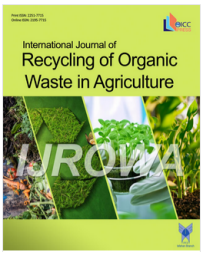

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Fermentation of poultry manure for improving its quality and safety as ruminant feed

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Abstract:
Purpose: Poultry manure (PM) is a concern for Bangladesh. The improvement of quality and safety is always desirable. The experiment assesses the quality and safety parameters of PM with the addition of *Saccharomyces cerevisiae* and indigenous microorganisms (IMO) in different fermentation conditions (aerobic, facultative anaerobic, and anaerobic) for 7 days.
Method: Fermentation condition × treatment factorial analyses were performed to explore their effect on the quality and safety of PM. The organoleptic quality (color, smell, and texture), pH, nutritional components (organic matter, crude fiber, crude protein, ether extract, nitrogen-free extract), in-vitro organic matter digestibility, metabolizable energy, mineral contents (phosphorus, potassium, sulfur), heavy metals (lead, copper), and microbial properties (total coliform count, *E-coli*, *Salmonella*) of fermented PM at 0, 3, 5, and 7 days were evaluated.
Results: All parameters of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing the fermentation time. All parameters were found desirable in 10% IMO treated PM. Organoleptic parameters (color, smell, and texture) were satisfactory in aerobic fermentation but other parameters were acceptable in anaerobic fermentation conditions. pH was dropped significantly ($P < 0.05$) with increasing the duration.
Conclusion: To summarize all properties, it could be noted that the quality and safety of PM were improved after 7 days of fermentation with a fermentation mixture of PM (90%) and molasses (10%) inoculating 10% IMO under an anaerobic condition which could be used for animal feeding.

Keywords: *Saccharomyces cerevisiae*; *E. coli*; Heavy metals; Indigenous Micro Organisms (IMO); *Salmonella*

1. Introduction

Since the start of the twentieth century, Bangladesh's poultry industry has provided an immense platform for job creation, quick profit, and cheap animal protein production (Rahman et al. 2017; Islam et al. 2022). A total of approximately 150,000 poultry farms exist in the country (Alam et al. 2019). In general, a chicken produces nearly 1 kg of manure with a variation in water content for every kg of feed consumption. As a result, it can be estimated that approximately 13.46 MT of poultry manure is generated every year in Bangladesh (Alam et al. 2019). As a huge amount of manure is produced by layer and broiler farms, less attention is paid to managing these two particular sectors of the poultry industry.

Due to the accompanying air, water, and soil pollution, raw chicken manure storage and disposal have turned into a hazard for the environment as the manufacturing of poultry products has increased. Poultry manure starts to break down right after excretion, releasing ammonia, which in excessive amounts can be harmful to the health and production of the poultry as well as the farm employees (Ghaly and Macdonald 2012). Additionally, manure can act as a breeding ground for disease-carrying microbes and a vector for the spread of illnesses among birds. On manure, flies and other unpleasant insects can grow, posing health risks and causing annoyance. Manure also produces an odor, which is brought on by the action of harmful bacteria (Ghaly and Macdonald 2012). Poultry manure is rich in nutrients like nitrogen and phosphate, as well as other ejected materials

like hormones, antibiotics, pathogens, and heavy metals. These compounds can contaminate groundwater resources and surface water through runoff and leaching (Gerber et al. 2007). This waste also contributes significantly to the increase in dangerous gas emissions (Komolafe and Sonaiya 2014; Tchoukanova et al. 2012). These emissions cause global warming that threatens our existence. Among other things, ensuring proper management of the manure and reducing environmental pollution are now major challenges. Smallholding farms make up the majority of ruminant farms. Farmers frequently experience a major problem with a lack of feed, both in terms of quantity and quality (Islam and Khan 2021). Additionally, quality protein feed is costly for farmers. The processing of substitute feed ingredients may offer a solution to the issues with feed availability and cost (Ritu et al. 2021; Kabir et al. 2021). The manure of poultry is one of the alternative protein feed ingredients that are of high quality and is widely available all year (Utama and Christiyanto 2021a). In particular, stocker and brood cattle, which form the foundation of the nation's cattle sector, benefit greatly from the additional protein that poultry manure provides. Manure has a high level of crude protein (up to 30%). When this manure can be recycled by fermentation, it can be used up to 10 times more effectively as cattle feed. Rumen microorganisms are also able to utilize other elements of the manure to build body protein consumed by cattle. Furthermore, the manure frequently contains different percentages of high-quality spillage chicken feed, which can sometimes greatly increase its nutritional value (Mkhombe and Hendrickx 2015).

