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Utilization of Bacterial Consortium as a Biodisinfectan to Reduce Ammonia (NH_3) Odor in Broiler Feces

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ABSTRAK

Populasi ayam broiler tahun 2021 mencapai 3,11 miliar ekor, limbah feses mencapai 0,15 kg/ekor setiap hari Populasi broiler tahun 2021 mencapai 3,11 miliar ekor, limbah feses mencapai 0,15 kg/ekor setiap hari dengan potensi penghasil amonia (NH_3) 54%. Untuk mengurangi konsentrasi ammonia (NH_3) dibutuhkan biodisinfektan yang diproduksi dari konsorsium bakteri. Penelitian ini melakukan uji terhadap feses yang diberikan biodisinfektan mencakup total bakteri, konsentrasi amonia, nitrit, nitrat dan pH feses. Uji total bakteri menggunakan uji *Total Plate Count* (TPC), konsentrasi nitrit dan nitrat di uji mengacu SNI 06-2484-2004, uji pH menggunakan pH meter digital dan uji konsentrasi amonia (NH_3) mengacu SNI 06-6989.30-2005. Analisis data menggunakan *Analisis of Variance* (ANOVA) taraf kepercayaan 95% dan uji lanjut Duncan menggunakan software SPSS 26.00. Hasil penelitian ini menunjukkan bahwa total bakteri biodisinfektan terbaik didapatkan pada perlakuan P3 (15% biodisinfektan). Kemudian Penurunan konsentrasi beberapa variabel pada feses perlakuan seperti nitrit yaitu 96,06%, nitrat yaitu 52,46%, amonia (NH_3) yaitu dari 46 ppm menjadi 6 ppm atau penurunan sebesar 76,6% serta nilai pH terendah yaitu pH 4.7 juga di dapatkan pada perlakuan P3 (15% biodisinfektan). Penurunan konsentrasi amonia (NH_3) sesuai dengan standar yang ditetapkan oleh Peraturan Menteri Lingkungan Hidup Indonesia Nomor 5 tahun 2014 yaitu 0,30 kg/ton atau setara dengan 30 ppm dan penurunan konsentrasi nitrit maupun nitrat sesuai dengan Peraturan Pemerintah Indonesia No. 82 tahun 2001.

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ABSTRACT

In 2021, the broiler chicken population reached 3.11 billion heads, generating 0.15 kg of fecal waste per head per day, with an ammonia (NH_3) production potential of 54%. To reduce ammonia concentration, biodisinfectants derived from a bacterial consortium are needed. This study tested the effect of biodisinfectants on feces by measuring total bacteria, ammonia concentration, nitrite, nitrate, and fecal pH. Total bacteria were analyzed using the Total Plate Count (TPC) method, while nitrite and nitrate concentrations followed SNI 06-2484-2004. pH was measured using a digital pH meter, and ammonia concentration was tested based on SNI 06-6989.30-2005. Data analysis was performed using Analysis of Variance (ANOVA) with a 95% confidence level and Duncan's multiple range test using SPSS 26.00. The results showed that the best treatment was P3 (15% biodisinfectant), which produced the highest total bacteria count. Nitrite concentration decreased by 96.06%, nitrate by 52.46%, and ammonia from 46 ppm to 6 ppm (a reduction of 76.6%). The lowest pH value (4.7) was also observed in the P3 treatment. The decrease in ammonia concentration complies with the Indonesian Minister of Environment Regulation No. 5 of 2014, which sets a limit of 0.30 kg/ton or 30 ppm. Additionally, the reductions in nitrite and nitrate align with Indonesian Government Regulation No. 82 of 2001. These findings indicate that bacterial consortium-based biodisinfectants effectively reduce ammonia levels and related parameters in broiler chicken fecal waste.

INTRODUCTION

The increase of Indonesian people population was followed by the high amount of food consumption, especially broiler meat. According to the Central Statistics Agency (2021), the consumption of broiler meat in Indonesia was 3.22 million tons in 2020 and will increase to 3.43 millions tons in 2021. The high consumption was depended on the total population of broiler chickens. Central Statistics Agency (2021) said that the poultry population in Indonesia has experienced a significant increase, they were 305.4 millions of domestic chickens, 345.2 millions of layer chickens and 3.11 billions of broiler chickens.

