Effort to reduce ammonia gas in the broiler chicken excreta with the addition of probiotic as substitute for antibiotic growth promoter

B P Mahardhika1,*, R Mutia1 and M Ridla1

1Department of Nutrition and Feed Technology, IPB University, Faculty of Animal Science, Bogor, Indonesia

* E-mail: brahamahardhika@gmail.com; phone:085212345169

Abstract. This study has been carried out to evaluate the use of drinking water-soluble probiotics as an alternative to Zinc bacitracin Antibiotic Growth Promoter (AGP) in an effort to reduce the concentration of ammonia excreta as a result of increased feed digestibility. The probiotic used contains $2.0 \times 10^7$ CFU mL$^{-1}$ Lactobacillus sp, $1.6 \times 10^7$ CFU mL$^{-1}$ Bacillus sp, and $7.4 \times 10^9$ CFU mL$^{-1}$ Streptomyces sp. The excreta sample was obtained from 15 male Lohmann strain broiler chickens aged 35 days from a total population of 300 chickens that had previously been reared since Day Old Chick. The chickens were distributed into three treatments and five replications in a metabolic cage with a size of $50 \text{ cm} \times 30 \text{ cm} \times 56 \text{ cm}$ for three days. This study used Completely Randomized Design (CRD) with Analysis of Variance (ANOVA). The addition of probiotic significantly reduced ($P <0.01$) water content and ammonia concentration of broiler chicken excreta. The addition of drinking water-soluble probiotics significantly increased ($P <0.01$) the feed intake and feed digestibility of broiler chicken. The use of probiotics was better than Zinc bacitracin in reducing excreta ammonia concentration and feed digestibility.

1. Introduction

Ammonia gas is one of the gases that causes air pollution and causes livestock health problems and even human health. Ammonia gas is the result of decomposition of nitrogenous waste material in poultry excreta such as uric acid, excreted proteins and other non-protein nitrogen (NPN) compounds that occur due to excreta microorganism activity [1]. Sources of ammonia emissions are from animal manure, fertilizer, and industrial processes [2]. The most ammonia gas emissions come from the livestock industry, which is 80% to 90%. Ammonia gas concentration greater than 20 ppm will reduce performance and affects broilers health [3]. Ammonia gas concentration that exceeds 25 ppm and has exposure for more than 8 hours will have a negative impact on human health [4].

Ammonia gas is formed due to the conversion of metabolic waste such as uric acid into ammonia gas with the help of the uricase enzyme. The main source of ammonia gas formation in poultry farming environments comes from excreta. Accumulation of nutrient levels that are wasted through excreta causes pathogenic bacteria to grow rapidly because the nutrient intake is fulfilled. The process of converting uric acid to ammonia is triggered by uricase enzyme activity. Efforts should be made to reduce the excretion of excreta and nutrient accumulation in excreta. The method that can be taken is...
by increasing the feed’s digestibility so that the absorption of nutrients is better, and many nutrients are not excreted.

Various methods have been proven to improve the feed digestibility, for example, the addition of Antibiotic Growth Promoter (AGP). In addition to increasing the digestibility of feed, the use of AGP can also improve the health of livestock from pathogenic bacteria in the digestive tract. A long period of AGP addition can cause resistance in livestock and leave residue in livestock organs [5]. Based on this reason, AGP is banned in almost all over the world started by the European Union [6]. This rule worries the farmers because feed efficiency and livestock health will deteriorate. The low feed efficiency will increase excreta production, so it is feared that the cage environment will be unhealthy due to increased in ammonia gas emissions. Another alternative that can be used to replace AGP is by giving probiotics. According to [7], probiotic is a multiplying living microbe in the digestive tract to maintain microflora balance.

