Research Article

Dynamics of Ammonia Gas Production from Feces of Laying Hens during Eco-enzyme Treatment of Vegetable Waste

Muhammad Ardas Daruslam[1], Muhammad Irfan Said[2]*, Nancy Lahay[3]

[1]Student of Animal Science and Technology Study Program, Faculty of Animal Science, Hasanuddin University, Indonesia;[2]Department of Animal Science, Faculty of Animal Science, Hasanuddin University, Indonesia;[3]Department of Animal Feed and Nutrition, Faculty of Animal Science, Hasanuddin University, Indonesia.

Abstract | Ammonia gas is one of the contributors to greenhouse gas emissions (GHE). This gas is mainly produced from livestock waste such as poultry. These emissions can be overcome by enzymatic treatment, one of which is known as eco-enzyme (Ec-En). A total of 2 types of study objects were evaluated in this research, namely (1) related to the characteristics of Ec-En produced from 2 different types of vegetable waste, Kale (Ka) and Spinach (Sp) and (2) examining the optimal ratio of Ec-En that can be applied to the poultry (laying hens) feces as ammonia gas reducer. A total of three vegetable waste source formulas were applied, namely: V_1 = Kale (Ka)(100%); V_2 = Spinach (Sp)(100%); V_3 = Ka(50%) + Sp(50%) to study the Ec-En character. In addition, four Ec-En dilution process formulas were used to evaluate the performance of Ec-En as a ammonia gas reducer during the fermentation process, namely: P_0 = without Ec-En (control); P_1 = 100% of Ec-En + 0% water (v/v), P_2 = 90% of Ec-En + 10% water (v/v) and P_3 = 80% of Ec-En + 20% water (v/v). Fermentation time uses three levels, namely: T_0 = 0 minutes; T_1 = 15 minutes and T_2 = 30 minutes. Fermentation time is the time after Ec-En is applied to the feces of laying hens. Data were analyzed statistically using ANOVA. The results showed that differences in vegetable waste sources had a significant effect ($p<0.05$) on Ec-En properties such as pH (<4), protease enzyme activity (0.3835-0.4259 U/mL) and total Lactate Acid Bacteria (LAB) (5.5-6.00 log CFU/mL). The Ec-En product produced from P_1 produces the best characteristics compared to other treatments. Application of P_1 for 15 minutes (T_1) of fermentation time was able to reduce ammonia gas in laying hen feces by (9.77-36.90 ppm).

Keywords | Ammonia, Eco-enzyme, Laying hens, Vegetable waste

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***Correspondence** | Muhammad Irfan Said, Department of Animal Science, Faculty of Animal Science, Hasanuddin University, Indonesia; **Email:** irfanunhas@gmail.com
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INTRODUCTION

Ammonia gas, a dangerous gas at high concentrations with long contact times. Ammonia is a contributor to Green House Emissions (GHE) (Kang *et al*., 2016). Ammonia gas pollution is increasing along with the increase in the livestock industry, especially poultry (Ministry of Agriculture, 2014). This increase in pollution has a big impact on human health so efforts need to be made to reduce it. Data shows that there is an increase in the poultry population, especially laying hens. In 2020, the population was 345,181 million, increasing to 368,190 million in 2021 (BPS, 2022). produces fecal waste of 0.06-0.15 kg/head/day. Every 100 g of feces will produce 0.54 ppm of ammonia gas. If calculated in total, the potential for ammonia gas production reaches 298,557 billion ppm/day. If the handling process is not appropriate, it can certainly

pose a threat to the environment. Ammonia gas is known as a very dangerous pollutant at high concentration levels. Apart from livestock, ammonia gas can also cause poisoning in plants (vegetation) (Fangmeier *et al*., 1994; Roelofs *et al*., 1985).

The impact of ammonia also turns out to be very detrimental to human health. The impact of ammonia on human health has been widely studied by (Schiffman *et al*. (2005) and Wing *et al*. (2000) as well as on the environment (Abouelenien *et al*. (2010) and Wang *et al* (2019). Several recent studies have been developed to Reducing emissions includes the use of sophisticated air purification technology and more efficient waste management strategies. The results of the study show that these two technologies are inefficient and have a direct negative impact on the environment enzymatic. One innovation that can be carried out is the application of eco-enzyme (Ec-En).

