Efforts to reduce ammonia gas in broiler chicken litter with the use of probiotics

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Abstract. Ammonia is gas that can reduce livestock performance. The decrease in ammonia litter gas can be done by increasing the nutrient digestibility and inhibiting the uricase enzyme activity in the excreta. One way that can be done is by using probiotic. The aim of this study was to evaluate the use of probiotics as an effort to reduce levels of ammonia gas in broiler chicken litter with its application through drinking water and spraying on the litter. The animals were distributed into 4 treatments and 5 replications each of which consisted of 20 broiler chickens (10 male and 10 female). The design used in this study was a completely randomized design (CRD). Data were analyzed by using Analysis of Variance (ANOVA). The treatment used in this study was T0 = basal diet, T1 = T0 + Probiotic in drinking water, T2 = T0 + Probiotics Spray on litter, T3 = T0 + T1 + T2. Data showed that application of probiotic spray on litter and the combination can reduce significantly (P<0.01) the concentration of ammonia litter broiler cage. There was no effect on the pH and moisture of broiler cage litter.

Keywords: Ammonia, moisture, pH, probiotic.

1. Introduction
Broiler chicken is one of the ingredients of quality food with animal protein and is favored by the people of Indonesia. The target of production can be achieved in a short time and is relatively easy to farm. Good quality of feed ingredient needed to increase efficiency production of broiler. Good quality and economical feed ingredients are very difficult to find in Indonesia. The effect of a low-quality feed ingredient could reduce digestibility of nutrients. Low digestibility of nutrients can increase the number of nutrients in the excreta.

Nutrient accumulation in excreta will be a source of various types of pathogenic microbes. The high protein content in excreta will increase the chances of the formation of ammonia gas. Emissions of ammonia gas in the poultry cage caused a chemical reaction between uric acid and water as well as the uricase enzyme from the gram-negative of bacteria [1]. Ammonia Gas has the character of a pungent odor. The highest concentration of ammonia (NH₃) in the cage can reduce broiler chicken performance and can interfere the farmer health. The accumulation of excreta is a source of ammonia that could potentially pollute the environment [2]. Ammonia levels more than 25 ppm can interfere the
health and performance of broiler chickens, triggers the growth of Newcastle disease (New Castle Disease/ND) and damage to the respiratory system (in long time) [3]. The negative impact of the high concentration of ammonia in broiler chickens at 50 ppm and 75 ppm can reduce body weight by up to 17% and 20%. A lot of the dangers posed by the formation of ammonia gas which is capable of poisoning livestock and humans. At the level of 50 ppm of ammonia gas can cause irritation of the eyes, nose and throat (exposure for 2 hours), 100 ppm can cause eye irritation and irritation of the respiratory tract, 250 ppm is the limit of tolerance for human (exposure to 30-60 minutes), 700 ppm is immediately irritating to eyes and throat, >1500 ppm can cause pulmonary edema, coughing, laryngospasm [4].

The application of probiotics is one of a method to improve the feed digestible that supplying Lactic Acid Bacteria (BAL) in the small intestine. Improved digestibility of feed can reduce the number of feces and the accumulation of nutrients in the excreta that triggers the growth of microbes and the production of ammonia gas. The use of probiotics as inhibiting the growth of pathogenic bacteria can be provided inside or outside digestive tract of animal. In the digestive tract of livestock, probiotics act as competitors of pathogenic bacteria in nutrients and attachment of the villi of the intestine so that the digestibility of the feed can be increased. Probiotics outside the body serve to inhibit the production of ammonia gas which occurs due to a chemical reaction between the uric acid with water and the enzyme uricase from the negative gram bacteria by the competition in the utilization of the nutrients remaining in the excreta. So this study was conducted to evaluate the use of probiotics as an effort to reduce levels of ammonia gas of broiler little chicken with the application through drinking water and spraying on the litter.

2. METHOD

2.1. Date and Place
This research was conducted from March 2019 to May 2019. The location of the maintenance of the broiler chicken at the Field Laboratory of Poultry Animal Nutrition Block C Faculty of Animal Science, IPB University.

2.2. Animal and Cage
The bird used in this study were Lohmann broiler strains which were reared from Day Old Chick (DOC) to the age of 35 days. The proportion of male and female chickens used was balanced. The number of broiler chickens used was 400 which were distributed into 5 treatments and 5 replications, each replication consisted of 20 chickens.