The high population of poultry, especially broiler chickens was followed by the high load of fecal waste. The amount of chicken manure excreted is 0.15 kg per chicken per day with 26% of dry matter so that can cause smelly gas (Fuchs *et al*., 2018). The accumulation of faecal waste will increase the concentration of nitrogen and surfide which are decomposed by microbes into ammonia gas (NH_3), nitrite, nitrate and sulfide gas. Among the various gases, ammonia gas (NH_3) has the highest percentage, reaching 54% of the total gas (Fuchs *et al*., 2018).

Gates *et al*. (2005) explained that the emission of ammonia gas (NH_3) from broiler chicken farms with a capacity of 25,000 heads is 0.45 ppm/head/day or around 11.25 ppm/day. It can be calculated that in 30 days of maintenance, the emissions of 337.5 ppm ammonia gas (NH_3) was released into the environment. According to Ritz *et al*. (2004), the level of ammonia gas (NH_3) in broiler chicken cages should not be more than 25 ppm. Ammonia (NH_3) levels of 20 ppm will increase tetelo disease and damage the respiratory system, ammonia (NH_3) levels of 25 ppm will cause a decrease in feed efficiency and cause infectious bursal disease. Ammonia (NH_3) levels of 25-125 ppm decrease feed consumption and feed efficiency, causing poisoning in broiler chickens including irritation of the trachea, inflammation of the air sacs, conjunctivity, and dyspnea as well as massive mortality (Ritz *et al.*, 2004; Reza *et al.*, 2022; Mahardhika *et al.*, 2019; Zhou *et al.*, 2021).

The serious impact caused by the ammonia gas (NH_3) exposure on the performance of broiler chickens, odor reduction efforts are needed. Efforts to control the odor can be conducted by spraying biological compounds to the feces, namely biodisinfectants which are produced from bacteria such as *Pseudomonas aeruginosa*, *Nitrosomonas* sp, *Nitrococcus*, *Strepthomyces sp* and *Micrococcus,* these bacteria can accelerate the process of oxidation of ammonia (NH_3), nitrites, nitrates and accelerate the decrease in the pH of broiler chicken feces (Al-Adham *et al*., 2013; Vilela *et al*., 2020; AlKahfi *et al*., 2021).

Manin *et al*. (2012) proven that various types of bacteria such as acid-producing bacteria, proteolytic bacteria and phosphate solubilizing bacteria can work synergistically to reduce emissions of ammonia gas (NH_3) in poultry feces. Lactic acid-producing bacteria can reduce pH of the feces, inhibit the activity of proteolytic enzymes and decrease the number and activity of gram-negative bacteria so that they can cut the cycle of ammonia gas (NH_3) formation in the feces (Santoso, 2001). Based on the researches, it is important to make a biodisinfectant formulated from a consortium of bacteria that has competitive properties to inhibit ammonia-producing microbes, so that it can suppress gas formation and reduce the concentration of ammonia gas (NH_3) in broiler feces. A bacterial consortium is a collection of several bacteria that live together in a medium. Bacterial consortia have complementary functions and can survive in various conditions.

METHODS

Preparation of bacterial consortium. The bacteria needed were prepared that are *Pseudomonas aeruginosa*, *Nitrosomonas* sp, *Nitrococcus* organic matter solvent bacteria *Strepthomyces* sp and *Micrococcus* phosphate solvent bacteria. All bacteria were mixed with the volume of 100 mL of each bacteri into 1 L of aqudest. The mixture of bacteria and aqudest was put into the MRS broth medium and was incubated for 48 hours, incubation using an incubator at a temperature of 37 ºC. The consortium then was ready for used (Verma *et al.*, 2022).