Eco-enzyme, a natural enzyme in the form of a liquid produced from natural ingredients (plants) through a fermentation process. This process can take three months. This enzyme is produced from several ingredients such as: sugar, organic waste, and water, in a ratio of (1:3:10) (Nazim and Meera, 2015). The Ec-En product acts as an effective remediation agent by utilizing enzymes and active microorganisms produced during the fermentation process to degrade harmful pollutants. This enzyme is rich in microorganisms such as lactic acid bacteria (*Lactobacillus* and *Leuconostoc*) and a type of yeast (*Pichia* and *Candida*) (Tong, 2011 and Win 2011). Several natural materials have been developed to become sources of raw materials in Ec-En production. One of them is waste from vegetable plants, especially kale (Ka) and spinach (Sp). Several researchers have previously studied the use of land kale and green spinach as raw materials. Effendi *et al*., (2015) have reported that Ka plants are able to reduce ammonia in freshwater cultivation wastewater by up to 48.6%. The results of other research have been reported by Endut *et al*., 2010), Ka can reduce ammonia nitrite by more than 50% in the aquaponic recirculation process. Other researchers have also reported that Ec-En from papaya plants (Pa) and Sp can reduce ammonia, nitrite and nitrate in water (Wikaningrum and Anggraina (2022). Based on several studies, this has shown the important role of plant waste as a source of enzymes The potential for Ec-En to be very important in reducing the production of ammonia gas. This research aims to evaluate the characteristics of Ec-En produced from 2 different types of vegetable waste (kale and spinach) and 2) examine the optimal ratio of Ec-En. can be applied to the feces of laying hens as a reducer of ammonia gas.

MATERIALS AND METHODS

Research Material

A total of 2 types of vegetable waste were used as raw materials for eco-enzyme (Ec-En), namely: Kale (Ka) (*Ipomoea reptans Poir*) and Spinach (Sp) (*Amaranthus hybridus* L) each 3 kg. Vegetable waste was obtained from the traditional market "Mappasaile", Pangkaje'ne District, Pangkep Regency, South Sulawesi Province, Indonesia. A total of 50 kg of laying hen feces (Hy-Line Brown strain) was obtained from the "Kandang Biru" livestock industry, Allu Hamlet, Baji Pamai Village, Maros Baru District, Maros Regency, South Sulawesi Province, Indonesia. Supporting ingredients include: 3 kg of molasses obtained from a sugar factory in Takalar district, South Sulawesi Province, Indonesia. Supporting equipment for Ec-En production and ammonia gas reduction tests include: Smart Sensor Ammonia Tester (AR-8500 NH3); Thermo Hygrometer (HTC-1); Square Glass Tube (dimension (modification); scales (RENHE) capacity 10 kg; plastic syringe (Asena) capacity 500 mL and plastic vacuum tube. All equipment was obtained from the Lab. Animal By-Products Processing Technology, Faculty of Animal Science, Hasanuddin University, Makassar city, Indonesia.

Research Methods

Production process of Ec-En: A total of 4.5 kg of Ka vegetable waste and 4.5 kg of Sp were ground using a grinder until crushed. The composition of the ingredients uses the formula (10: 3: 1), where the formula uses 10 liters of water + 3 kg of each vegetable waste + 1 kg of molasses. The ingredients are mixed homogeneously and then put into a fermentation container. The fermentation process is carried out at room temperature and under anaerobic conditions. The fermentation process for the formula is 3 months.

Preparation of eco-enzyme: A total of 150 g of laying hen feces was put into a Square Glass Tube and left for 20 minutes until the feces produced ammonia gas. A total of 500 mL of Ec-En solution was put into a plastic syringe and then sprayed into a Square Glass Tube. The ammonia gas reduction production process was observed for 0, 15 and 30 minutes. Testing the condition of ammonia gas was analyzed with the Smart Sensor Ammonia Tester Detector (AR-8500 NH3).

Research design and statistical analysis: The research was designed experimentally using a Completely Randomized Design (CRD) with a 4x3x3 factorial pattern, where the research used 4 treatments combined with the dilution process; P_0 = without Ec-En (control); P_1 = 100% of Ec-En + 0% water (v/v), P_2 = 90% of Ec-En + 10% water (v/v) and P_3 = 80% of Ec-En + 20% water (v/v). A total of 3 fermentation times were applied, namely: T_0 = 0 minutes; T_1 = 15 minutes and T_2 = 30 minutes. Each treatment was repeated 3 times. Treatments that showed a real effect were then tested using the Duncan's Multiple Range Test (DMRT) significant difference test at the 5% level (Gaspersz, 1991). Data from the characterization of the Ec-En product were analyzed descriptively, while data from the ammonia gas

reduction test were analyzed statistically using ANOVA.

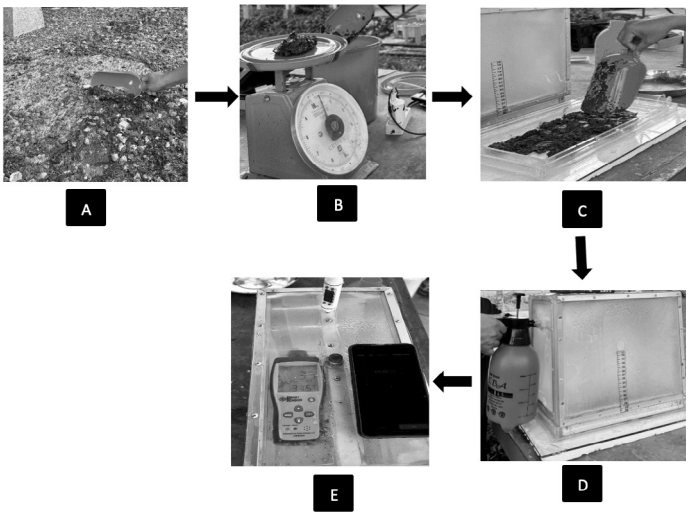

Figure 1: Design of the eco-enzyme application process

Parameter and Data Analysis

pH value: The pH value of the eco-enzyme is measured using a calibrated pH meter. Measure the degree of acidity (pH) by inserting 100 mL of eco-enzyme into a beaker, then inserting the tip of the pH meter sensor; the tool will display the pH value of the measured liquid.

