Short Communication

Characterization Plant-Enzyme from Fruit and Vegetable Waste as Ammonia Reducer in the Laying Hen Industry

Urfiana Sara[1,4], Muhammad Irfan Said[2]*, Wempie Pakiding[2], Sri Purwanti[3]

[1]*Faculty of Animal Science, Hasanuddin University, 90245, Indonesia;* [2]*Department of Animal Science, Faculty of Animal Science, Hasanuddin University, Indonesia 90245;* [3]*Department of Animal Feed and Nutrition, Faculty of Animal Science, Hasanuddin University, Indonesia 90245;* [4]*Agricultural Development Polytechnic of Gowa, Indonesia 92171.*

Abstract | Ammonia gas emissions are an environmental problem in Indonesia. The study aims to evaluate the character and properties of Pl-Enz as ammonia gas reducing agents, especially in the laying hen livestock industry. The combination of raw materials consists of: En_1 (100% of Fw); En_2 (75% of Fw + 25% of Vw); En_3 (50% of Fw + 50% of Vw); En_4 (25% of Fw + 75% of Vw) and En_5 (100% of Vw). The results of the study showed that there was a significant difference ($p<0.05$) between the commercial enzyme (En_0) and the treatment enzyme (En_1, En_2 ,En_3 , En_4 and En_5) for all parameters tested (Ec; pH value; Pea,Tot-LAB), and Lac. The results of the analysis of the difference test between treatments showed a significant difference ($p<0.05$) (En_1 Vs En_2 Vs En_3 Vs En_4 and En_5) especially in the parameters Ec, Pea, Tot-LAB and Lac. The final results of the study showed that En_5 showed characteristics associated with potential ammonia reduction and warrants further applied validation.

Keywords | Emmision, Ammonia, Enzyme, Fruit waste, Vegetable waste

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***Correspondence** | Muhammad Irfan Said, Department of Animal Science, Faculty of Animal Science, Hasanuddin University, Indonesia 90245; **Email:** irfanunhas@gmail.com
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INTRODUCTION

The laying hen industry is a crucial sector in providing animal protein, but its production activities generate waste that can cause environmental problems, particularly ammonia (NH_3) emissions. Ammonia gas is formed from the decomposition of nitrogen in chicken manure, specifically from uric acid compounds broken down by microorganisms. Ammonia emissions from poultry farms are known to contribute to air pollution, environmental degradation, and nutrient loss from livestock waste. Furthermore, high ammonia concentrations in poultry houses can negatively impact the health of chickens and workers, such as respiratory problems and reduced livestock productivity. Ammonia concentrations exceeding approximately 25 ppm have been reported to affect poultry health and air quality in the house. Therefore, effective and environmentally friendly technologies are needed to reduce ammonia gas emissions in laying hen production systems (Jiang *et al*., 2021).

Various approaches have been implemented to control ammonia emissions in livestock farming, such as litter management, improved ventilation, the use of additives, and biological waste treatment. Several studies have shown that biological processes such as microbial nitrification or changes in manure pH can reduce ammonia volatility by converting NH_3 into the more stable ammonium (NH_4^+)

form. However, most of the technologies used still rely on chemicals or conventional management methods, which have limitations in terms of cost, sustainability, and potential environmental impact. On the other hand, the use of organic waste such as fruit and vegetable peels to produce plant enzymes through fermentation is gaining attention due to their content of various hydrolytic enzymes such as proteases, lipases, and amylases. However, research specifically examining the characterization of plant enzymes from these wastes and their mechanisms of action in suppressing ammonia gas formation in livestock environments is still very limited. Therefore, further research is needed to evaluate the potential of plant enzymes as a biological alternative in mitigating ammonia emissions in the laying hen industry (Salim *et al*., 2014; Harahap and Ginting, 2021; Hasanah and Gultom, 2023; Pit Ay *et al*., 2024; Tokpohozin *et al*., 2015), biodisinfectants (Ginting *et al*., 2021; Hasnudi *et al*., 2022; Rizqan *et al*., 2023; Vidalia *et al*., 2023a), livestock wastewater cleaning agents (Herdin *et al*., 2023; Nurjana *et al*., 2023), and natural medicines for scabies or mange in livestock (Mursyid *et al*., 2022).