Production of biodisinfectants according to the treatments. Biodisinfectants were made using 4 treatments, namely: P0 (negative control) with a formulation of 60% aquadest, 10% rice water, 10% coconut water, 10% maltodextrin and 10% molasses; P1 with a formulation of 55% aquadest, 10% rice water, 10% coconut water, 10% maltodextrin, 10% molasses and 5% bacterial consortium; P2 with a formulation of 50% aquadest, 10% rice water, 10% coconut water, 10% maltodextrin, 10% molasses and 10% bacterial consortium; P3 with a formulation of 45% aquadest, 10% rice water, 10% coconut water, 10% maltodextrin, 10% molasses and 15% bacterial consortium. Each treatment was produced in 3 replicates. The ingredients were mixed homogeneously, then were fermented in drums for 2 weeks aerobically (Al-Adham *et al*., 2013; Vilela *et al*., 2020; AlKahfi *et al*., 2021).

Counting of total bacteria. The density of total bacterial cells in the biodisinfectant medium was calculated using TPC (Total Plate Count) method. Cell density was calculated in agar plate media PCA prepared by dissolving 4.96 gr, in 120 mL of distilled water. Then the media was autoclaved at 121ºC with a pressure of 1.5 atm for 15 minutes. The media were poured into eight petri dishes aseptically. Each treatment of biodisinfectan was taken as much as 1 mL and then diluted with 9 mL of distilled water. The mixture was homogenized then dilution was carried out in stages up to three times or 10^{-3}. Then, 100 µL of the diluted was taken and inoculated into agar plate media, the inoculant was leveled with Drygalski (spread method) then incubated at room temperature for 24 hours. Each cell density was calculated by the Colony Forming Unit (CFU) method using a colony counter (Ekamaida, 2017; Townsend & Naqui, 1998).

Application of the biodisinfectant to the broiler chicken faeces. This application was used 6 treatments and 3 replications for each treatment. The treatments included P0 (negative control) without biodisinfectant, P1 (5% biodisinfectant), P2 (10% biodisinfectant), P3 (15% biodisinfectant), P4 (20% biodisinfectant), P5 (25% biodisinfectant) and P6 (30% biodisinfectant). The application of biodisinfectant for each treatment was carried out by spraying biodisinfectan all treatment it on the piles of feces once a week. After spraying the feces samples from each treatment were taken weekly to measure ammonia (NH_3), nitrite, nitrate and pH levels.

Testing of nitrite concentration. Testing for nitrite concentration based on the reference from Indonesian National Standard SNI No. 06-2484-2004. This method can measure the concentration of nitrite in water samples in the range of 0.01-1.0 mg/L NO_2-N at a wavelength of 543 nm (Dwirastina & Ditya, 2019; Indonesian National Standard, 2004).

Testing of nitrate concentration. Testing for nitrate concentration based on the references from Sohail & Adeloju (2016) and the Indonesian National Standard (2004). This method can measure the concentration of nitrate in water samples up to 10 mg/L NO_3-N at a wavelength of 410 nm.

Calculation of the pH value of broiler chicken feces. pH measurement was carried out using a pH meter (Hana) in the every week for four weeks. As much as 40 grams of fecal material that has been treated was put into 400 mL of aquadest in a beaker, plug the tip of the pH meter (Hana) into the sample solution until a constant pH level is obtained (Shukla *et al*., 2007; Galster, 1991).

Testing the concentration of ammonia (NH_3). Testing the concentration of ammonia (NH_3) based on the reference from Indonesian National Standard SNI 06-6989.30-2005. Testing the concentration of ammonia (NH_3) was carried out with phenate spectrophotometer. This test can measure ammonia (NH_3) in the range of 0.1-0.6 mg/L NH_3-N at a wavelength of 640 nm (Indonesian National Standard, 2005).

RESULT AND DISCUSSION

Total Biodisinfectant Bacteria

Based on the ANOVA analysis of the TPC test results, the total bacteria were significantly different for each treatment ($P<0.05$). The test results showed that the highest number of bacterial colonies was found in the P3 treatment (Table 1) which showed that the bacterial colonies were *Pseudomonas aeruginosa* 8.8 x 10^7 CFU/mL, *Nitrosomonas* sp bacteria 7.8 x 10^7 CFU/mL, *Streptomyces* sp bacteria 5.4 x 10^7 CFU/mL, *Micrococus* bacteria 6.3 x 10^7 CFU/mL and *Nitrococus* 7.8 x 10^7 CFU/mL, so that P3 treatment was applied to broiler faecal waste. The five types of bacteria form a consortium that works synergistically in degrading chemical compounds (Safitri *et al*., 2015). Goldford *et al*. (2018) stated that associated bacterial communities had a better effect than single isolates.

