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Regulation of odor gas emission and performance by probiotic Bacillus in livestock industry

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Abstract

Livestock operations have shifted from small farms to industrial facilities. Industrialized farms have benefits with improved the efficiency of animal management however there are problems with these operations, such as infectious disease and waste disposal. In the case of waste disposal, especially odors such as ammonia (NH3) and hydrogen sulfide (H2S) are problematic on farms. NH3 and H2S emissions can have severe negative effects on farm workers, such as chronic or acute pulmonary disorders, as well as on domestic animals like swine and poultry.

Probiotics are a potential solution for this critical problem. Bacillus-based probiotics complex are generally recognized as useful microorganism to decrease the malodor and enhance the growth performance in livestock. Various studies of dietary supplementation with Bacillus have been conducted on monogastric animals. The dietary supplementation of Bacillus spp. showed several beneficial effects in swine as follows; the reduction of noxious gases (NH3, H2S and mercaptan) emission, and the improvement of growth performance parameters such as average daily gain, average daily feed intake and feed conversion ratio. It has been reported that feed supplementation with Bacillus spp. was definitely effective to improve the growth performance and egg production quality in chickens.

Moreover, NH3 and H2S emissions from poultry manure were dramatically decreased after dietary Bacillus spp. supplementation. Some authors have suggested that the beneficial effects of Bacillus supplementation may be boosted by the addition of other probiotics, such as Lactobacillus spp. However, probiotics have a disadvantage as feed additives, namely the inconsistency in results caused by variation in dietary compositions, dose levels, strains, and environmental factors. Additional studies of complex probiotics are needed to find appropriate combinations of microbial sources, that satisfy both odor reduction and growth performance requirements in monogastric animals.

Keywords: Odor; Gas emission; Bacillus; Ammonia; Hydrogen sulfide; livestock

Abbreviations: ADG: Average Daily Gain; ADFI: Average Daily Feed Intake; FCR: Feed Conversion Ratio; DFM: Direct Fed Microbial; DM: Dry matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; GE: Gross Energy; ME: Metabolic Energy; PKM: Palm Kernel Meal

Introduction

Livestock operations have transitioned over time from small farms to industrial facilities. Industrialized farms have improved the efficiency of animal management. However, there are problems with these large-scale operations, such as infectious disease and waste disposal [1]. Waste disposal can cause environmental issues, including soil erosion and the production of global greenhouse gases and air pollutants [2,3].

In terms of air pollutants, various emissions such as ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), nitrous oxide (N_2O), volatile organic compounds (VOCs), and other odors are released from livestock production facilities [4]. These emissions are not only a nuisance to people living in nearby residential areas [5] but can also result in health problems for farm workers. Ammonia and H_2S have shown critical negative effects on farm workers, including chronic or acute pulmonary disorders, as well as on domestic animals like swine and poultry.

Ammonia is generated from livestock barns, open feedlots, and manure storage facilities on farms, as well as during manure handling, treatment, and spreading. Ammonia dissolves readily in water (e.g., swine urine and drinking water) where it ionizes to form an ammonium ion. The atmospheric pressure and temperature affect ammonia solubility in water from dissolved or suspended materials [6]. On the other hand, ammonia produced in poultry facilities is created by urea and uric acid degradation [7].

Another source of odor in livestock production is H_2S, which has been recognized as harmful to humans, and animals in deep-pit production systems [8,9]. Hydrogen sulfide is formed under anaerobic conditions by bacteria reducing sulfate to sulfide;

sulfide then combines with hydrogen ions to form hydrogen sulfide [10]. Pigs are affected by different levels of hydrogen sulfide. Severe distress, eye irritation, and drooling can be caused by concentrations of 100 ppm. Pigs exposed to 250 ppm of H_2S may exhibit cyanosis, convulsions, and death [11].

Farm workers are also affected negatively by hydrogen sulfide exposure. Humans can detect a smell like rotten eggs when exposed to 0.1 to 5 ppm of H_2S, even though these levels are not toxic. Eye and respiratory irritation in humans can occur at H_2S levels of 100 ppm. High levels of H_2S, (e.g., 150 to 200 ppm) cannot be detected by humans due to olfactory paralysis. At levels >200 ppm, H_2S affect the nervous system and levels >1,000 ppm result in immediate collapse and respiratory paralysis [12].

There are several possible solutions to mitigate the environmental pollution from animal housing. Excretion of nitrogen and phosphorus can be reduced by formulating diets that improve nutrient digestibility [13]. Feed utilization and dry matter intake can be improved by fine grinding and pelleting, which reduce the size and increase the surface area of grains, thereby increasing the potential for interaction with digestive enzymes [13].