Enzyme activity of protease: A sample of 0.2 mL of enzyme solution was added to 1 mL of Tris-HCl buffer pH 7, then 1 mL of 20 mg/mL casein was added, then incubated at 37°C for 30 minutes. After that, 1 mL of 0.1 M TCA was added. The control was carried out using the same procedure as the sample but without the incubation process; TCA was immediately added to stop the enzyme activity. The sample supernatant (filtrate) and control were separated by centrifugation at 10,000 g for 5 minutes. Take 1.5 mL of the filtrate from protease hydrolysis, 2.5 mL of 0,4 M Na_2CO_3, and 1 mL of 50% Follin, and let it sit for 30 minutes. After that, the absorption at the maximum wavelength was measured using a UV-Vis spectrophotometer. T standards and tyrosine blanks were prepared using the same procedure as the hydrolysis filtrate. The blank used is tris-HCl buffer pH 7. Enzyme activity is expressed in units (U), defined as the amount of enzyme required to liberate 1 µmol of tyrosine per minute at pH 7 and 37°C. Enzyme activity can be calculated using the following equation: Protease activity (U) = µmol tyrosine/t, where, µmol tyrosine = tyrosine concentration obtained via the tyrosine standard curve; t: time (minutes).

Lactic acid bacteria (LAB) total: Calculation of LAB total using the SPC (Standard Plate Count) method as much as 1 mL of the sample was diluted into 9 mL of 0.85% physiological NaCl from 10^{-1} – 10^{-4}, then the last three dilution series (10^{-2}, 10^{-3}, and 10^{-4}) 1 mL was taken and inoculated using a pour plate on MRS Agar medium supplemented with 1% $CaCO_3$ in a petri dish, then incubated for 48 hours in an incubator at 37°C. The growth of lactic acid bacteria on MRS Agar medium supplemented with $CaCO_3$ is characterized by a clear zone around the bacterial colony. The grown LAB were counted, and the total number was calculated using the Total Plate Count (TPC) method (Amaliah *et al*., 2018). Number of cells = v x n, where v=number of samples grown, n=number of colonies in the cup, f = dilution factor.

Temperature kinetics (°C): Temperature measurements were taken 20 minutes after the feces were covered using a hood. The temperature data was collected by observing temperature data on the Ammonia Tester Sensor (AR-8500 NH_3) at the 0 minute, 15th minute, and 30th minute after spraying.

Humidity kinetics (%): Humidity measurements were carried out 20 minutes after the feces were covered using a hood. The humidity data was collected by observing humidity data on a Thermo hygrometer (HTC-1) at the 0th minute, 15th minutes, and 30th minutes after spraying.

RESULTS AND DISCUSSION

Characteristics of Ec-en

pH Value: The complete comparison results of pH test values for Ec-En products are presented in Figure 2. The results of statistical analysis using ANOVA show that the different types of vegetable waste materials or their combinations do not show a significant effect ($p<0.05$) on the pH value of Ec-En. The pH value is on average 3.37-3.38. A pH value below 4 indicates that the Ec-En product produced is in an acidic condition.

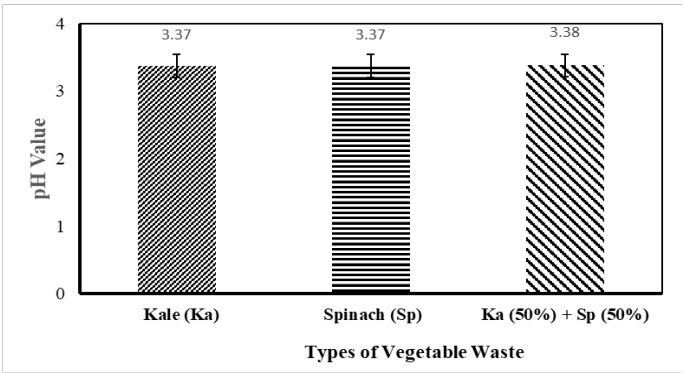

Figure 2: Comparison of the pH value of eco-enzymes produced from kale (*Ipomoea reptans Poir*) (Ka) and spinach (*Amaranthus hybridus L*) (Sp) vegetable waste and their combination.