Table 1. Total Number of Bacteria using the TPC Method

Sample	Types of bacteria	Total colonies
PO	*Pseudomonas aeruginosa*	1.2 x 10^7 CFU/mL
	Nitrosomonas sp	4.2 x 10^7 CFU/mL
	Streptomyces sp	5.2 x 10^7 CFU/mL
	Micrococcus	1.1 x 10^7 CFU/mL
	Nitrococus	6.4 x 10^7 CFU/mL
P1	*Pseudomonas aeruginosa*	1.5 x 10^7 CFU/mL
	Nitrosomonas sp	2.1 x 10^7 CFU/mL
	Streptomyces sp	2.3 x 10^7 CFU/mL
	Micrococcus	4.2 x 10^7 CFU/mL
	Nitrococus	2.1 x 10^7 CFU/mL
P2	*Pseudomonas aeruginosa*	8.1 x 10^7 CFU/mL
	Nitrosomonas sp	4.4 x 10^7 CFU/mL
	Streptomyces sp	7.1 x 10^7 CFU/mL
	Micrococcus	8.8 x 10^7 CFU/mL
	Nitrococus	6.3 x 10^7 CFU/mL
P3	*Pseudomonas aeruginosa*	8.8 x 10^7 CFU/mL
	Nitrosomonas sp	7.8 x 10^7 CFU/mL
	Streptomyces sp	5.4 x 10^7 CFU/mL
	Micrococcus	6.3 x 10^7 CFU/mL
	Nitrococus	7.8 x 10^7 CFU/mL

Salim *et al*. (2014) stated that *Nitrosomonas* sp and *Nitrococus* bacteria are able to break down ammonia (NH_3) and hydrogen sulfide (H_2S) compounds. Furthermore Robles-Porchas *et al*. (2020) added that *Nitrosomonas* sp and *Nitrococus* bacteria are able to turn feces into biological flocks thereby accelerating the decomposition of organic matter. According to Li *et al*. (2018) other bacteria that can function as a breakdown of hydrocarbon compounds are *Micrococcus* bacteria, and are able to lower the pH of organic matter to reach pH 4.5, low pH can suppress the growth of ammonia-producing bacteria (NH_3).

Patmawati (2022) reported that the addition of *Pseudomonas* sp bacteria to tofu waste could reduce ammonia (NH_3) levels from 8.191 mg/L to 2.836 mg/L within 10 days, and the pH of the waste decreased from 5.92 to 4.25. This is reinforced by the opinion of Lam *et al.* (2011) which explains that ammonium can be converted by *Pseudomonas* sp bacteria into nitrites and nitrates in the nitrification process, then nitrates are reduced in the bacterial cells. The results of AlKahfi *et al.* (2021) explained that *Pseudomonas* sp bacteria were able to reduce the ammonia (NH_3) level of wastewater by 88.81% from 750.48 mg/kg to 84.02 mg/kg within 8 days. Combining two types of bacteria, namely *Nitrococcus* sp and *Pseudomonas* sp. are able to reduce waste ammonia (NH_3) levels from 13.6 mg/L to 1.02 mg/L. Indonesian Environment No. 5 of 2014, namely the ammonia (NH_3) content of 10 mg/L (Banin *et al*., 2021; Pramana & Utama, 2018; Indonesian National Standard, 2004).