Based on these problems, the use of fruit and vegetable peel waste as a source of plant enzymes has the potential to be an innovative solution in controlling ammonia emissions in the livestock industry. This organic waste can be fermented to produce liquid enzymes containing various enzymatic activities and microorganisms that can accelerate the degradation of organic matter in livestock manure. A more efficient biodegradation process is expected to reduce the accumulation of nitrogen compounds that are precursors to ammonia gas formation. In addition, this approach also supports the principle of a circular economy by utilizing agro-industrial waste into value-added and environmentally friendly products. Therefore, characterization of plant enzymes produced from fruit and vegetable peel waste is important to determine their physicochemical properties, enzymatic activity, and potential in reducing ammonia emissions. The hypothesis of this study is that plant enzymes from fruit and vegetable peel waste have biocatalytic activity that can accelerate the degradation process of organic matter in livestock waste, thereby reducing the formation and emissions of ammonia gas in the environment of laying hen farms (Anderson *et al*., 2020; David *et al*., 2015; Bist *et al*., 2023).

MATERIALS AND METHODS

Materials

The main raw material of Pl-Enz is fruit waste (Fw) and vegetable waste (Vw). Fruit waste is obtained from the skin of the fruit. The types of fruit used include: watermelon (Wm), orange (Or), banana (Bn), and papaya (Pa), while vegetable waste is obtained from the stems of vegetables such as kale (Ka), green mustard (Gm), spinach (Sp), and chayote (Ch).

Methods

The research was designed using a Completely Randomized Design (CRD) with a 6x3 one-way pattern. A total of 5 treatment combinations + 1 control were used as treatments. Each treatment was repeated 3 times. Plant enzymes were obtained from 2 types of raw materials, namely fruit waste (Fw) and vegetable waste (Vw). The treatments applied consisted of: (1) En_0 (commercial plant enzyme) as a control; : (2) En_1 (100% Fw); (3) En_2 (75% Fw + 25% Vw); (4) En_3 (50% Fw + 50% Vw); (5) En_4 (25% Fw + 75% Vw) and (6) En_5 (100% Vw). The formulation of each material is presented in Table 1. Research parameters include: 1) Ethanol content (Ec) (%); 2) pH value; 3) Protease enzyme activity (Pea) (U/mL); 4) Total of lactic acid bacteria (Tot-LAB) (CFU/mL) and 5) Lactic acid content (Lac)(%). The data obtained were processed using analysis of variance (ANOVA) based on a Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

The results of the analysis related to the characteristics of Pl-Enz from several raw material (Fw and Vw) formulations are presented in full in Table 2.

Based on Table 2, ethanol content, it can be seen that the En_5 (100% Vw) treatment contains the highest ethanol content. The results of statistical analysis (ANOVA) showed that the value was significantly higher ($p<0.05$) compared to the control (En_0) and the other four treatments (En_1-En_4). En_0 (control) contains the lowest ethanol content compared to the treatments. The value was not significantly different ($p>0.05$) from En_1(100% Fw), but was significantly lower ($p<0.05$) than En_2 to En_5. The ethanol content in this study ranged from 13-17%. The 17% ethanol concentration in this study did not meet the effective threshold as a disinfectant, as ethanol's antiseptic activity is generally optimal at concentrations ≥60–70%. Therefore, ethanol is not positioned as a primary disinfection agent. The mention of ethanol as one of the "best" formulation indicators refers to its role as a marker of fermentative metabolic intensity, which correlates with the production of other metabolites, particularly organic acids. At this concentration, ethanol only has the potential to provide a partial antimicrobial effect against certain microbes and contributes to general odor reduction, but not through a direct mechanism of NH_3 reduction. Thus, the primary factors for ammonia reduction remain determined by organic acid production and pH reduction, which affect the NH_3/NH_4^+ balance.

Table 1: Formulation of raw materials used to Make Pl-Enz from each treatment.