Enzymes can also be used to increase nutrient availability in animal feed. Enzymes can supplement the host's endogenous enzyme production, increasing the availability of nutrients, improving the digestibility of fibrous material, and decreasing any anti-nutritional factors present in feed ingredients [14]. For example, protease can degrade protein sources such as soybean meal and improve protein digestibility [13]. Other indirect contributors to improving swine house environments include antibiotics, probiotics, and organic acids. Low crude protein formulations using synthetic amino acids can also be used to reduce N excretion.

Probiotics can protect young animals against enteropathogenic disorders and improve growth performance [15]. Studies have shown that probiotics can create a gastrointestinal tract environment that is unfavorable to pathogenic growth [16]. Probiotics can decrease intestinal microbial catabolism and have a protein sparing effect, leading to reduced nitrogen flows [17].

A number of Bacillus strains could be used for feed additive in livestock industry. *Bacillus* are aerobic or facultative anaerobic, gram-positive, rod-shaped, and spore-forming bacteria. The spore-forming habit of *Bacillus* is highly beneficial for long term storage without a loss of activity, compared with non-spore-forming bacteria. Spores also have the ability to survive low pH, harsh environments, meaning their probiotic properties can benefit the small intestine [18].

Bacillus in swine can help to improve gut health and immunity for piglets and reduce environmental pollutants such as odor gas emissions from pig manure [19]. Upadhaya et al. [20] proposed that the reduction of fecal NH_3 emissions was observed when *Bacillus*-including feed was supplied to pigs, suggesting the improvement of nutrient digestibility by probiotics. However, Wang et al. [21] reported that it has no influence to enhance nutrient digestibility but indicates the effectiveness for the reduction of slurry NH_3 emissions.

The roles of *Bacillus* in poultry are similar to those in swine. Various effects have been observed in poultry fed with *Bacillus*, including histological changes in the intestine of broilers, increased villus height and villus height to crypt depth ratio, improved nutrient digestibility and absorption capacity of the small intestine [22], reduced digesta viscosity caused by soluble non-starch polysaccharides (which affect nutrient availability and absorption) [23], improved quality of meat and eggs [24], and reduced NH_3 emissions from manure [25].

Effects of Bacillus spp. in swine

Reduction of ammonia (NH_3) and hydrogen sulfide (H_2S) excretion

Ammonia and hydrogen sulfide are negative substances on farm workers as well as animals in swine production and cause environment pollution [26]. Nguyen et al. [27] found that the supplementation of Bacillus-containing feed showed an advantageous effect to decrease NH_3 emissions but have no effect on the reduction of other gases (H_2S and mercaptan). A recent study [27], showed that the addition of the increase in *Lactobacillus* inhibited pathogenic microorganisms and improved nutrient digestibility, resulting in reduced fecal NH_3 emissions.

Growing pigs fed diets with Bacillus *licheniformis* and Bacillus *subtilis* for 15 weeks, showed improved performance and reduced gas emissions due to increased fecal Lactobacillus counts and improved utilization of sulfur-containing amino acids [28]. It was concluded that the increase in Lactobacillus reduced intestinal pH through the production of organic acids, and that the bacitracin (bacteriocin) secreted by B. licheniformis inhibited the microbes that produce urease, thereby reducing NH_3 gas emissions. These results are supported by our research data, which showed that pigs fed diets with B. *subtilis* complex probiotics produced lower NH_3 and H_2S emissions after a three-week growing period (unpublished data). These results suggest that three weeks of feeding is needed for probiotic adherence in the gut to have positive effects in swine.

According to Balasubramanian et al. [29], when probiotics containing Bacillus *coagulans*, B. *licheniformis*, and B. *subtilis* were fed to growing and finishing pigs over 16 weeks, no reduction in fecal noxious gas (NH_3, H_2S) emissions was observed. Yan et al. [30] found that increased nutrient digestibility reduced the substrate for microbial fermentation in the large intestine, which resulted in a decrease in fecal gas emissions. Chen et al. [31] showed that dietary *Bacillus* supplementation decreased NH_3 emissions, however, other odor substances such as H_2S and mercaptan did not decrease.

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Bacillus spp. as probiotics can also affect the production of malodorous substances such as skatole. Skatole is a malodorous compound in meat and fecal that causes an off-flavor, so called “boar taint” [32]. Sheng et al. [33] demonstrated that dietary B. *subtilis* natto and B. *coagulans* supplementation decreased the skatole content of meat and feces. Doerner et al. [34] found that the reduced number of *Clostridium* in the feces of pigs fed *Bacillus* spp. was consistent with a lower skatole concentration in the meat and feces; *Clostridium* in feces is involved in the conversion of tryptophan to skatole.