The cage used in this study was a colony cage measuring 2 m × 1 m. The material used was bamboo. The base of the cage (litter) used was chaff. The cage was equipped with 2 bulbs of 100 watts, a place to feed and drink.

2.3. Diet
The diet used was the basal diet of pre-starter, starter, and finisher period. The diet was arranged based on the standard needs of the broiler [5]. Data on feed ingredient and nutrient content of basal diet are shown in table 1 and table 2.

2.4. Probiotics Composition
Probiotic used was from PT STM Tunggal Jaya (Jakarta) with the content of lactic acid bacteria of Lactobacillus sp. 10⁹ CFU mL⁻¹ and Bacillus sp 10⁹ CFU mL⁻¹. Before the application, probiotic was dissolved in mineral water with a ratio of 1:1 then fermented for 4 days. Dosage of Probiotic application in drinking water was 2 mL of probiotics in 1 L of drinking water while the dose for spraying was 80 mL per cage (2 m × 1 m)
Table 1. Feed Ingredient of the basal diet

| Ingredients         | Pre starter (%) | Starter (%) | Finisher (%) |
|---------------------|-----------------|-------------|--------------|
| Corn                | 58.70           | 56.00       | 59.00        |
| Rice Brand          | 0.00            | 4.59        | 6.55         |
| Soybean meal        | 19.40           | 22.50       | 16.50        |
| Meat Bone Meal      | 6.00            | 6.60        | 6.00         |
| Corn Gluten Meal    | 10.53           | 5.25        | 6.50         |
| CPO                 | 3.30            | 2.81        | 3.00         |
| CaCO₃               | 0.90            | 0.70        | 0.80         |
| NaCl                | 0.20            | 0.20        | 0.20         |
| Premix              | 0.50            | 0.50        | 0.50         |
| DL-Methionin        | 0.22            | 0.30        | 0.40         |
| Lysin               | 0.20            | 0.45        | 0.45         |
| Tryptophan          | 0.05            | 0.10        | 0.10         |

Table 2. Nutrient content of basal diet

| Nutrient          | Pre starter (%) | Starter (%) | Finisher (%) |
|-------------------|-----------------|-------------|--------------|
| DM %              | 90.26           | 90.13       | 89.94        |
| ME (Kkal kg⁻¹)    | 3203.18         | 3058.49     | 3104.95      |
| CP %              | 23.09           | 22.21       | 20.17        |
| EE %              | 2.72            | 2.74        | 2.94         |
| CF %              | 2.37            | 2.82        | 2.98         |
| Lysin %           | 1.05            | 1.33        | 1.16         |
| Methionin %       | 0.60            | 0.61        | 0.68         |
| Ca %              | 0.99            | 0.98        | 0.95         |
| P %               | 0.45            | 0.52        | 0.49         |
| Na %              | 0.16            | 0.17        | 0.16         |
| Cl %              | 0.19            | 0.20        | 0.20         |

DM = Dry Matter, Mc = Metabolizable Energy, CP = Crude Protein, EE = Ether Extract, CF = Crude Fiber, Ca = Calcium, P = phosphor, Na = Natrium, Cl = Chloride

2.5. Procedure

2.5.1. Preservation. Broiler chickens were reared for 35 days. On the first day, the chickens were fed with a sugar-water solution 10%; on the second day, they were fed with anti-stress vitamin. The dosage of probiotic application in drinking water was 2 mL of probiotics in 1 L of drinking water while the dose for spraying was 80 mL per cage (2 m × 1 m). Feed and drinking water was given in ad libitum.

2.5.2. Ammonia Measurement. The ammonia litter level was measured by using an ammonia meter. The ammonia meter before use was calibrated for 10 minutes. Ammonia level measurement was carried out during the day from 11.00 am - 12.00 am to get the highest ammonia level due to evaporation that occurred due to heat. Ammonia measurement was carried out first by turn off the exhaust fan and cooling fan for 30 minutes so the air around the cage did not spread. The ammonia meter used was equipped with an ammonia gas catching hose which was directed close to the litter. It was observed the increase in ammonia gas until the highest level of the ammonia gas was obtained.
2.5.3. **pH Measurement.** pH was measured by using a pH meter. The method to measure pH by dissolving the broiler chicken litter into the water with a ratio 1:10. Then the pH of litter and water mixture was measured by using pH meter.