Raw materials	En_0	En_1	En_2	En_3	En_4	En_5
Fruit waste (Fw)						
Water Melon (Wm)(%)	-	25	18.75	12.5	6.25	0
Orange (Or)(%)	-	25	18.75	12.5	6.25	0
Banana (Bn)(%)	-	25	18.75	12.5	6.25	0
Papaya (Pa)(%)	-	25	18.75	12.5	6.25	0
Sub Total of Fw	-	100	75	50	25	0
Vegetable waste (Vw)						
Kale (Ka)(%)	-	0	6.25	12.5	18.75	25
Green mustard (Gm %)	-	0	6.25	12.5	18.75	25
Spinach (Sp)(%)	-	0	6.25	12.5	18.75	25
Chayote (Ch)(%)	-	0	6.25	12.5	18.75	25
Sub Total of Vw (%)	-	0	25	50	75	100
Total (%)		100	100	100	100	100
Formulation Ratio of Pl-Enz						
(Fw + Vw) : Mo : Wat = (3 : 1 : 10)						
(Fw + Vw)	-	1/3 x 3 x 900 g = 900 g				
Molases (Mo)	-	1/3 x 1 x 900 g = 300 g				
Water (Wat)	-	1/3 x 10 x 900 g = 3,000 mL				
Fruit Waste (Fw)						
Water Melon (Wm)(g)	-	225	168.75	112.5	56.25	0
Orange (Or)(g)	-	225	168.75	112.5	56.25	0
Banana (Bn)(g)	-	225	168.75	112.5	56.25	0
Papaya (Pa)(g)	-	225	168.75	112.5	56.25	0
Sub Total of Fw (g)	-	900	675	450	225	0
Vegetable Waste (Vw)						
Kale (Ka)(g)	-	0	56.25	112.5	168.75	225
Green Mustard (Gm)(g)	-	0	56.25	112.5	168.75	225
Spinach (Sp)(g)	-	0	56.25	112.5	168.75	225
Chayote (Ch)(g)	-	0	56.25	112.5	168.75	225
Sub Total of Vw (g)	-	0	225	450	675	900
Total (g)	-	900	900	900	900	900
Formulation of Pl-Enz						
(Fw + Vw) (g)		900	900	900	900	900
Molases (Mo) (g)	-	300	300	300	300	300
Water (Wat) (mL)	-	3,000	3,000	3,000	3,000	3,000

The pH values of the eco-enzymes in this study can be seen in Table 2. Data analysis showed that En_1 (eco-enzyme 50% fruit waste + 50% vegetable waste) had the highest pH value. This value was significantly higher (*P<0.05*) than the control (En_0), but not significantly different (*P>0.05*) from En_1, En_2, En_4, and En_5. The lowest pH value was found in the control (En_0), which was significantly lower (P<0.05) than En_1 to En_5. The pH range in this study was 2.9 to 3.3. The pH value is a measure of the acidity or alkalinity of a solution, with values ranging from 1 to 14. The pH value of a solution can be an indicator of the level of enzyme activity or the amount of organic acids produced. The five types of eco-enzymes in this study had relatively high levels of acidity. Several previous studies have also shown that the fermentation process generally results in high concentrations of eco-enzyme liquids.

Table 2, protease activity shows the protease activity of eco-enzymes prepared from a mixture of fruit and vegetable waste at varying percentages. En_1 (eco-enzyme made from 100% fruit waste) had the highest protease activity compared to the control and other treatments.

Links Researchers

Table 2: Characteristics and properties of Pl-Enz produced from Fw and Vw in different formulations.

Parameter	Formulation					
	En_0	En_1	En_2	En_3	En_4	En_5
Ec (%)	13.250±0.500[a]	15.500±0.577[ab]	15.875±0.250[b]	15.000±0.000[c]	15.000±0.000[c]	17.000±0.000[d]
pH Value	2.942±0.049[a]	3.207±0.101[b]	3.215±0.145[b]	3.277±0.141[b]	3.190±0.133[b]	3.142±0.095[b]
Pea (U/mL)	21.237±0.674[ab]	21.957±0.971[a]	21.310±0.205[ab]	20.470±0.664[b]	20.747±0.601[b]	21.117±0.30[ab]
Tot-LAB (CFU/mL)	($1.5x10^2$± $7.1x10^1$)[a]	($3.55x10^6$± $2.1x10^5$)[b]	($1.25x10^7$± $7.1x10^5$)[c]	($1.60x10^7$± 0.000)[d]	($7.95x10^7$± $2.1x10^6$)[e]	($2.30x10^5$± $1.1x10^5$)[a]
Lac (%)	1.590±0.097[a]	2.009±0.205[b]	2.584±0.194[cd]	2.364±0.105[c]	2.793±0.093[de]	2.981±0.200[e]

Note: [a-d] Different superscripts on the same row indicate significant differences (p<0,05). Plant enzyme= Pl-Enz; Fw= Fruit waste; Vw= Vegetable waste; En_0 (commercial enzyme (control); En_1 (100% of Fw); En_2 (75% of Fw + 25% of Vw); En_3 (50% of Fw + 50% of Vw); En_4 (25% of Fw + 75% of Vw) and En_5 (100% of Vw); Ec= ethanol content; Pea= protease enzyme activity; Tot-LAB= total lactic acid bacteria; Lac= lactic acid content.

This value was significantly higher (*P<0.05*) than treatments En_3 and En_4, but not significantly different (*P>0.05*) from the control, En_2, and En_5. The lowest protease activity was found in eco-enzyme En_3 (eco-enzyme made from 50% fruit waste + 50% vegetable waste). This value was significantly lower (P<0.05) than En_1, but not significantly different (*P>0.05*) from the control, En_2, En_3, En_4, or En_5. Eco-enzyme activity in this study ranged from 20.4 to 22.0 U/mL.