This result is related to the research results of Win (2011),

the criteria for a perfect eco enzyme if the pH content is <4. These results indicate that the eco enzyme produced still meets standards. The highest pH value was obtained from a combination of eco-enzyme kale and spinach. In contrast, the single eco-enzymes of kale and spinach have relatively similar pH values. The difference in pH value is influenced by the type of material. Low pH values tend to have high organic acid content. This is in accordance with the study of Etienne *et al*., (2013), the high organic acid content of citric acid and acetic acid) is caused by low pH values. Organic acid levels are produced from the fermentation process for 3 months. In addition, the activity of bacteria such as Lactobacillus which is involved during fermentation produces lactic acid as a metabolic product. These results are related to the results of research conducted by Larasati *et al*., (2020), acetic acid was obtained from the metabolism of bacteria in vegetable waste. This process is anaerobic metabolism, namely bacterial fermentation to obtain energy from sugar. The by-products produce acetic acid and alcohol.

Enzyme activity of protease

The test results related to the protease enzyme activity in Ec-En during complete treatment are presented in Figure 3. Figure 3 shows that the protease enzyme activity in Ec-En produced from different base materials is determined in units per milliliter (U/mL). On average, the enzyme activity produced from Ka material produces an activity of 0.4043 U/mL. Furthermore, material from Sp produces an activity of 0.4259 U/mL and the combination of (Ka+Sp) (V_3) produces an activity of 0.3835 U/mL. Based on these results, it shows that the highest enzyme activity is Ec-En from Sp material compared to the combination (Ka+Sp). The analysis results show that spinach eco-enzyme has the highest protease enzyme activity among the three types of eco-enzyme produced. The lowest enzyme activity is the combination (V_3).

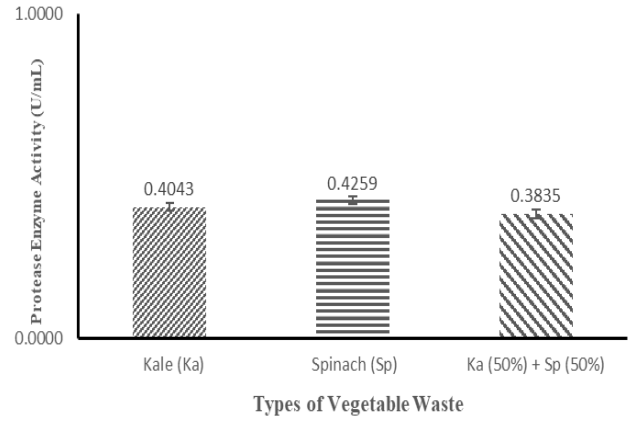

Figure 3: Comparison of the protease enzyme activity (U/mL) of eco-enzymes produced from kale (*Ipomoea reptans Poir*) (Ka) and spinach (*Amaranthus hybridus L*) (Sp) vegetable waste and their combination.

However, the results of statistical analysis showed that there was no difference in enzyme activity from the different materials used ($p>0.05$). These results show that although Ka and Sp are good sources of protease enzymes when used separately, however, the combination of the two (Ka+Sp) does not produce a synergistic effect, so the combination is not always effective in producing high activity. According to Saranraj *et al*., (2017), protease enzyme activity is greatly influenced by temperature and pH during fermentation. The fermentation process increases enzyme activity. This process is influenced by the environment, especially acid with a temperature of 22-25°C (Mokoena 2005). This is related to research conducted that the pH of the eco-enzyme produced is <4. Another researcher, Sousa *et al*., (2017) also explained that the protease enzyme has acidic properties (pH 2.0-6.0) and plays an important role in enzyme activity to achieve maximum results. One of the enzyme activities is influenced by the pH value because certain enzymes will only work in breaking down substrates at a certain pH. Protease enzymes can be produced from the activity of lactic acid bacteria (LAB). This bacteria plays a role in the Ec-En fermentation process to produce the enzymes amylase, protease and lipase. The results of biochemical analysis showed the presence of acetic acid, sugar, protein, alcohol, and enzyme activity in the enzymes protease, amylase, and lipase (Samriti *et al*., 2019). According to Win (2011), LAB in molasses and vegetable waste will produce activity during the fermentation process. In the fermentation process, bacteria multiply and consume nutrients to meet their needs. Nutrients can be obtained by producing extracellular enzymes such as proteases, which break down proteins into amino acids.

Lactic Acid Bacteria (LAB) Total

The total LAB contained in Ec-En produced from kale and spinach vegetable waste and their combination presented in Figure 4.