Probiotics can increase feed efficiency and digestive tract health as well as benefit the environment. The use of drinking water-soluble probiotics with incubation measurements of 1 hour and 24 hours can reduce ammonia concentration in the faeces and litter of broiler chickens [8]. The use of drinking water-soluble probiotics can reduce ammonia concentration broiler chickens litter from 31.36 ppm to 20 ppm [9]. Decreased ammonia levels due to probiotic addition are due to increased feed digestibility, especially the crude protein. According to [10], the addition of probiotics can increase the growth of broiler chickens, nutrient digestibility and apparent metabolic energy corrected by nitrogen (AMEn) and the composition of microflora in the cecum. According to [3], increased digestibility, and the composition of microflora in the digestive tract due to the addition of the probiotics have an impact on the reduction in ammonia gas derived from the decomposition of nitrogenous wastes in excreta. Addition of additives such as antibiotics, probiotics, prebiotics, and symbiotics can reduce ammonia by reducing pathogenic bacteria’s activity in the digestive tract [11].

2. Methods

2.1. Material

2.1.1. Animal and Cages

This study used 15 male Lohmann strain broiler chickens aged 35 days obtained from a total population of 300 chickens that had previously been reared since Day Old Chick. The chickens were distributed into three treatments and five replications in a metabolic cage with 50 cm × 30 cm × 56 cm for three days. During the DOC phase, chickens were kept in a colony cage (2 m × 1 m) with a base of rice husks. One sample of broiler chickens in each test was transferred to the metabolic cage in 35 days old for an excreta collection. Treatment was given from the beginning of maintenance.

2.1.2. Probiotic

This study used probiotics with constituent microorganisms in the form of lactic acid bacteria Lactobacillus sp, and Bacillus sp, and moulds of Streptomyces sp. The probiotic used was tested at the Indonesian Biotechnology and Bioindustry Research Center (2019) which has 2.0 × 10⁷ CFU mL⁻¹ Lactobacillus sp, 1.6 × 10⁷ CFU mL⁻¹ Bacillus sp, and 7.4 × 10⁶ CFU mL⁻¹ Streptomyces sp. The form of probiotic is liquid. The probiotics were dissolved into mineral water in a 1: 1 and fermented for four days–five days before use. The dose was 2 mL probiotics per litre of drinking water.

2.1.3. Diet

The diet used in this study was basal feed for finisher period broilers based on the recommendation by [12]. In the antibiotic treatment, AGP supplemented in was the form of 0.04% zinc bacitracin. Data on the composition of feed ingredients and nutrient content of broiler chicken diet are presented in Table 1.
Table 1. The composition of feed ingredients and nutrient content of broiler chicken diet

| Material               | Basal   | AGP    |
|------------------------|---------|--------|
| Yellow Corn            | 59.00   | 59.00  |
| Rice Brand             | 6.55    | 6.55   |
| Soy Bean Meal          | 16.50   | 16.50  |
| Meat Bone Meal         | 6.00    | 6.00   |
| Corn Gluten Meal       | 6.50    | 6.50   |
| Crude Palm Oil         | 3.00    | 3.00   |
| CaCO3                  | 0.80    | 0.80   |
| NaCl                   | 0.20    | 0.20   |
| Premix                 | 0.50    | 0.50   |
| DL-Methionine          | 0.40    | 0.40   |
| Lysine                 | 0.45    | 0.45   |
| Tryptophan             | 0.10    | 0.10   |
| Total                  | 100     | 100    |
| Zinc bacitracin (%)    | 0       | 0.04   |

Nutrient

| Nutrient                  | Basal | AGP  |
|---------------------------|-------|------|
| Dry Matter %              | 89.94 | 89.94|
| Metabolizable Energy (Kkal kg⁻¹) | 3004.95 | 3004.95 |
| Crude Protein %           | 20.17 | 20.17|
| Ether Extract %           | 2.94  | 2.94 |
| Crude Fiber %             | 2.98  | 2.98 |
| Lysine %                  | 1.16  | 1.16 |
| Methionine %              | 0.68  | 0.68 |
| Calcium %                 | 0.95  | 0.95 |
| Phosphor %                | 0.49  | 0.49 |
| Natrium %                 | 0.16  | 0.16 |
| Chloride %                | 0.20  | 0.20 |