This study showed that protease activity was not significantly different between the control and treatments, at around 20.0 U/mL. Previous studies have shown more varied results. Research by Ratih *et al.* (2023) found that eco-enzymes made from fruit peel waste (banana, pineapple, orange, papaya, and mango) had a protease activity of 15.41 U/mL-1 at pH 3.45. Another study by Arun and Sivashanmugam (2017) found protease enzyme activity in eco-enzymes from pineapple and orange peels of 44.039 U/mL at pH 6. In contrast to Abdullah *et al.* (2023) found lower protease activity in eco-enzymes mixed with palm sugar, namely 0.962 U/mL at pH 7.

Data analysis of showed that the highest total LAB content (Table 2) was found in treatment En_4 (Eco-enzyme 75% vegetable waste + 25% fruit waste), significantly higher (*P<0.05*) than the control and other treatments. En_0 had the lowest total LAB content. This value was significantly lower (*P<0.05*) than En_1, En_2, En_3, and En_4, but not significantly different (*P>0.05*) from treatment En_5. The range of total LAB content in this study was 1.50 x 102 to 8.00 x 107 CFU/mL. Lactic acid bacteria in eco-enzyme play an important role, particularly those specific for reducing ammonia gas in livestock fecal waste. Studies related to total LAB content in eco-enzyme are still quite limited. Mahdia *et al.* (2022) found a total bacterial count of 1.9 x 106 CFU/mL in orange peel eco-enzyme, although the total LAB population was not detailed. Another study by Setiawati *et al.* (2023) found a total LAB count of 2.34 log CFU/mL in eco-enzyme from spinach cuttings. Putri *et al.* (2024) also found LAB counts in eco-enzyme from a mixture of beef cattle feces and rice straw, ranging from 6.76 x 105 to 14.13 x 105 CFU/mL after 7 days of fermentation.

The lactic acid levels of eco-enzyme derived from various fruit and vegetable waste mixtures (Table 2). Analysis results showed that En_5 contained significantly higher levels of lactic acid (P<0.05) compared to the control and other treatments. Treatment En_0 contained significantly lower levels of lactic acid (*P<0.05*) compared to the other treatments. Lactic acid levels in this study ranged from 1.5% to 3.0%. The difference between LAB cell count and lactic acid accumulation suggests that the relationship between the two is not always linear. This could be due to differences in the ability of each strain to produce lactic acid (Silva *et al.*, 2018). In En_5, each cell likely has a higher lactate production capacity than En_4, so even though the cell count is not very large, acid accumulation remains high. Furthermore, En_5 may have entered the early stationary phase, where cell growth begins to slow but acid production continues. Conversely, En_4 is likely still in the exponential phase, so more energy is used for cell growth than acid formation. Differences in fermentation type also play a role, as homofermentative LAB produce more lactate, while heterofermentative LAB produce a mixture of other metabolites, resulting in lower lactic acid production (Krumova *et al.*, 2024).

CONCLUSION

Based on the research results, En_5 demonstrated the highest ethanol (17.00%) and lactic acid (2.981%) levels compared to other treatments, with pH and protease activity not significantly different from the highest values, and total LAB still within the active fermentative range. Conceptually, the determination of En_5 as the best eco-enzyme is based on a combination of biochemical parameters most consistent with the mechanism of ammonia volatility reduction: relatively high acid production, a moderate enzymatic profile, and stable fermentation dynamics. However, this conclusion remains inferential because in situ functional validation has not been performed on laying hen feces as the primary source of ammonia emissions. Therefore, the effectiveness of En_5 in reducing ammonia gas needs to be confirmed through further field testing.

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NOVELTY STATEMENT

Based on findings from previous research projects, published data and papers do not yet contain specific information regarding the characteristics of plant enzymes. The data presented in this article has not been widely published.

AUTHOR'S CONTRIBUTION

Conceptualization: Urfiana Sara., Muhammad Irfan Said., Sri Purwanti and Wempie Pakiding; investigation: Urfiana Sara., Muhammad Irfan Said; data analysis and interpretation: Urfiana Sara., Muhammad Irfan Said., Sri Purwanti; original draft preparation: Muhammad Irfan Said., and Sri Purwanti; review and editing: Muhammad Irfan Said., Wempie Pakiding. Project administration: Urfiana Sara., Muhammad Irfan Said; funding acquisition, Muhammad Irfan Said., Urfiana Sara., the authors have read and approved the published version of the manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this 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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