Fermentation technology is the biological process in which various microorganisms such as yeast, bacteria, and fungi are involved in the conversion of complex substrates into simple compounds. Organic acid and alcohol are the main products of fermentation. In this process, there is the liberation of secondary metabolites like antibiotics, enzymes, and growth factors (Kuila and Sharma 2018). Fermentation of poultry manure is considered an appropriate option for the management of such waste (Shurson 2018; Alam et al. 2019; Han et al. 2018). Proper microbial and enzyme sources can increase the degradation of raw materials. Biological treatment of feedstock can be a cost-effective and environmentally friendly method to optimize the production process and reduce ammonia emissions by treating waste such as poultry manure (Rubežius et al. 2020). Indigenous micro-organisms (IMO) are a good source of the innate microbial consortium that inhabits the surfaces of all living things inside and outside, which have the potential for nitrogen fixation, bioleaching, biodegradation, bio-composting, and as well in the production of growth hormones (Kumar and Gopal 2015). Yeast fermentation could also potentially be used to enhance their nutritive value as animal diets, especially the protein and mineral contents of these products (Shurson 2018).

The study of ruminant feed preparation (Sultana et al. 2020) and *in-vivo* trials using poultry manure have been investigated (Elemam et al. 2009; Khan et al. 2016; Obeidat et al. 2011; Mohammadi et al. 2014; Han et al. 2018; Tadayon et al. 2017; Obeidat et al. 2019). The outcomes from the research support this research project as beef cattle feeding for the enhancement of nutritional properties required for beef cattle production. Considering the above evidence, the present experiment was undertaken to improve the quality and safety of poultry manure as ruminant feed through the fermentation process.

2. Material and methods

2.1 Location of the study

The experiment and nutritional analysis (organoleptic parameters, pH, organic matter, crude fiber, crude protein, ether extract, nitrogen-free extract, in-vitro organic matter digestibility, and metabolizable energy) were determined in the laboratory of the Department of Animal Science, Bangladesh Agricultural University. Heavy metals (Pb and Cu) were analyzed at the Interdisciplinary Institute for Food Security, Bangladesh Agricultural University. Minerals (P, K, and S) were analyzed at the Department of Soil Science, Bangladesh Agricultural University. Microbial analysis (Coliform, *E.coli*, and *Salmonella*) was performed in the laboratory of the Department of Microbiology & Hygiene, Bangladesh Agricultural University.

2.2 Collection of materials

The collection of poultry manure was done from the poultry farm, at Bangladesh Agricultural University. The containers and bakery yeast used to ferment poultry manure were purchased from Ganginapar, Mymensingh. The containers were washed thoroughly, dried, and marked according to the treatments. Molasses was purchased from Swadeshi Bazar, Mymensingh. IMO was produced from the fermentation of boiled rice according to the guidelines of (Jan et al. 2020).

2.3 Study design

Fermentation condition × treatment factorial analyses were performed to explore their main and interaction effects over the fermentation time. Poultry manure was fermented at 37°C (Chun et al. 2020) for 7 days under aerobic, facultative anaerobic, and anaerobic conditions to reduce offensive odors, pathogenic organisms, and heavy metals, and to improve the nutritional quality. The treatments were followed to conduct the study:

Treatments:

T_0 = Only poultry manure (100%)

T_1 = Poultry manure (90%) + molasses (10%)

T_2 = Poultry manure (90%) + molasses (10%), the addition of Saccharomyces cerevisiae (10%)

T_3 = Poultry manure (90%) + molasses (10%), the addition of IMO (10%)

The ingredients were measured on a dry matter basis.

2.4 Fermentation

Yeast and IMO were mixed with molasses and then added to the poultry manure. After that, the poultry manure was kept in the container. For anaerobic fermentation, the containers were sealed with the container's lid properly so that air could not enter. In facultative anaerobic fermentation, tissue papers were placed on the containers. All containers were incubated at a fixed temperature of 37°C for 7 days in an

Figure 1. Parameters of color assessment.

air-dry oven. The samplings were performed at 0, 3, 5, and 7 days. The semi-solid samples, containing on average 60% moisture, were collected in zipper bags.

2.5 Organoleptic test

Organoleptic tests with non-parametric analysis were carried out using a scoring method in which color, smell, and texture were observed and evaluated using a comparative scale. In the experiment, the panelists' numbers amounted to 5 people, with 7 classes of comparison scales for the assessment of the parameters. This method was followed according to Utama and Christiyanto (Utama and Christiyanto 2021a).

Color assessment:

Score 1: Deep black; Score 2: Black; Score 3: Dark brown blackish; Score 4: Dark brown; Score 5: Brown; Score 6: Light brown; Score 7: Yellow-brown (Fig. 1).

Smell assessment:

Score 1: Odorless ammonia; Score 2: Very little smell of ammonia; Score 3: Slight smell of ammonia; Score 4: Characteristic smell of ammonia; Score 5: Smell of ammonia is slightly pungent; Score 6: Smell of ammonia stings; Score 7: Smell of ammonia is very pungent;

Texture assessment:

Score 1: No blobs; Score 2: Very few blobs; Score 3: Slight blob; Score 4: Medium; Score 5: More blobs; Score 6: Very many blobs; Score 7: Lump it all together.