2.2. Procedure

2.2.1. Measurement of Feed Digestibility and Excreta Moisture
Measurement of feed digestibility was carried out using a modified method by [13] the total excreta collected method. Broilers were kept in a metabolic cage with feed adaptation. After that, broilers were fasted 24 hours to empty the digestive tract to avoid the previous feed’s influence. Drinking water was given ad libitum. Broiler chickens were fed and collected the excreta every day for three days. Every day, feed intake and excreta fresh weight measurement were taken. The collected excreta was put in the freezer for 48 hours. After that, the excreta was dried in an oven in 60°C and 105°C. The dry weight of excreta and excreta moisture was calculated using the [14] method and feed digestibility.

2.2.2. Measurement of Ammonia Excreta Concentration
Ammonia measurement was carried out using ammonia meters. Before use, the ammonia meter was calibrated for 10 minutes. Ammonia gas was measured every day around 11.00 pm–12.00 pm. Ammonia measurement was carried out in each metabolic cage of broiler chickens. The device was equipped with an ammonia gas catching hose close to the excreta storage container in a metabolic cage.
2.3. Experimental Design and Data Analysis

The experimental design used was a completely randomized design (CRD) with three treatments and five replications. This study used Analysis of Variance (ANOVA) with the DMRT (Duncan Multiple Range Test) tests if obtained significantly different data. The mathematical model used is based on (15) as follows:

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

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

3. Results and Discussion

3.1. Environmental Conditions

This study used a comfortable temperature for poultry in the morning and at night, but the temperature is higher during the day. Humidity in the metabolic cage was relatively high. Data on temperature and humidity on the metabolic cages used in this study presented in Table 2.

| Table 2. Temperature and humidity on the metabolic cages |
|----------------|----------------|----------------|----------------|
| Day | Parameter | Morning | Afternoon | Night |
| 1 | Temperature | 25.00 | 32.50 | 24.00 |
| | RH | 87.00 | 80.00 | 88.00 |
| 2 | Temperature | 26.50 | 30.00 | 23.50 |
| | RH | 86.00 | 80.00 | 90.00 |
| 3 | Temperature | 24.30 | 33.50 | 23.00 |
| | RH | 85.00 | 79.00 | 90.00 |
| Average | Temperature | 25.27 | 32.00 | 23.50 |
| | RH | 86.00 | 79.67 | 89.33 |

RH= Relative Humidity, Morning (05.00–06.00), Afternoon (11.00–12.00), Night (18.00–19.00)

The temperature in the metabolic cages in this study tended to be a comfortable temperature for the poultry except during the daytime. This study used standard maintenance of broiler chickens based on [16] that the optimal temperature of broiler chicken maintenance in the finisher (22 days–35 days) was not more than 26°C with 60% humidity.

3.2. Feed Digestibility and Ammonia Excreta Concentration

The addition of drinking water-soluble probiotics significantly (P<0.01) reduced the moisture and ammonia excreta concentration of broiler chickens. The addition of Zinc bacitracin AGP significantly (P <0.01) reduced the ammonia excreta of broiler chickens. The addition of drinking water-soluble probiotics significantly (P <0.01) increased feed intake and feed digestibility of broiler chicken. Data on ammonia excreta and feed digestibility of broiler chicken are presented in Table 3.
Table 3. Ammonia excreta and feed digestibility of broiler chicken