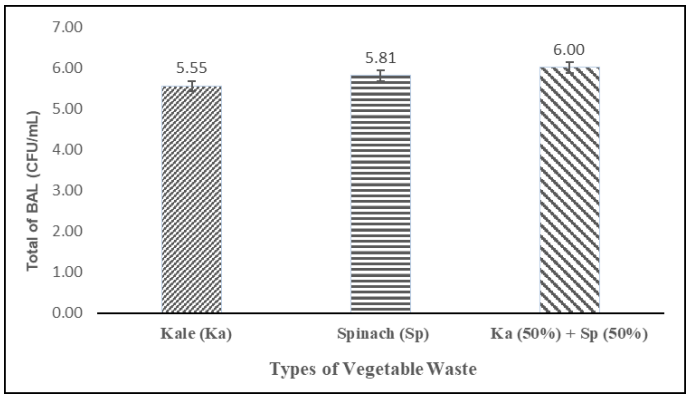

Figure 4: Comparison of the total of lactic acid bacteria (LAB) (CFU/mL) of eco-enzymes produced from kale (Ka) (*Ipomoea reptans Poir*) and spinach (*Amaranthus hybridus L*) (Sp) vegetable waste and their combination.

Figure 4 shows the total bacteria (CFU/mL) in Ec-En produced from three different ingredients. Materials from Ka and Sp each produced total bacteria of 5.55 and 5.81 log CFU/mL, while (Ka+Sp) produced 6.00 log CFU/mL. The combination (Ka+Sp) produces the highest number of bacteria compared to the others. These results indicate that the combination of these materials is able to create a more favorable environment for bacterial growth. During the fermentation process, medium conditions and LAB activity are influenced by temperature and pH (Pelczar and Chan, 2005). Acidic conditions (pH<4) cause LAB to produce bacteria that can work optimally. High amounts of LAB can be caused by the fermentation process which produces organic acids as metabolic products. Total LAB can increase with increasing fermentation time. This can be influenced by substrate availability. Conversely, if there is a decrease in the substrate during the fermentation process, there will be a decrease in the number of bacteria (Kiani *et al*., 2008). According to Larasati *et al.* (2020), in the fermentation process, acetic acid is produced from the metabolic process of bacteria which are naturally found in fruit and vegetable waste. The anaerobic metabolic process is usually called the fermentation process. Fermentation is an attempt by bacteria to obtain energy from carbohydrates under anaerobic conditions (without oxygen) and with by-products in the form of alcohol or acetic acid (depending on the type of microorganism). Fungi and some bacteria produce alcohol in fermentation, while most produce acetic acid.

Characteritics of Gas Ammonia

Ammonia gas production: Changes in ammonia gas production from laying hen feces after complete application of Ec-En (spinach)(Sp) are presented in Figure 5.

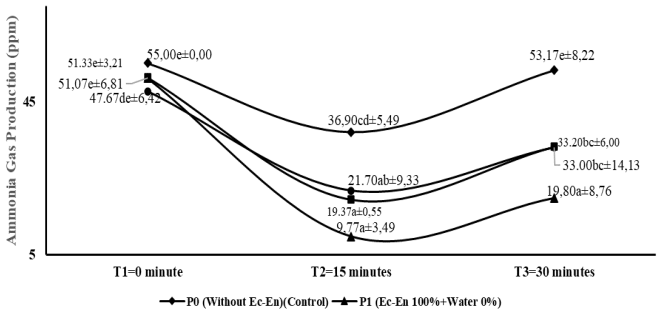

Figure 5: Changes in ammonia gas production (ppm) from laying hen feces after applying eco-enzyme (Ec-En) from vegetable waste (spinach)(Sp) with different amounts of diluent.

The results of statistical analysis of the data in Figure 5 show that differences in fermentation time have a significant effect ($p<0.05$) on ammonia production. in fermentation conditions from 0 to 15 minutes, all Ec-En treatments (P_0, P_1, P_2 and P_3) experienced a decrease in ammonia gas production. However, at the 30th minute measurement there was an increase again. The highest level of reduction in gas production was in the P1 dilution treatment (100% of Ec-En + 0% water). This shows that P_1 treatment is most effective in reducing ammonia gas compared to the others (P_0; P_2; and P_3). Apart from that, these results also show that bacterial activity in reducing ammonia gas only occurs at a fermentation time of 15 minutes. Ammonia fluctuations are triggered by microclimate factors, including increased temperature and humidity. According to Bleizgys *et al.* (2023), ammonia emissions are strongly influenced by air temperature and relative humidity, with emission levels tending to increase as temperature increases and then decrease as humidity increases. Furthermore, Moreira *et al.* (2006) revealed that increased urease activity is very sensitive to feces temperature and is the cause of high concentrations of ammonia in feces, where feces temperature is very dependent on air temperature. Lupis *et al.* (2010) added that ammonia is produced when urea in urine is broken down by the urease enzyme found in feces and soil, producing ammonia gas and carbamine acid. This process can continue for several hours, depending on the amount of urea and urease available as well as meteorological conditions such as temperature and humidity. In overcoming this problem, eco-enzymes play an important role by changing the chemical and biological conditions of feces thereby reducing urease activity. Kujur *et al.* (2013) revealed that the protease enzyme in eco-enzyme is a hydrolytic enzyme that controls the decomposition of various biological macromolecules and can accelerate the biodegradation process. According to Salim *et al.* (2014), the use of eco-enzymes in feces management can cause a decrease in ammonia levels in the air. This process can be explained by the fact that eco-enzymes are not only able to increase the population and activity of microorganisms, but these eco-enzymes can help degrade urea and uric acid into ammonia. Of course, eco-enzymes have a big contribution to improving the quality of air temperature which will ultimately be able to reduce the concentration of ammonia in the air. The study of Nemet *et al.* (2021) strengthened the role of microorganisms in decomposition, showing that the enzymes they produce effectively break down organic matter, reducing ammonia production. The importance of these microorganisms is most apparent in undiluted eco-enzymes because high concentrations of microorganisms allow for more effective breakdown of ammonia. For example, lactic acid bacteria, which have the special ability to decompose nitrogen. On the other hand, dilution of eco-enzyme reduces decomposition activity due to reduced enzyme activity per unit volume. Research by Fardes *et al.*, (2020) illustrates that eco-enzyme is effective in reducing ammonia gas. This enzyme is obtained from active microorganisms in high concentration substrates. Extracellular enzymes synthesized by microorganisms can decompose high molecular weight compounds into low molecular