2.6 Quality and safety properties

pH, nutritional components, in-vitro organic matter digestibility, and metabolizable energy were analyzed according to the procedure of Sarker et al. (2022). To determine the minerals (P, K, and S) and heavy metals (Pb, and Cu), 5 g of semi-solid samples, containing on average 60% moisture, were digested in 5 ml $HClO_4$ and 10 ml HNO_3 at 180°C for 2 hours. The digested samples were filtered, and the filtrated samples were volumed up to 20 ml and then the liquid samples were used for P, K, S, Pb, and Cu determination. P, K, and S were estimated by following (Kutu et al. 2019; Domínguez et al. 2019; Wu et al. 2019). Pb and Cu were determined by following (Utama and Christiyanto 2021a). The total coliform count was performed by the procedure of Soare et al. (2022) and *E.coli* and *Salmonella* were conducted by following Utama and Christiyanto (2021b).

2.7 Statistical analysis

At a 5% significance level, the Duncan multiple-range test was employed to check for differences between sample means. To determine the differences between the total mean values, the mean comparison test was also used. In this experiment, the following model was used:

Yijk = μ + Ai + Bj + (AB) ij + ϵijk i = 1. . . .a; j = 1. . . b; k = 1. . . n Where,

Yijk = Observation k in level i of factor A and level j of factor B

μ = The overall mean

Ai = The effect of level i of factor A

Bj = The effect of level j of factor B

3. Results and discussion

3.1 Organoleptic parameters

- Color

The color of the different treatments in different fermentation conditions changed over the fermentation time (Fig. 2). At 0 days, all treatments excluding T_0 improved their color due to the addition of molasses on them. After 7 days of incubation, T_3 treatment under aerobic conditions showed the darkest color among other treatments. The value was close to 2 which represented that it had a black color. The reason for changing color in poultry manure during the period is the increment of temperature during fermentation (Utama and Christiyanto 2021a). At this time, sugars and proteins reacted and bound together with increasing temperature. The microorganisms caused changes in the temperature during the fermentation that generated heat and trapped oxygen. In aerobic fermentation, ventilation was enabled to increase of the temperature rapidly within 7 days (Ouyang et al. 2014). Therefore, the color change in aerobic fermentation was more intense than in facultative anaerobic and anaerobic fermentation. Utama and Christiyanto (2021a) stated that after 9 weeks of facultative anaerobic fermentation, the fermented poultry litter color was brown.

- Smell

From Fig. 3, it was noticed that the smell of all treatments in different fermentation conditions reduced after 7 days of the fermentation period. But at 3 and 5 days, the smell of all treatments under anaerobic and facultative anaerobic conditions was increased compared to the aerobic condition due to the activities of microorganisms and the production of ammonia that could not be emitted in the environment. The smell came from the functions of microbial metabolism during the process of fermentation (Jha and Berrocoso 2016). Wang et al. (2018) presented that fermentation can produce an ammonia smell when nitrogen-containing compounds are degraded by the microorganisms. The smell of fermented poultry manure was caused by the activities of microorganisms in fermentation. The lowest smell was observed in aerobic conditions, especially in T_3 among other treatments and fermentation conditions. The value was close to 2 which meant that it had very little smell of ammonia. Aerobic fermentation reduced strong odors due to the emission of NH_3 and volatile organic compounds

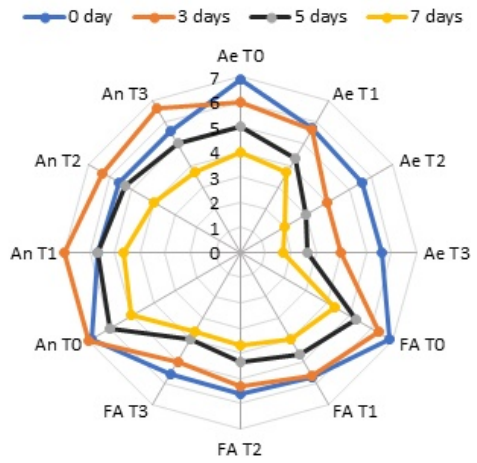

Figure 2. Color of the different treatments in different fermentation conditions and duration.
Where, Ae = Aerobic, FA = Facultative anaerobic, An = Anaerobic; T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Scoring list: Score 1: Deep black; Score 2: Black; Score 3: Dark brown blackish; Score 4: Dark brown; Score 5: Brown; Score 6: Light brown; Score 7: Yellow-brown.

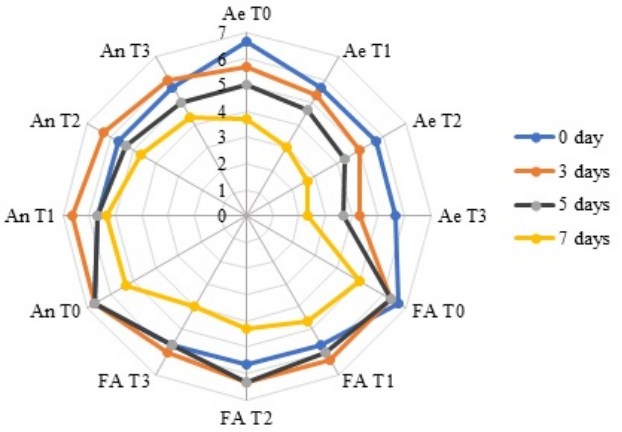

Figure 3. Smell of the different treatments in different fermentation conditions and duration.
Where, Ae = Aerobic, FA = Facultative anaerobic, An = Anaerobic; T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Scoring list: Score 1: Odorless ammonia; Score 2: Very little smell of ammonia; Score 3: Slight smell of ammonia; Score 4: Characteristic smell of ammonia; Score 5: Smell of ammonia is slightly pungent; Score 6: Smell of ammonia stings; Score 7: Smell of ammonia is very pungent.