Faecal Nitrite Concentration

Nitrite has toxic properties if it is more than the tolerance threshold. Nitrite is toxic because it oxidizes Fe^{2+} in the hemoglobin, thereby reducing the blood's ability to bind oxygen, the low ability of blood to bind oxygen causes tissue damage and death of chickens (Kordi & Tancung, 2007; Baghbanzadeh & Decuypere, 2008). Based on ANOVA analysis of nitrite concentrations, the results were significantly different (P <0.05) for each treatment (Table 2), the highest nitrite levels were found in the P0 treatment, that are 2.2 mg/L, 1.9 mg/L, 1.9 mg/L and 1.4 mg/L respectively from the first day to the fourth day. While the lowest nitrite levels were found in the P6 treatment that are 1.0 mg/L, 0.5 mg/L, 0.5 mg/L and 0.3 mg/L respectively from the first day to the fourth day. If it is percentaged from the highest nitrite concentration, which is 2.9 mg/L to the lowest nitrite concentration, which is 0.3 mg/L, it is obtained a 96.06% of nitrite reduction. Regulation of the Government of the Republic of Indonesia No. 82 (2001) explains that the threshold or tolerance value for nitrite in the environment that it is not dangerous is 0.6 mg/L.

Table 2. Faecal Nitrite Concentration for Each Treatment from the First Week to the Fourth Week

Treatment	**Nitrite concentration (mg/L)**			
	Week 1	**Week 2**	**Week 3**	**Week 4**
P0	2.2 ± 0.34^{a}	2.9 ± 0.25^{a}	2.9 ± 0.23^{a}	2.4 ± 0.13^{a}
P1	2.5 ± 0.51^{a}	2.3 ± 0.31^{b}	1.9 ± 0.12^{b}	1.7 ± 0.09^{b}
P2	1.9 ± 0.29^{b}	1.5 ± 0.15^{c}	1.6 ± 0.11^{c}	1.4 ± 0.08^{c}
P3	1.5 ± 0.19^{c}	1.2 ± 0.20^{d}	1.1 ± 0.09^{d}	0.8 ± 0.05^{e}
P4	1.3 ± 0.12^{c}	0.9 ± 0.13^{d}	1.7 ± 0.08^{c}	0.7 ± 0.04^{e}
P5	1.0 ± 0.07^{d}	1.0 ± 0.05^{d}	0.7 ± 0.09^{e}	0.8 ± 0.02^{e}
P6	1.0 ± 0.09^{d}	0.5 ± 0.02^{e}	0.5 ± 0.01^{e}	0.3 ± 0.01^{f}

Note:

- At Week 1, P_0 (2.2 ± 0.34^{a}) and P_1 (2.5 ± 0.51^{a}) were not significantly different because they had the same superscript (a).
- However, P_2 (1.9 ± 0.29^{b}) was significantly different from P_0 and P_1 because they had different superscripts (b).
- Similarly, P_3, P_4, P_5, and P_6 showed significant differences with P_0 and P_1.

It can be concluded that biodisinfectan with the greater of reduction in nitrite concentration in fecal waste will be used. There was a decrease in the nitrite concentration of faecal waste from each treatment, due to the presence of nitrite-degrading bacteria present in the biodisinfectant. According to Nurlita & Utomo (2011), the bacteria which function to decompose harmful chemical compounds in fecal waste are *Nitrosomonas* sp. and *Nitrobacter* sp. *Nitrosomonas* sp. plays a role in oxidizing ammonia compounds (NH_3) to nitrite, while *Nitrobacter* sp. plays a role in oxidizing nitrite to nitrate in the process of the nitrogen cycle. Nitrite is a transition material that occurs in the nitrogen cycle, this compound is produced from the biochemical oxidation process of ammonium, but is unstable under aerobic conditions and is released as nitrogen gas (Nurlita & Utomo, 2011; Sedlacek *et al*., 2016).

Nitrifying bacteria such as *Nitrosomonas* sp. and *Nitrobacter* sp. functions to accelerate the decomposition of ammonia (NH_3) into nitrite and nitrite into nitrate, so that with the rapid decomposition of ammonia (NH_3), ammonia (NH_3) does not last long in the air and that the concentration will drops. Madigan *et al*. (2008) explained that in the nitrification process, the oxidation of ammonia (NH_3) to nitrite is carried out by *Nitrosomonas* and *Nitrosococcus* bacteria, while in the nitratation process the oxidation of nitrite compounds to nitrite is carried out by the *Nitrobacter* group of bacteria. The ability of *Nitrosomonas*, *Nitrosococcus* and *Nitrobacter* to oxidize ammonia (NH_3) to nitrite and nitrate cause by their capable of producing enzymes namely ammonia monooxygenase and hydroxylamine oxidoreductase (Daims *et al*., 2016).