weight compounds and are able to break down polymer structures. This process not only increases the efficiency of ammonia production but also reduces energy consumption and waste. Meanwhile, dilution of eco-enzyme reduces the number of active enzymes and microorganisms per unit volume, thereby reducing its effectiveness in decomposing ammonia. Therefore, undiluted eco-enzyme is more effective in reducing ammonia than diluted. At high concentrations, microorganisms will be active in carrying out a faster and more comprehensive degradation process. The process of reducing ammonia gas in chicken feces is very effective in treatment P_1. According to Nazim (2013), higher Ec-En concentrations and longer fermentation times result in a more significant reduction in ammonia production. A high eco-enzyme concentration not only indicates that the acidity level is still ideal for lowering the pH of feces but is also an important condition for effective waste management. The eco-enzyme fermentation process, which involves the intake of nutrients from vegetable waste such as fiber, vitamins, and minerals, plays a key role in this. Lactic acid bacteria along with other microorganisms utilize these nutrients to produce enzymes and organic acids. These acids are effective in lowering environmental pH; more specifically, they not only inhibit the formation of ammonia from nitrogen contained in feces but also accelerate the process of decomposition of organic matter. This helps make the waste management process more environmentally friendly. In addition, with a decrease in pH, the resulting acidic conditions reduce ammonia volatilization, which contributes to a reduction in ammonia emissions into the air. In line with the research results of Yustiani *et al.* (2023) and Tang *et al.* (2011), using high doses of eco-enzyme directly in river water is effective in acidifying the water and improving waste quality. Interestingly, these pH changes affect microbial activity, which can facilitate or inhibit their growth. Ratzke *et al.* (2018) found that these changes significantly affected bacterial populations; on the one hand, acidic properties can dissolve complex organic materials that are usually insoluble, becoming soluble (Sambaraju *et al.*, 2020). The ammonia decomposition process is also enhanced by the activity of the protease enzyme contained in eco-enzyme. Parmar *et al.*, (2001) revealed that protease enzymes have a significant role in reducing the amount of sludge solids in cultivation. Interestingly, Roman *et al.*, (2006) showed that this enzyme facilitates anaerobic digestion and breaks down larger organic particulate materials into smaller particles, increasing the surface area available for the bacteria responsible for degradation. In addition, Puigagut *et al.*, (2011) and Elsamadony (2019) found that the presence of organic acids in eco-enzymes, which function as a carbon source and biocatalytic activity, helps in the process of dissolving insoluble minerals into soluble forms, shows how Ec-En can reduce total ammonia nitrogen and total phosphorus in cultivation sludge. According to Husain *et al.* (2008), enzymes work by converting substrates into insoluble compounds. The reduction of ammonia gas in feces occurs due to the presence of protease enzymes as protein molecules in Ec-En (Anonymous, 2024; Rasit *et al.*, 2019). Protease enzymes also help stabilize organic materials into substances that are more easily dissolved and decomposed (Rasit *et al.*, 2019).

Temperature Kinetics (°C): Changes in feces temperature kinetics of laying hens after application of Ec-En (spinach)(Sp) with different amounts of diluent are completely presented in Figure 6.

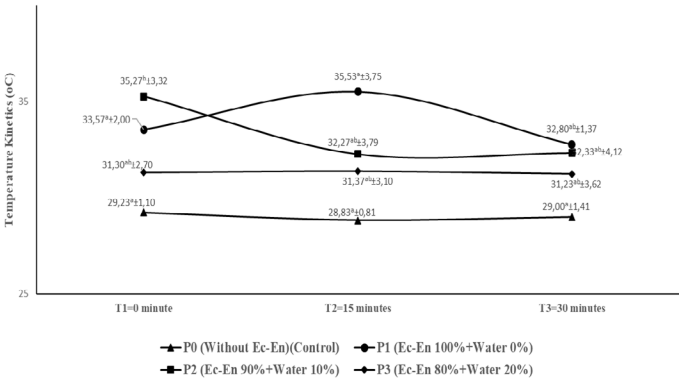

Figure 6: Changes in temperature kinetics (°C) from laying hen feces after applying eco-enzyme (Ec-En) from vegetable waste (spinach)(Sp) with different amounts of diluent.