into the environment (Colomer-Mendoza et al. 2012). Utama and Christiyanto found a pungent ammonia smell in fermented broiler litter after 9 weeks of fermentation in facultative anaerobic conditions (Utama and Christiyanto 2021a). Moreover, Shurson (2018) highlighted that yeast fermentation facilitates odor reduction in fermented products. Andreev et al. (2017) mentioned that fermentation can reduce odor due to the degradation of materials associated with odor by fermented microorganisms. Joshi et al. (2019) prioritized the importance IMO to eliminating the foul odor during fermentation as they have a mixture of microbiomes.

- Texture

All treatments under all fermentation conditions improved their texture after 7 days of fermentation. However, the texture deteriorated in anaerobic and facultative anaerobic fermentation after 3 days and improved again after 7 days (Fig. 4). The best value of texture was attributed to T_3 in aerobic conditions which was close to 1 representing no blobs in the sample. Fermentation is a kind of process in which physical, chemical, and biological changes occur (Peng and Guo 2015; Suningsih et al. 2019). The reasons for improving the texture in aerobic conditions and T_3 treatments were the evaporation of moisture and the functions of varieties of microflora in the samples. Moreover, the factor causing the changes in the texture during fermentation was the temperature changes that led to changes in the manure structure. There was a hot environment in the fermentation process that softened the structure of the manure. Utama and Christiyanto (2021a) found a little texture of blobs in the fermented broiler litter after 9 weeks of fermentation in facultative anaerobic conditions. Moreover, Irfan et al. (2017) presented that manure had a juicy, slight blob texture in an anaerobic fermentation process.

- pH of fermented poultry manure

The pH of the different treatments in different fermentation conditions changed with increasing period (Fig. 5). The pH value was lessened in all treatments after 7 days. The lowest pH was found in anaerobic fermentation and T_3 treatment which was below 5.

The acidic pH state in the product was caused by the microbial community and biodiversity in the fermentation system. Carbohydrate addition also contributed to the significant reduction in pH caused by organic acid generation (Liu et al. 2012). The main cause of pH decline was lactic acid produced during fermentation (Kung et al. 2018). Lactic acid generation appears to be greater under anaerobic conditions than under aerobic conditions (Smetanková et al. 2012). Indigenous microbes are the accumulation of aerobic and anaerobic microflora, with anaerobic digestion playing a significant role in organic compound bioconversion (Kumar and Gopal 2015). El-Jalil et al. (2008) reported that inoculated microbes to chicken waste mixtures proliferated rapidly and caused the pH to drop below 4.0 in a matter of days , and Khalib et al. (2018) obtained a similar outcome.

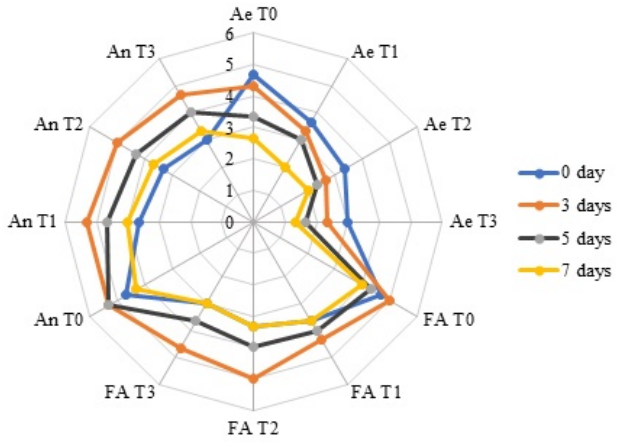

Figure 4. Texture of the different treatments in different fermentation conditions and duration.
Where, Ae = Aerobic, FA = Facultative anaerobic, An = Anaerobic; T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Scoring list: Score 1: No blobs; Score 2: Very few blobs; Score 3: Slight blob; Score 4: Medium; Score 5: More blobs; Score 6: Very many blobs; Score 7: Lump it all together.

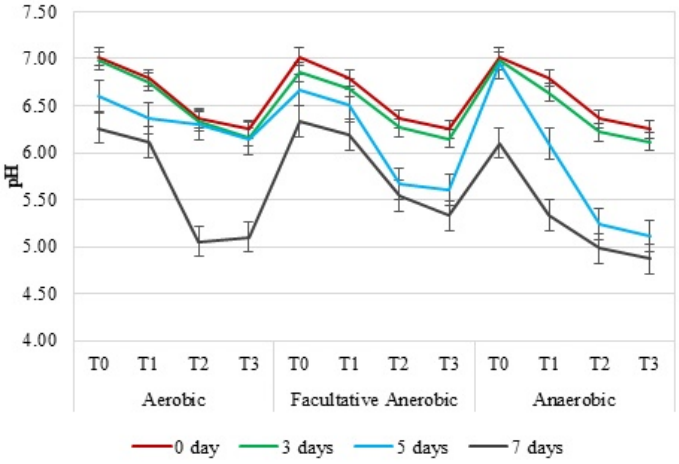

Figure 5. pH of different treatments in different fermentation conditions and time.