Growth Performance in swine

Nguyen et al. [27] reported that dietary supplementation with probiotics-based *Bacillus* in weaning pigs linearly improved average daily gain (ADG) and average daily feed intake (ADFI) on days 0 to 7 of the experiment, as well as ADG and feed conversion ratio (FCR) on days 8 to 21. According to research by Lan et al. Kim et al. [28], dietary supplementation with B. licheniformis and B. subtilis complex probiotics (Bioplus YC) in growing pigs for 15 weeks resulted in improved growth performance in some periods but there was no significant difference from the control over the study period as a whole. The reasons for improved performance may be explained by changes in intestinal microorganisms and an increase in the secretion of digestive enzymes.

Hu et al. [35] observed that the ADG and FCR of piglets were improved and diarrhea occurrence was reduced when weaning pigs were fed B. subtilis KN-42 for 26 days. Greater bacterial diversity in the intestinal environment indicated an increase in the relative number of Lactobacillus and reduction in the relative number of E. coli in the feces. Wang et al. [21] also reported that ADG tended to increase linearly and ADFI increased as the levels of probiotic (Bioplus 2B®) increased, however, no linear or quadratic effects were observed in FCR.

In growing and finishing pigs, dietary direct-fed microbial (DFM) supplementation has been shown to have negative effects on growth performance. Growing and finishing pigs have better digestibility, improved immunity, and increased resistance to intestinal disorders [36]. Balasubramanian et al. [29] reported that dietary supplementation of three probiotic Bacillus strains (B. *coagulans*, B. *licheniformis*, and B. *subtilis*) did not show a positive effect on the ADG and FCR without affecting ADFI in growing and finishing pigs. Upadhaya et al. [37] reported that there were significantly effective to ADG and ADFI, when two probiotic complexes (B. *licheniformis* and B. *subtilis*) was supplied to growing and finishing pigs as feed additive during the experimental period.

According to Patarapreech et al. [38], both types of probiotic additives (Bacillus *subtilis* + Sanizyme®) improved the nutrient utilization of feed components [dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and gross energy (GE)] in the starter and grower periods, as well as growth performance (ADG, ADFI, FCR). Such improvements in nutrient digestibility may be due to the enzymes secreted by Bacillus spp., such as lipase, cellulose, amylase, and protease [39].

The results of probiotic complex supplementation are not consistent. The use of Bacillus-based probiotics in diets fed to finishing pigs did not affect ADG, FCR [40], ADFI, and FCR [31]. Davis et al. [39] also reported that two probiotic Bacillus strains (B. licheniformis and B. subtilis) were ineffective in improving the growth performance in growing and finishing pigs, when they were supplied in feed during test period.

Moreover, Sheng et al. [32] also found that the dietary supplementation of two probiotic complex (B. *subtilis* natto and B. *coagulans*) did not show a remarkable improvement of growth performance in growing pigs, but indicate dramatical effects in terms of meat quality, antioxidant function, and the skatole content of meat. The effects of Bacillus-based probiotics are influenced by a variety of factors, (e.g., age of the pigs, diet composition, differences in strains of bacteria, dosage levels, and breeding environment) [41].

Effects of other beneficial microbials

Growing pigs fed a diet with 10% palm kernel meal (PKM) and added probiotics (B. *subtilis* and Saccharomyces *cerevisiae*), showed a reduction in fecal NH_3, total mercaptans, and H_2S content [42]; pigs fed a diet without PKM produced less mercaptans than pigs fed diets with PKM. The addition of probiotics to a non-PKM diet had a significant effect on ADG and FCR, but the addition of probiotics to a diet with PKM did not have a positive effect on performance. These results may be due to the presence of non-starch polysaccharides in PKM creating a viscous environment in the gut.

Chen et al. [43] found the dietary supplementation of three probiotic complex (Lactobacillus *acidophilus*, S. *cerevisiae*, and B. *subtilis*) enhance ADG, when it was provided to the growing pigs for six weeks. In addition, fecal NH_3-N excretion was reduced when pigs were fed a probiotic complex, however, there was no effect on volatile fatty acid (VFA) production. Chen et al. [31] reported that dietary supplementation probiotics combination (B. *subtilis*, B. *coagulans*, and L. *acidophilus*) in finishing pigs reduced fecal NH_3-N production and improved ADG, however, there was no effect on ADFI or FCR. In their study, digestibility of N was not increased, therefore, the reduction in fecal NH_3-N may not have resulted from nutrient digestibility but rather changes in intestinal microflora.