Faecal nitrate concentration

Nitrate is the product of the oxidation of nitrites carried out by the *Nitrobacter* group of bacteria. Based on the ANOVA analysis of the results of the analysis of nitrate concentrations, the results were significantly different ($P<0.05$) for each treatment (Table 3). The highest nitrate concentrations were found in the P0 treatment. Nitrate concentrations from the first week to the fourth week was always increased, but it was different from P1, P2, P3, P4, P5 and P6 which decreased from the first week to the fourth week. This is because the P0 was not given biodisinfectant, so the oxidation process carried out by bacteria did not exist. P1 to P6 decreased because biodisinfectants were given with different concentrations, the more reduction of nitrite concentration was obtained from the more biodisinfectant concentrations given. The different between the highest (47.32 mg/L) and the lowest (9.02 mg/L) of nitrate concentration is 52.46%.

Tabel 3. Faecal Nitrate Concentration from the First Week to the Fourth Week

Treatment	Nitrate concentration (mg/L)			
	Week 1	Week 2	Week 3	Week 4
P0	40.18 ± 2.30^{a}	40.75 ± 3.12^{a}	43.19 ± 2.11^{a}	47.32 ± 5.23^{a}
P1	42.78 ± 3.45^{a}	37.34 ± 4.01^{ab}	39.41 ± 3.04^{b}	35.58 ± 2.34^{b}
P2	33.90 ± 1.12^{b}	30.89 ± 2.15^{b}	32.45 ± 1.45^{c}	30.05 ± 3.10^{c}
P3	30.45 ± 2.07^{c}	28.00 ± 2.49^{c}	25.49 ± 2.19^{d}	26.32 ± 1.90^{d}

Treatment	Nitrate concentration (mg/L)			
	Week 1	Week 2	Week 3	Week 4
P4	25.03 ± 3.10[cd]	25.45 ± 1.34[c]	23.07 ± 1.83[d]	22.39 ± 1.59[e]
P5	21.09 ± 2.05[d]	19.26 ± 2.55[d]	17.00 ± 1.90[e]	18.89 ± 3.66[e]
P6	18.02 ± 1.33[e]	14.29 ± 3.11[e]	9.89 ± 2.41[f]	9.02 ± 2.61[f]

Note:

- In Week 1, P_0 (40.18 ± 2.30[a]) and P_1 (42.78 ± 3.45[a]) were not significantly different because they had the same superscript (a).
- However, P_2 (33.90 ± 1.12[b]) was significantly different from P_0 and P_1. In Week 4, P_6 (9.02 ± 2.61[f]) was significantly different from all other treatments.

Republic of Indonesia Government Regulation No. 82 of (2001) stated that the tolerance limit for nitrate is 10 mg/L (Patty *et al*., 2015; Nahm, 2003; Prilia & Kamil, 2011). According to Yu & Chandran (2010), the main impact of high nitrate in the environment, soil and water can cause a decrease in dissolved oxygen. Furthermore, Deng *et al.* (2016) explained that oxygen is one of the micronutrient elements that is very important for the survival, physiological and metabolic processes in the living bodies. The low of oxygen dissolved in the environment of broiler chickens is caused by the high concentrations of nitrate. It will cause poultry experience hypoxemia or ascites syndrome (Owen *et al*., 1995; Ruiz-Feria, 2009). The results of Nurmeiliasari's research (2010) explained that hypoxemia in broiler chickens will cause excess fluid accumulation in the peritoneal cavity so that the pulmonary artery pressure increases. This condition can cause death due to lack of oxygen supply for the body's metabolic needs.