3.2 Nutrient components

- Organic matter

The organic matter (OM) of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing period (Table 1). OM was reduced in all fermentation conditions and treatments over time. In all fermentation conditions, OM was comparatively lower in T_3 than in other treatments. 55.40% was the lowest value found in T_3 under anaerobic conditions. Furthermore, both main effects showed significant differences ($P < 0.05$) while fermentation conditions were at 7 days and treatments were on all days. Leng (2014) reported that a diversified microbial population in fermentation causes the enhancement of organic matter digestion for their feed. T_3 had the lowest organic matter due to inoculating IMO, and a variety of microflora. These microbiomes utilized organic matter as their food and improved the fermentation condition.

- Crude fiber

The crude fiber (CF) of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing time (Table 2). CF was reduced in all fermentation conditions and treatments by improving the fermentation period. In T_3, the anaerobic condition showed the lowest value of 9.54%, in between facultative anaerobic (11.15%) and aerobic (11.10%) conditions. However, the main effects showed a significant difference ($P < 0.05$) at 3, 5, and 7 days, respectively. Microorganisms during fermentation can break down the fiber by their enzymatic action as a source of carbohydrates (Liang et al. 2008; Adebo et al. 2022) Fiber degradation by microbes in the fermentation process also improves the ease of digestion due to the solubilization of hemicellulose and cellulose contents (Adebo et al. 2022). Chun et al. (2020) found decreased crude fiber in food wastes after 5 days of fermentation with 0.02% yeasts.

- Crude protein

The crude protein (CP) of the different treatments in different fermentation conditions changed ($P < 0.05$) with increasing time (Table 3). CP was enhanced in all fermentation conditions and treatments over the incubation period but the most improvement was observed in T_3 treatment (31.80%) under anaerobic conditions at 7 days. T_2 (25.76%) was also improved in the same fermentation conditions in contrast to other conditions. Surprisingly, in this parameter, a difference ($P < 0.05$) was observed in the interaction effects between fermentation and treatment at 5 and 7 days including the main effects of treatments at 3, 5, and 7 days, respectively. During fermentation, the microorganism used protein to build up its cells at the early stages of the process. The main effects (fermentation condition and treatment) appeared a significant difference at 0, 3, and 7 days. During this process, some organisms die off and are regained as the source of protein. Besides, the improvement of protein materials was also resulted from the breakdown of the organic carbon compounds (Jusoh et al. 2013; Khalib et al. 2018). The increment of crude protein in fermentation was reported by (Mukherjee et al. 2016; Khalib et al. 2018; Chun et al. 2020; Haque et al. 2022).

- Ether extract

The ether extract (EE) of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing period (Table 4). The improvement of EE was notified in all the fermentation conditions and treatments over the period. The highest EE at 7 days was 7.17% in T_3 under anaerobic fermentation while at 0 days, it was only 2.72%. The result shows that there was a significant difference ($P < 0.05$) of mean values in the main effects of

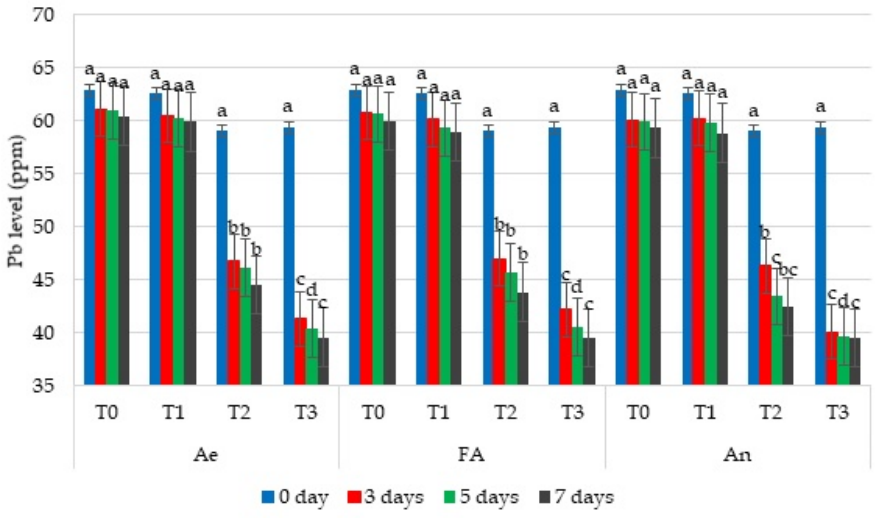

Figure 6. Effects of fermentation condition and time on Pb content in treatments.
Different letters on bars indicates significant difference ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Ae = Aerobic; FA = Facultative anaerobic; An = Anaerobic.

fermentation conditions at 3, 5, and 7 days and treatments at 0, 3, 5, and 7 days with the interaction effects between them at 3, 5, and 7 days, respectively. The improvement in fat content may partially be attributed to the decrease in carbohydrate content during fermentation (Adebo et al. 2022). Mukherjee et al. (2016), Akinola et al. (2017), and Inyang and Zakari (2008) reported on the advancement of crude fat in fermented products. Oluseyi and Temitayo (2015) also supported the results.