2.5.4. **Moisture Measurement (%).** Moisture was measured by AOAC [6] method using oven 60 °C and Oven 105 °C. Litter was aerated first and then dried on oven 60 °C for 48 hours. After that, it was continued by analyzing the sample using Oven 105 °C for 3 hours. Total moisture was measured using calculation of analysis result from oven 60 °C and 105 °C.

2.5.5. **Experiment Design and Data Analysis.** The experiment design used in this study was a completely randomized design (CRD) with 5 treatments and 5 replications. Each replication consisted of 20 male and female broilers in the same proportion. The data were analyzed by Analysis of Variance (ANOVA). If the data obtained by the different statistical method was continued with the Duncan test. The mathematic model based on Steel and Torrie [7]:

\[ X_{ij} = \mu + \tau_i + \epsilon_{ij} \]  

\( X_{ij} \) = The value of the observation on the probiotic treatment and replication  
\( \mu \) = General mean  
\( \tau_i \) = Effect of the probiotic treatment  
\( \epsilon_{ij} \) = Error of treatment  
\( i \) = The number of treatment  
\( j \) = The number replication

2.5.6. **Treatment and Parameters.** The treatment in this study was T0 = basal diet, T1 = T0 + Probiotics dissolved in drinking water, T2 = T0 + Probiotics Sprayed to the litter, T3 = T0 + T1 + T2. The parameters observed were the concentration of ammonia of litter, pH of litter and moisture of litter.

3. **Result and Discussion**

3.1. **Environmental Conditions**  
Environmental conditions of the cage can play an important role in the performance and health of livestock. The environmental condition of the cage is illustrated from the data on the temperature and humidity of the cage presented in table 3.

The temperature and humidity in this study was the optimal temperature for broilers, which is in line with the statement [8] that the uniform temperature and humidity of the cage during the brooding period will generate good broiler performance. The increase of the brooding period was 14 days, with the initial temperature setting was 28°C - 32°C and humidity of 60% - 80%. The average temperature and humidity of broiler chickens aged 1 day - 3 days, 4 days - 6 days, 7 days - 14 days, 15 days - 21 days and 22 days - 35 days recommended for optimum production of growth were 32 °C, 31°C, 30°C, 28°C, 26°C with 60% humidity [9].

3.2. **Litter Condition of 35 Days old Broiler Chicken**  
The addition of probiotics by means of spraying it on the litter and a combination of spraying on the litter by significantly dissolving it in drinking water (P <0.01) can reduce ammonia litter level and did not influence pH and moisture of litter. The data on the effect of probiotics on ammonia level, pH and moisture of litter can be seen in table 4.
### Table 3. Temperature and relative humidity of the cage during the research

| Time   | Parameter | Morning | Afternoon | Evening | Night |
|--------|-----------|---------|-----------|---------|-------|
| Week 1 | Temperature | 27.52   | 33.52     | 29.71   | 29.38 |
|        | RH        | 64.17   | 53.17     | 64.83   | 68.00 |
| Week 2 | Temperature | 25.57   | 32.55     | 26.77   | 25.98 |
|        | RH        | 79.83   | 67.50     | 80.15   | 76.00 |
| Week 3 | Temperature | 25.25   | 31.95     | 27.40   | 25.33 |
|        | RH        | 81.50   | 71.50     | 75.67   | 81.00 |
| Week 4 | Temperature | 26.83   | 31.83     | 25.38   | 25.62 |
|        | RH        | 80.58   | 70.00     | 81.00   | 80.17 |
| Week 5 | Temperature | 26.44   | 32.56     | 26.60   | 24.00 |
|        | RH        | 84.00   | 70.20     | 77.80   | 81.20 |
|        | Temperature | 26.33   | 32.61     | 27.10   | 25.97 |
| Average| RH        | 78.13   | 66.76     | 76.00   | 76.88 |

RH = Relative humidity, morning from 06.00-07.00, afternoon from 12.00-13.00, evening from 18.00-19.00, Night from 23.00-24.00

### Table 4. Effects of herbal probiotics on ammonia level, pH and moisture of litter of 35-days-old broiler chickens

|        | T0          | T1          | T2          | T3          |
|--------|-------------|-------------|-------------|-------------|
| Ammonia (ppm) | 31.36±3.88c | 20±2.29b   | 13.26±3.33a | 9.84±3.46a |
| pH     | 7.52±0.20   | 7.46±0.20   | 7.48±0.04   | 7.40±0.21   |
| Moisture (%) | 27.72±7.28 | 26.40±12.20 | 36.03±17.81 | 34.62±9.13 |