The results of statistical analysis using ANOVA on the data (Figure 6) show that the differences in retail treatment in Ec-En and fermentation time produced a significant effect ($p<0.05$) on the reaction temperature. Changes in temperature occur along with changes in the Ec-En concentration level used. Changes in the applied Ec-En concentration resulted in different temperature change trends. As time went by, from 0 to 30 minutes, treatment P0 (control) showed a stable decrease in temperature from start to finish. Treatment P1 started with a higher temperature at 0 minutes compared to the control and experienced an increase in temperature at 15 minutes, and at 30 minutes, the temperature fell again. This shows that the full administration of eco-enzyme is very effective in reducing ammonia in the 15th minute. According to Zaferanloo *et al.*, (2014), enzyme activity increases with increasing temperature until it reaches the optimal temperature. However, increasing the temperature above the optimal temperature will cause enzyme work to decrease (Baehaki *et al.*, 2008). This is in line with research conducted by Souza *et al.* (2017) who characterized acid protease as working at an optimum pH of 5.0 and an optimum temperature of 55°C. The administration of eco-enzymes to feces has a crucial role in the composition process, especially in increasing temperature which has an impact on ammonia concentrations. According to Muliarta *et al.*, (2023), microorganisms in eco-en-

zymes accelerate the decomposition of organic matter, which not only increases temperature but also composting efficiency by reducing the volume and weight of feces. This effect helps stabilize ammonia, reducing its volatility so that it becomes less dangerous. Furthermore, the same study showed that eco-enzyme spraying increased leachate water temperature, indicating its potential as a composting activator. The high temperatures produced by the activity of microorganisms can increase oxygen consumption and speed up the decomposition process. Research by Priyambada *et al*. (2018) revealed that the optimal temperature for microorganisms is very important to optimize the process of decomposing organic materials. Expanding on this analysis, Nemet *et al*. (2021) added that temperature variations mark the progression of organic materials through important phases such as mesophilic, thermophilic, cooling and ripening. When using eco-enzymes, whether diluted or not, temperature is very important in determining how well the enzyme works. In other words, undiluted eco-enzymes that have lots of microorganisms work better in maintaining stable activity even though the surrounding temperature changes. This is because the thick microbial biomass provides protection, helping to maintain stable conditions for the enzyme to function. On the other hand, diluted eco-enzymes are more easily affected by temperature changes. The small number of microorganisms can cause the ability and stability of the enzyme to decrease. In contrast to eco-enzymes, if they are diluted the amount of organic compounds per unit volume will tend to decrease. This causes the acid content to decrease which will ultimately reduce the pH value. This is important because enzymes such as protease, amylase, and lipase, help break down organic compounds that work most effectively under certain pH conditions. Therefore, when the pH value changes due to the dilution process, the effectiveness of this enzyme will also be affected. As explained by Arun *et al*. (2015). The influence of temperature and pH on eco-enzymes is very important. For example, in processing organic waste in industry or agriculture, choosing the right concentration and temperature conditions of eco-enzymes can increase the speed and efficiency in breaking down waste. This not only saves time and costs, but also makes the process more environmentally friendly, as per studies (Vidyawati *et al*. 2019). Wang *et al*. (2023) revealed that the optimal conditions for ammonia degradation are at a temperature of around 38˚C, with an effectiveness level of 85.3%. Although the maximum temperature achieved by eco-enzyme with 100% administration is 35˚C, this value remains close to 38˚C, indicating that this temperature is effective for degrading ammonia.

Humidity kinetics (%): Humidity kinetics of laying hens after application of eco-enzyme (spinach)(Sp) with different amounts of diluent are completely presented in Figure 7.

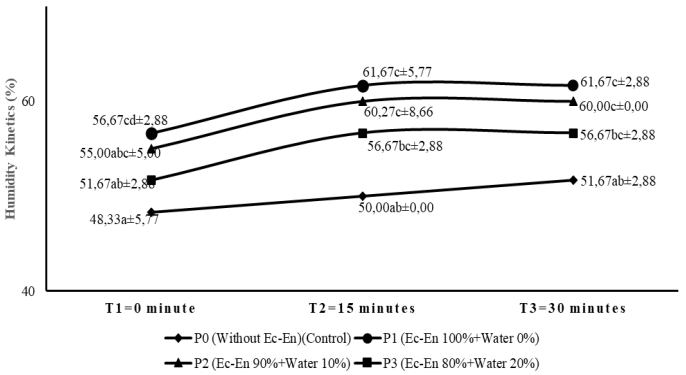

Figure 7: Changes in humidity kinetics (%) from laying hen feces after applying eco-enzyme (Ec-En) from vegetable waste (spinach)(Sp) with different amounts of diluent