- Nitrogen free extract

The nitrogen-free extract (NFE) of the various treatments under varied fermentation conditions changed considerably ($P < 0.05$) as the time increased (Table 5). The values of NFE decreased with fermentation length in all fermentation conditions and treatments, with T_3 (6.89%) having the lowest value at 7 days under anaerobic circumstances. The value of NFE at 0 days was 18.13%, which was reduced 2.2 times after 7 days of fermentation. The primary impacts of fermentation conditions at 3, 5, and 7 days, as well as treatments at 0, 3, 5, and 7 days, differed significantly ($P < 0.05$). At the same time, the interaction effects between them showed a difference significantly ($P < 0.05$)

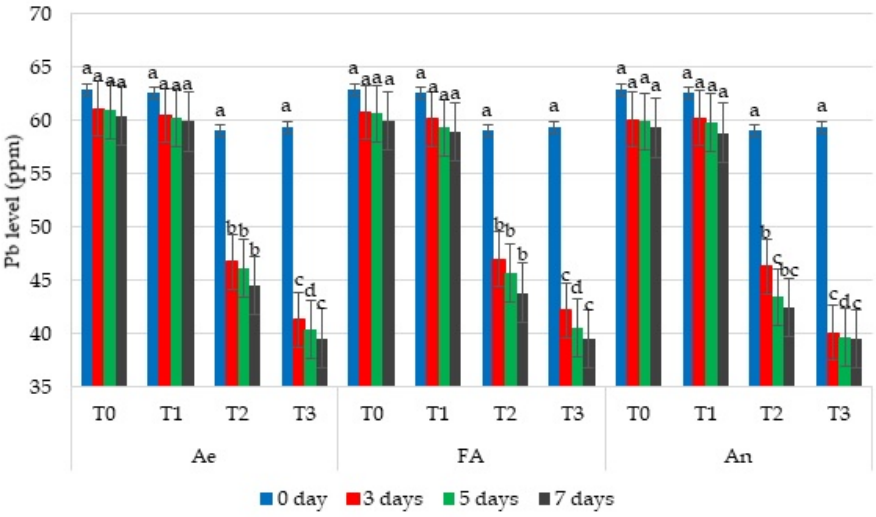

Figure 7. Effects of fermentation condition and time on Cu content in treatments.
Different letters on bars indicates significant difference ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Ae = Aerobic; FA = Facultative anaerobic; An = Anaerobic.

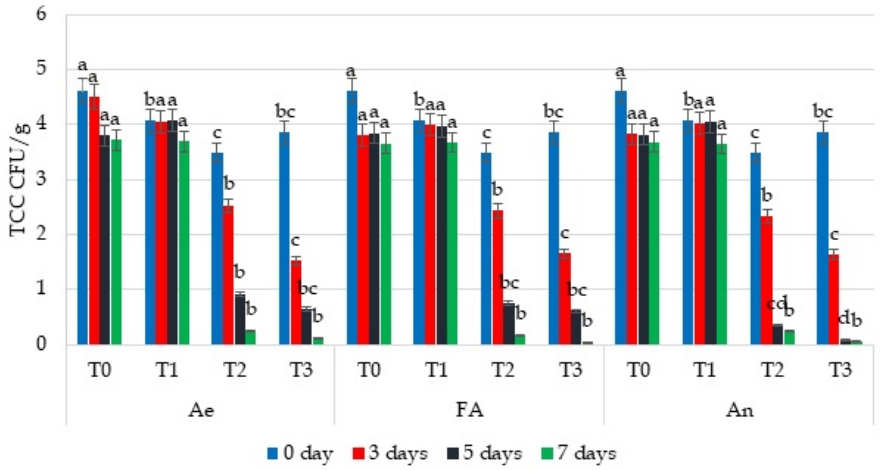

Figure 8. Effects of fermentation condition and time on total coliform count in treatments.
Different letters on bars indicates significant difference ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Ae = Aerobic; FA = Facultative anaerobic; An = Anaerobic.

on all days except 0 days. Nitrogen-free extracts can be defined as soluble carbohydrate (CHO) sources containing starches and sugars with small amounts of other materials. The enzymatic action and metabolism of the microorganisms led to a reduction in soluble CHO concentration. A decline in CHO was also caused by the α-amylase activity secreted by the microflora (Akinola et al. 2017). Akinola et al. (2017), Inyang and Zakari (2008), and Onwurafor et al. (2014) justified the result found in this experiment.