Superscript in the same column describe statistic significantly level, T0 = basal diet, T1 = T0 + Probiotics dissolved in drinking water, T2 = T0 + Probiotics sprayed on the litter, T3 = T0 + T1 + T2

Ammonia is a gas from the decomposition of nitrogen waste in excreta, such as uric acid, unabsorbed proteins, amino acids and other non-protein nitrogen (NPN) compounds due to the activity of microorganisms in feces [10]. Moisture in the litter indicates water content that a good condition for the growth of a microbe. The use of probiotics by spraying can inhibit the chemical reaction of the formation of NH₃ between uric acid and water and the uricase enzyme from gram (-) bacteria. Lactic acid bacteria that sprayed on the litter which has been exposed to feces before, it will inhibit the formation of the uricase enzyme that converts uric acid in broiler chickens to ammonia gas. The pH and moisture content of the litter had no influence due to the spraying probiotics because the dosage of probiotics spraying was very small, only 80 ml in 2 square meters of the cage. The water content in probiotics will evaporate every day. Probiotic was able to produce bacteriocin which will be able to suppress gram-negative microbial growth so that the production of uricase enzymes are suppressed, inhibiting the conversion of excreta uric acid to ammonia [11]. The addition of ingredients such as antibiotics, prebiotics, probiotics, and symbiotics can reduce ammonia formation by decreasing bacterial activity in the digestive tract [12].

Supplementation of soluble probiotics in drinking water can reduce the concentration of ammonia gas up to 36.22%, by spraying and combination can reduce ammonia gas 57.72% - 68.89%. The decrease in ammonia gas concentration occurs because the administration of probiotics can increase the digestibility of nutrients, especially protein digestibility so that the number of nutrients wasted in feces will decrease. Nutrients are needed by microbes in feces to grow so that increasing the number of nutrients will increase the chance of increasing the population of pathogenic microbes that are able to convert uric acid in feces into ammonia gas by the formation of the uricase enzyme.
4. Conclusion
Probiotic can significantly (P<0.01) reduce the ammonia level of litter on the 35-days-old broiler cage. The best treatment to reduce ammonia levels was sprayed on litter and the combination. There was no influence on Moisture and pH of litter by application of probiotic.

5. Reference
[1] Yusrizal F, Manin Y and Noverdiman 2012 The use of probiotic and prebiotic (symbiotic) derived from palm kernel cake in reducing ammonia emission in the broiler house Proc. The 1st Poul Int. Sem. 9(1) 333-343
[2] Bittman S and Mikkelson R 2009 Ammonia emissions from agricultural operations: livestock. Better Crops 93(1) 28-31
[3] Ritz C W, Fairchild B D and Lacy M P 2004 Implications of ammonia production and emissions from commercial poultry facilities: a review. J. Appl. Poult. 13 684-692
[4] Agency for Toxic Substance and Disease Registry (ATSDR) 2004 Toxicological profile for ammonia (Atlanta, GA: ATSDR) p 223.
[5] Leeson S and Summers J D 2005 Commercial Poultry Nutrition. 3rd Edition (Canada, US: Nottingham University Press)
[6] AOAC 2005 Official Methods of Analysis 18th edition (Gaithersburg, MD: Association Of Analytical Chemists).
[7] Steel R G D and J H Torrie 1993 Prinsip dan Prosedur Statistika (Pendekatan Biometrik) Penerjemah Sumantri B (Jakarta, ID: Gramedia Pustaka Utama)
[8] Setiawan I and Sujana E 2009 Bobot Akhir, Persentase Karkas dan Lemak Abdominal Ayam Broiler yang Dipanen Pada Umur Yang Berbeda. Seminar Nasional Fakultas Peternakan UNPAD.
[9] Pokphand C 2005 Manual Managemen Broiler CP 707 http://www.charoendpokphand.com
[10] Charles R T and Hariono B 1991 Pencemaran lingkungan oleh limbah peternakan dan pengelolaannya Bull. FKG-UGM X(2) 71-75
[11] Riza H, Wizna R and Yusrizal Y 2015 Peran Probiotik dalam Menurunkan Amonia Feses Unggas JPI 17(1) 19-26
[12] Yusrizal and Chen T C 2003 Effect of adding chicory fructans in feed on fecal and intestinal microflora and excreta volatile ammonia Int. J. of Poult. Sci. 2(3) 188-194