Feces pH value

The degree of acidity (pH) is a unit of degree of acidity or alkalinity which is given a value of 1 to 14 with pH 7 as a neutral unit. The normal pH in chicken feces and litter is in the range of pH 7.09 – 7.4 (Metasari *et al*., 2014). Glatz & Pym (2013) also reported that broiler feces are wet in a pH ranging from 8.39 – 8.5 so it is very good for the development of ammonia (NH_3) producing bacteria. Based on the results of ANOVA analysis, it is related to the fecal pH value of broiler chickens treated with biodisinfectants, the results were significantly different (P<0.05) from each treatment (Table 4). The pH value of each treatment from the first week to the fourth week with the highest pH (9.5) obtained in the P0 treatment at the thirth and the fourth week. The lowest pH value (4.7) was obtained in the fourth week of P6 treatment.

Table 4. The pH Value of each Treatment from the First Week to the Fourth Week

Treatment	pH Value			
	Week 1	Week 2	Week 3	Week 4
PO	7.2 ± 0.5[a]	8.6 ± 1.1[a]	9.5 ± 1.4[a]	9.5 ± 0.9[a]
P1	8.1 ± 0.9[a]	9.0 ± 0.9[a]	8.4 ± 0.8[a]	7.9 ± 1.2[b]
P2	7.5 ± 1.2[a]	7.9 ± 0.6[b]	7.2 ± 0.6[b]	7.0 ± 0.4[b]
P3	7.6 ± 0.6[a]	7.2 ± 1.4[b]	6.9 ± 0.9[b]	6.8 ± 0.7[bc]
P4	6.5 ± 0.9[b]	6.0 ± 0.9[c]	6.2 ± 0.7[c]	5.7 ± 0.2[c]
P5	6.6 ± 0.7[b]	5.8 ± 0.5[c]	5.0 ± 0.4[d]	5.0 ± 0.1[d]
P6	6.0 ± 0.6[b]	5.5 ± 0.5[c]	4.9 ± 0.3[d]	4.7 ± 0.3[d]

Note:

- At Week 1, P_0 (7.2 ± 0.5[a]), P_1 (8.1 ± 0.9[a]), P_2 (7.5 ± 1.2[a]), and P_3 (7.6 ± 0.6[a]) were not significantly different.
- However, P_4 (6.5 ± 0.9[b]), P_5 (6.6 ± 0.7[b]), and P_6 (6.0 ± 0.6[b]) were significantly different from P_0, P_1, P_2, and P_3.
- At Week 4, P_6 (4.7 ± 0.3[d]) and P_5 (5.0 ± 0.1[d]) were significantly different from all other treatments.

Mikkelsen (2009) explained that pH 4.5 can reduce the reaction rate of ammonia (NH_3). The research of Jaya *et al*. (2022) reported that the decrease of ammonia gas concentration (NH_3) in the wastewater was quite stable at a pH 4.5-6, so it can be concluded that pH conditions can affect the speed of the reaction and also determine what substances will be formed in the oxidation process. Ribera-Guardia & Pijuan (2017) added that the lower pH or the more acidic waste related to the lower concentration of ammonia gas concentration (NH_3). This is shows that the oxidation reaction is going well. The condition of pH 5 can reduce the level of ammonia in the waste from 0.021% to 0.002%. Furthermore, the research by Khaerunnisa *et al*. (2013) which analyzed the effect of pH on the formation of ammonia gas (NH_3) in faecal waste found that pH 6 would reduce the activity of ammonia (NH_3) bacteria and at pH 5.5 the activity of ammonia-producing bacteria (NH_3) was totally stop. Luther (2015) added that if the pH value is below 6.5, the activity of ammonia-producing bacteria (NH_3) will decrease and if the pH is below 5.0, the organic matter fermentation process will be stop.

Faecal ammonia (NH_3) concentration

Ammonia (NH_3) is one of the gaseous elements in excreta produced through the decomposition of nitrogenous waste materials in the form of uric acid, unabsorbed protein, amino acids and other non-protein nitrogen (NPN) caused by the activity of microorganisms in the feces (Manin *et al*., 2012). The concept of the formation of poultry ammonia (NH_3), namely uric acid into S (+)-Allantoin catalyzed by the enzyme urease that requires H_2O + ½ O_2 and produces CO_2 S (+)-Allantoin is converted to allatoic acid catalyzed by the enzyme S (+)-Allantoinase to produce H_2O then the allantoic acid is converted to S (+)-Ureidoglycolate catalyzed by the enzyme Allantoate amidohyrolase requires $2H_2O$ and produces CO_2 + $2NH_3$. Then S (+)-Ureidoglycolate is converted to Glyoxylate + Urea catalyzed by the enzyme of S (+)-Ureidoglycolase. Urea is converted to $2NH_3$+CO_2 by the urease enzyme to produce H_2O (Alagawany *et al*., 2016; Riza *et al*., 2015; Prasetyanto, 2011).