- In-vitro organic matter digestibility

The in-vitro organic matter digestibility (IVOMD) of the different treatments in different fermentation conditions changed ($P < 0.05$) with increasing time (Table 6). IVOMD was raised with fermentation duration in all treatments and fermentation conditions. Though the satisfactory result was found in T_3 (71.81%) under anaerobic conditions at 7 days, the results in facultative anaerobic (69.77%) and aerobic (70.21%) conditions were almost close to anaerobic conditions. One main effect, the treatment showed a significant difference ($P < 0.05$) on all days. In-vitro organic matter digestibility was determined to assess the feed value of the ingredient and it also determined the state of the fermentation in the rumen. IVOMD was enhanced with the addition of microorganisms due to the improvement in protein content and ash. In addition, dry matter (loss) was induced to increase the IVOMD in the fermented product (Cao et al. 2010). Sahoo and Walli (2008), Goiri et al. (2009), and Cao et al. (2010) reported improved IVOMD similar to the results of this research.

- Metabolizable energy

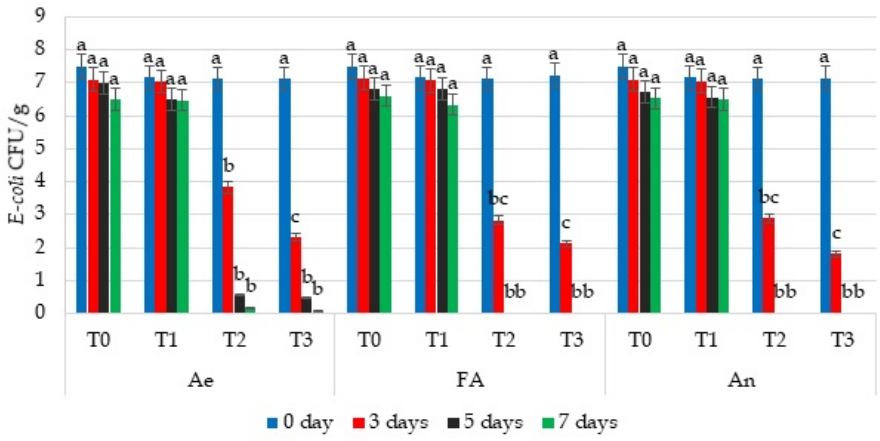

Figure 9. Effects of fermentation condition and time on E-coli in treatments
Different letters on bars indicates significant difference ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Ae = Aerobic; FA = Facultative anaerobic; An = Anaerobic

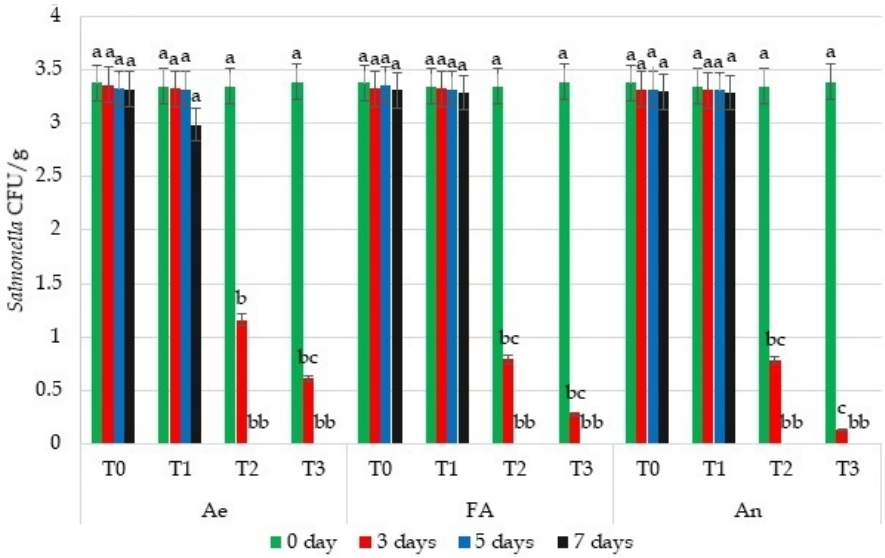

Figure 10. Effects of fermentation condition and time on Salmonella in treatments.
Different letters on bars indicates significant difference ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). Ae = Aerobic; FA = Facultative anaerobic; An = Anaerobic

The metabolizable energy (ME) of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing time (Table 7). The incubation period reduced the ME value in all treatments and fermentation conditions. Anaerobic conditions provided the lowest value, specifically in T_3 (2528.32 kcal/kg DM) after 7 days of the incubation period. However, only treatment (T) showed a significant difference ($P < 0.05$) on all days. ME was decreased during fermentation due to soluble carbohydrate utilization as microorganisms grow (Cherdthong 2020). Wittayakun et al. (2019) mentioned that ME was reduced with the improvement in crude fat and crude protein content. A similar result was found in this experiment while Haque et al. (2022), Chun et al. (2020) and Mihiret (2009) supported the findings.

