068-9
- Sheng, Z-Q. and Yuan, J-F., 1998. Study on industrial process on commercial orgnic fetilizers. *Pl. Nutr. Fertil. Sci.*, **4**: 117-122.
- Sultan, A., Ali, R., Khan, R., Khan, S., Chand, N. and Tariq, A., 2019. Nutritional evaluation of two

sorghum varieties in broiler fortified with phytase. *Pakistan J. Zool.*, **51**: 1183-1185.

- Wang, J., Du, X., Zhang, Y., Li, T. and Liao, X., 2016. Effect of substrate on identification of microbial communities in poultry carcass composting and microorganisms associated with poultry carcass decomposition. J. Agric. Fd. Chem., 64: 6838-6847. https://doi.org/10.1021/acs.jafc.6b02442
- Wang, H-X., Xu, J-L., Liu, X-J., Sheng, L-X., Zhang, D., Li, L-W., Wang, A-X., 2018. Study on the pollution status and control measures for the livestock and poultry breeding industry in northeastern China. *Environ. Sci. Pollut. Res. Int.*, **25**: 4435-4445. https://doi.org/10.1007/s11356-017-0751-2
- Yang, Y-M., Liu, H-L., Yang, Z-F., Xi, B-D. and Zhang, X-F., 2005. Methods and techniques in the control of nitrogen loss during the composting a review. J. Beijing Norm. Univ. (Nat. Sci)., 41: 213-216.
- Zhao, C., Li, H-M., Wei, Y-S., Zhong, J., Zheng, J-X., Han, S-W. and Wan, H-F., 2014. Effects of turning frequency on emission of greenhouse gas and ammonia during swine manure windrow composting. *Environ. Sci.*, **35**: 533-540.
- Zhang, P-Y., Shen, Y-J. and Liu, S-Q., 2015. Volatile gas emissions from different types of animal manure during aecobic fermentation process. *J. Agro-Environ. Sci.*, **34**:1378-1383.