The results of the ANOVA analysis on the concentration of ammonia (NH_3) showed a significant decrease (P<0.05) except for P0, because P0 there was not biodisinfectants. A significant reduction in the concentration of ammonia (NH_3) was seen in the P1, P2, P3, P4, P5 and P6 treatments (Figure 1). The lowest concentration of ammonia (NH_3) was found in P6 that was from 18 ppm in the first week to 6 ppm in the fourth week. The percentage of ammonia reduction from the highest concentration (46 ppm) to the lowest (6 ppm) 76.6%.

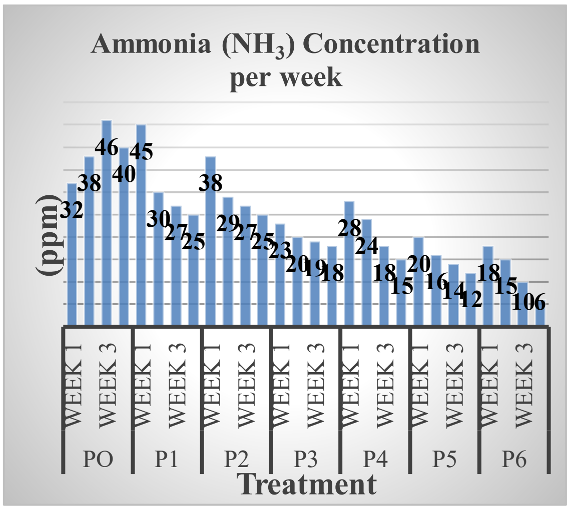

Figure 1. Ammonia concentration of each treatment in the first week to the fourth week

Ammonia gas (NH_3) produced by the feces and urine of broiler chickens can contaminate the environment and affect the livestock and human health. The concentration of ammonia (NH_3) 5 ppm is the lowest concentration of ammonia to smell, the concentration of ammonia (NH_3) 6 ppm can cause irritation of the eye mucosa and respiratory tract, the concentration of ammonia (NH_3) 11 ppm can reduce the productivity of chickens, the concentration of ammonia (NH_3) 25 ppm is the maximum that can be tolerated for 8 hours, ammonia concentration (NH_3) 35 ppm can only be tolerated for 10 minutes and ammonia concentration (NH_3) 40-50 ppm can cause pain, decreased appetite, reduced blood oxygen levels up to chicken mortality (Kristensen *et al*., 2000; Rigoni *et al*., 2017; Li *et al*., 2020).

CONCLUSION

This research successfully developed a biodisinfectant using a bacterial consortium. ANOVA results showed significant differences ($P<0.05$) in total bacteria, with the best outcome in treatment P3. The bacterial counts were: *Pseudomonas aeruginosa* (8.8×10^7 CFU/mL), *Nitrosomonas sp.* (7.8×10^7 CFU/mL), *Streptomyces sp.* (5.4×10^7 CFU/mL), *Micrococcus sp.* (6.3×10^7 CFU/mL), and *Nitrococcus sp.* (7.8×10^7 CFU/mL). Nitrite and nitrate levels decreased significantly ($P<0.05$), with nitrite dropping 96.06% (2.9 mg/L to 0.3 mg/L) and nitrate decreasing 52.46% (47.32 mg/L to 9.02 mg/L), both meeting Indonesian quality standards (PP No. 82/2001). The best pH and ammonia reduction were in P6, with ammonia decreasing 76.6% (46 ppm to 6 ppm).

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AUTHOR CONTRIBUTIONS

The authors read and approved the final manuscript.

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