Effects of Bacillus spp. in poultry

Reduction in ammonia (NH_3) and hydrogen sulfide (H_2S) excretion

In the poultry industry, Bacillus spp. probiotics are widely used. A reduction in NH_3 gas emissions from excreta was observed for poultry fed metabolic energy (ME)- and crude protein (CP)-reduced diets [44]. Poultry fed probiotic-supplemented diets also showed reduced NH_3 gas emissions compared with those fed diets

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without probiotics. Decreasing the CP content of the feed reduces the amount of synthetic amino acids supplied, thereby reducing the amount of N that is excreted by the poultry [45]. In addition, the feeding of probiotics can lead to increased nutrient utilization and changes in the balance of intestinal microorganisms, which can reduce NH_3 gas emissions.

According to research by Jeong et al. and Kim et al. [25], broilers fed a diet supplemented with B. *subtilis* C-3102 for five weeks, showed a reduction in NH_3 due to an increase in the number of *Lactobacillus* and reduction in the number of pathogenic bacteria. However, there were no effects on H_2S, mercaptan, or acetic acid production.

Ahmed et al. [46] reported that the supplementation of feed containing Bacillus *amyloliquefaciens* showed the effect of NH_3 reduction in feces during raising term. The observed reduction in NH_3 emissions from broiler excreta may be due to increased nutrient utilization and changes in intestinal microbiota. Another reason is that B. *amyloliquefaciens* reduced the pH of the feces. A reduced concentration of E. *coli* and improved utilization of sulfur amino acids in the intestine could reduce the conversion of fecal ammonium to volatile ammonia.

Tang et al. [47] indicated that inclusion of B. *amyloliquefaciens* product in laying hens reduced NH_3 production in a six-week feeding trial; the number of cecal Lactobacillus was increased, but the number of E. coli and Salmonella bacteria and NH_3 gas emission was reduced.

Performance in Poultry

The use of antibiotics in the poultry industry to control pathogenic infections, such as necrotic enteritis, has been banned in some places due to concerns about consumer safety. In such cases, *Bacillus* spp. have been used to improve performance through positive changes in intestinal microbiota.

Bacillus *subtilis* was added to a ME- and CP-reduced diet to evaluate the effects of probiotic supplementation related to energy and protein [44]. Poultry fed diets with reduced energy and protein content showed a decreased in ADG and FCR. However, animals fed diets with probiotics showed significant improvements in ADG and FCR in the growing and finishing periods. These performance improvements did not appear immediately; three weeks were required for normal enzyme production that produced effects.

A recent study by Jeong et al. & Kim et al. [25] found that broilers fed a diet with B. *subtilis* C-3102 showed improved ADG and FCR, however, there was no effect on meat quality. In this study, Lactobacillus counts in the cecum, ileum, and excreta were significantly increased, and E. *coli* counts in the cecum and excreta were decreased with dietary B. subtilis supplementation.

Ahmed et al. [46] reported that ADG, ADFI, and FCR were improved when broilers were fed a diet with a B. *amyloliquefaciens* probiotic; serum IgG and IgA were also increased. Tang et al. [47] reported that laying hens fed a diet with B. *amyloliquefaciens* commercial product for six weeks had better egg production, eggshell strength, and eggshell thickness than hens that received a non-supplemented diet.

Bacillus *amyloliquefaciens* has the ability to produce extracellular enzymes, such as cellulose, α-amylases, protease, and metalloproteases. Those enzymes can help to increase the efficiency of digestion and absorption of nutrients [48]. Bacillus *amyloliquefaciens* also produce bacteriocins, such as subtilin, which have antibacterial effects against pathogenic microorganisms [49].

Effects with other beneficial microbial

Probiotics have been used to reduce NH_3 emissions, improve performance, and maintain livestock product safety in the poultry industry, most commonly with a Bacillus spp. complex. In one study, a combination of Pichia *guilliermondii*, B. *subtilis*, and Lactobacillus *plantarum*, at a ratio of 1:2:1, reduced NH_3 gas emissions by 46% *in vitro* test [50]. This probiotic complex significantly decreased crude protein digestibility, pH, NH_3-N, urease, and uricase activity. Furthermore, the number of microorganisms responsible for fermenting carbohydrates to produce short chain fatty acids was increased.

Conclusion

In conclusion, dietary Bacillus spp. probiotic supplementation in monogastric animals can reduce NH_3 and H_2S production depending on the conditions. In terms of performance, there were various effects of supplementation level, viability, and composition of probiotic species, diet formulation, age of animals, livestock house environment, and so on. Nutrient digestibility can be improved by the enzymes or bacteriocin produced by *Bacillus* spp.

In addition, supplementation with *Bacillus* spp. can help reduce fecal odor production, gas emission, and improve the performance of monogastric animals. Additional studies of complex probiotics that satisfy both odor reduction and performance requirements for monogastric animals are recommended.

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