Based on the results of statistical analysis (ANOVA), the data in Figure 7 shows that differences in the amount of dilution and fermentation time have a significant effect ($p<0.05$) on the humidity kinetics of Ec-En. These results show that all treatments experienced an increase in humidity kinetics from the 15th minute to the 30th minute. The most significant increase occurred at 15 minutes in all treatments. These results indicate that the initial reaction to eco-enzyme undergoes responsive changes in conditions. Treatment P_2 showed that the humidity was 60.27% and 60.00% at the 30th minute, and P_3 showed a slight decrease in humidity from the 15th minute, namely 56.67%, and was consistent until the 30th minute. This decrease is caused by the dilution of eco-enzyme which affects the efficiency of increasing humidity at a certain level. This shows that the addition of water with a higher concentration level to the eco-enzyme reduces the ability of the eco-enzyme to retain humidity. Humidity plays a significant role in metabolic processes, especially affecting the function of protease enzymes. Eco-enzymes play an important role in decomposing poultry feces, primarily through their effect on humidity. Increased humidity facilitates more intense microbial activity in feces, which is accelerated by the use of eco-enzymes. This process not only increases decomposition but also produces significant heat. This heat promotes evaporation, effectively reducing humidity and avoiding anaerobic conditions that can inhibit the decomposition process. Furthermore, this reduction in humidity by the heat generated increases efficiency in fecal management within the hood, addressing the problem of excessive or insufficient humidity. Factors such as high temperature and humidity, which promote further decomposition, are highly dependent on carbon availability and microbial metabolic activity, as explained by Fang *et al*., (2022). Higher temperatures, which result from microbial activity, accelerate evaporation of humidity from feces, resulting in an overall reduction in humidity. This phenomenon, as explained by Bleizgys *et al*., (2023), causes

an increase in ammonia concentration as temperature rises, while increasing humidity actually decreases ammonia concentration. Therefore, reducing humidity is key to reducing ammonia, which tends to evaporate more in more humid conditions. Humidity reduction by eco-enzyme helps stabilize ammonia, minimizing the risk of ammonia evaporation into the hood. Bleizgys *et al*., (2023) also emphasized the strong correlation between humidity and ammonia emissions. They explained that ammonia evaporation is a diffusion process, where gas molecules move from areas of high concentration to areas of lower concentration until they are evenly distributed. This process is a mass transfer between the fluid on the mud surface and the surrounding air flow, which shows the importance of humidity regulation in reducing ammonia emissions. Low humidity results in reduced solubility of nutrients in the substrate, while higher humidity exceeds the optimum humidity and can reduce the effectiveness of enzyme work. This causes the degradation process to be slower (Dias *et al*., 2007). Diluted and undiluted eco-enzymes affect enzyme action. pH and humidity have a positive correlation with the enzymatic activity of dehydrogenase and urease. High humidity supports enzyme activity because enzymes need water to help the decomposition process of chemicals such as ammonia. Undiluted eco-enzymes may be more effective in maintaining the internal humidity required for microbial activity, thereby remaining efficient in decomposing ammonia even in dry environmental conditions. Meanwhile, dilution increases the liquid volume, which causes external humidity fluctuations to further affect the water balance in the mixture, potentially disrupting effective enzyme concentrations and reducing their ability to decompose ammonia. According to Dias *et al*., (2007), low humidity results in reduced solubility of nutrients in the substrate, while higher humidity beyond the optimum humidity can reduce the effectiveness of enzyme work. This causes the degradation process to be slower. The optimum humidity for the protease enzyme is around 65% Haque *et al*., (2016). The results showed that the humidity in the eco-enzyme treatment was still in the optimal humidity range compared to the control.

CONCLUSION

Eco-enzymes (Ec-En) can be produced from kale (Ka) and spinach (Sp) vegetable waste or their combination (Ka+Sp). The different types of materials used produce different Ec-En characteristics (pH, enzyme activity and total LAB). Treatment P_1 (100% of Ec-En + 0% of water) produces better characteristics than the other treatments (P_2 and P_3) and the control (P_0) in terms of ammonia gas production, kinetic temperature and kinetic humidity. The fermentation time of 15 minutes after the spraying process shows that there is a maximum reduction in ammonia gas in the feces of laying hens.

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NOVELTY STATEMENT

So far, the application of eco-enzymes is limited to being a bioactivator in making organic fertilizer. The application of eco-enzymes as ammonia gas reducers in poultry feces has not been widely published. This research provides a solution for reducing ammonia gas produced from poultry feces.

AUTHOR CONTRIBUTION

Conceptualization, M.A.D., M.I.S., and N.L.; investigation, M.A.D.; analysis and interpretation of data, M.A.D., M.I.S., N.L; original draft preparation, M.A.D., and N.L; review and editing, M.I.S., N.L. project administration, M.A.D.; funding acquisition, M.A.D. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

There is no conflict of interest between researchers and authors in the article preparation and publication process for this article.

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