| Parameter                  | T0               | T1               | T2               |
|----------------------------|------------------|------------------|------------------|
| The Ammonia of Excreta (ppm)| 37.33 ± 4.51A    | 31.01 ± 2.31B    | 25.00 ± 1.74C    |
| The Moisture of Excreta (%)| 76.35 ± 5.95A    | 67.76 ± 1.68B    | 67.42 ± 0.92B    |
| Dry Mater of Excreta (%)   | 23.65 ± 5.95A    | 32.24 ± 1.68B    | 32.58 ± 0.96B    |
| Feed Intake (g)            | 106.81 ± 3.78A   | 112.85 ± 5.52A   | 138.04 ± 4.57B   |
| Excretion of Excreta (g)   | 38.08 ± 2.66     | 35.50 ± 6.46     | 36.83 ± 1.92     |
| Feed Digestibility (%)     | 64.34 ± 2.28A    | 68.49 ± 2.28AB   | 73.30 ± 1.53B    |

T0 = control (basal diet), T1 = T0 + AGP (zink bacitrasin 0.04%), T2 = T0 + drinking water soluble probiotic.

The use of Zinc bacitracin antibiotics and drinking water probiotics can reduce ammonia excreta concentration. The decrease in ammonia was due to an increase in feed digestibility. Ammonia excreta concentration was related with the feed digestibility with a coefficient of determination of 0.506. The relationship between feed digestibility and ammonia excreta concentration can be seen in Graph 1.

![Ammonia (ppm)](image)

**Figure 1.** The relationship of feed digestibility and ammonia excreta

An increase in feed digestibility will reduce ammonia excreta concentration. An increase in ammonia excreta was due to an increase in the balance of microflora in the digestive tract. The addition of probiotics in drinking water can increase the total population of lactic acid bacteria in the digestive tract to improve the ileum morphology by increasing the length of villi and ileum crypt depth (17). The more nutrients ingested shows, the lesser waste of nutrients in the faeces. If more nutrients are left in the faeces, it will increase uric acid conversion to ammonia by the uricase enzyme. According to (18), probiotics can restrain the activity of decomposing proteins in faeces and litter so that a decrease in ammonia gas concentration can occur. Probiotics contain lactic acid bacteria which can produce protease enzymes (19). Proteolytic bacteria such as Bacillus sp inhibit uric acid conversion to ammonia by using it as a nutrient (20).

The use of Zinc bacitracin antibiotics and drinking water-soluble probiotics can reduce excreta water content. The decrease in the excreta water content will be in line with the decrease in ammonia gas (3). The dry excreta of broiler chickens indicate that the chickens are not in a diarrhoea condition. Wet excreta is caused by the digestive tract in a less favourable condition due to pathogenic bacteria so that the chickens will have diarrhoea or wet excreta. The water content obtained in this study was classified into normal excreta water content based on (12) which is 60%–80%.

Feed digestibility is related to feed intake. The use of drinking water-soluble probiotic on the broiler chicken can increase feed intake during feed digestibility measurements. The more feed consumed and not excreted through faeces will increase in feed digestibility. An increase in feed
intake as a result of probiotic supplementation was due to an increase in feed digestibility. An increase in feed digestibility was due to the addition of probiotics that can improve the balance of microflora and improve intestinal morphology so that feed can be digested more quickly.

The use of water-soluble probiotics can decrease ammonia excreta concentration more than the Zinc bacitracin antibiotic. This condition happened because Zinc bacitracin is a bactericidal antibiotic as the only sensitive to suppression of gram-positive microbial pathogen populations. According to (17), the addition of probiotics can increase the total bacterial population and a total population of lactic acid in ileum without affecting Salmonella and E.coli populations as well as the addition of Zinc bacitracin antibiotic. The broiler feed digestibility process with water-soluble probiotics was better than Zinc bacitracin in decreasing the ammonia concentration.

4. Conclusion
The addition of drinking water-soluble probiotics can reduce the ammonia concentration in broiler chicken excreta as a result of increased feed digestibility. The addition of probiotics through drinking water was more effective in reducing ammonia excreta concentration compared with the use of Zinc bacitracin antibiotic growth promoter.

5. References
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