- Minerals

The phosphorus (P), potassium (K), and sulfur (S) of the different treatments in different fermentation conditions changed significantly ($P < 0.05$) with increasing time (Table 8, 9, 10). It was observed that P, K, and S decreased with increasing time in all treatments and fermentation conditions. Surprisingly, P, K, and S showed similar kind of results, with lowest values in anaerobic conditions, particularly, in T_3. Depletion of the mineral is associated with the metabolic activities of organisms in fermentation that hydrolyzed the complex bond of metal-phytate to release the free minerals and take up for their body functions (Nnam and Obiakor 2003). The P, K, and S requirements for beef cattle were 0.17–0.39%, 0.70%, and 0.15% in the ration for 24 hours, respectively. P is essential for cartilage and bones. It is also required for the formation of nucleic acids, phospholipids, and high-energy phosphate esters. K is a major cation of the intracellular fluid in the animal body. It is responsible for the regulation of osmotic pressure. S is a component of amino acids like methionine and cysteine, the vitamin biotin, and the hormone insulin. It plays a key role in several key enzymes. Difo et al. (2014) found that K and S levels were reduced in cowpeas after 48 hours of fermentation, while Mihiret (2009) also observed reduced P levels in fermented sorghum after the same fermentation time. Mihiret (2009) reduction was attributed to the loss during decantation and utilization of the elements in the metabolic process. The scenario also occured due to mineral use by fermenting microflora and the mineral leaching into the fermentation water (Difo et al. 2014).

3.3 Heavy metals

- Lead and copper

The lead (Pb) and copper (Cu) of the different treatments in different fermentation conditions changed ($P < 0.05$) with increasing period (Fig. 6 and 7). It was observed that Pb and Cu decreased with increasing time in all treatments and fermentation conditions. Pb was found almost similar in facultative anaerobic (39.53 ppm) and aerobic conditions (39.52 ppm) conditions, especially in T_3, after 7 days of fermentation. Cu was found the lowest in T_3 (around 70 ppm) under anaerobic conditions at 7 days. The main effect, treatment only showed a difference ($P < 0.05$) at 3, 5, and 7 days, respectively. Pb and Cu metal contamination can be derived from the activities of nature and humans. Poultry feed is generally produced from grain and chemicals containing Pb and Cu is used to improve the color and texture of poultry feed. Pb and Cu metal content in the poultry feed material were not wholly digested when consumed, so they were excreted with droppings (Berata et al. 2016; Utama and Christiyanto 2021a). The requirement of the Pb and Cu in beef cattle is 30 ppm and 10 ppm, respectively (NRC 2000). The Pb levels were adjusted after fermentation, but the Cu was so high that it requires to be lessened to a safe level. Fermentation reduces the bioavailability and mobility

of heavy metals in the components (Liu et al. 2018) for the growth of microorganisms and the change in chemical speciation (Zhuang et al. 2011). In a solid-state fermentation of 56 hours, the Pb and Cu contents were reduced to 227 and 82 mg/L, respectively (Zhuang et al. 2011). Utama and Christiyanto (2021a) stated that Pb and Cu levels were reduced in poultry litter after 63 days of fermentation.

3.4 Bacteriological analysis of fermented poultry manure

- Total Coliform count, *E-coli* and *Salmonella*

The total coliform count (TCC), *E. coli*, and *Salmonella* bacteria of the different treatments in different fermentation conditions changed ($P < 0.05$) with increasing time (Figure 8, 9, 10). It was noticed that there was an interaction between treatments and fermentation time to reduce their levels ($P < 0.05$) in all fermentation conditions. In TCC, T_3 imparted the lowest value under facultative anaerobic (0.05 CFU/g) and anaerobic (0.06 CFU/g) conditions at 7 days, in contrast to other treatments and aerobic conditions. *E. coli* could not be detected under facultative anaerobic and anaerobic conditions in both treatments (T_2 and T_3) after 7 days of incubation. A similar result was found in *Salmonella* when aerobic conditions were included. Throughout the incubation period, the bacterial count reduction was associated with pH drop and molasses addition. The elimination of total coliform count, *E. coli*, and *Salmonella* may also be related to acidity caused by lactic acid bacteria. *Lactobacilli* are well-known for producing high levels of bacteriocins and organic acids, which aid in the elimination of bacteria like Coliform, *E. coli*, and *Salmonella* (Ruiz-Barrera et al. 2018). The presence of molasses in the samples, a source of soluble carbohydrates, caused lactobacilli to thrive in the acidic environment, eliminating all harmful bacteria. Lactobacilli enhance to accumulation of lactic acid, which reduces the pH level of the medium and prevents the growth of gram-positive and gram-negative bacteria. These accumulated acids enter the cells of microbial pathogens, inhibiting microbes' growth and causing cell death (Ramirez et al. 2011). Ruiz-Barrera et al. (2018) narrated that the elimination of pathogenic bacteria (Coliform, *E. coli*, and *Salmonella*) was observed and the addition of the probiotic yeast at 7% and 15% as a wet basis resulted in the reduction of these pathogenic bacteria to undetectable levels by day . The study carried out showed that 48 hours of fermentation of cattle manure reduced the index of coliform bacteria to an acceptable level and eliminated *E. coli* 10 times lesser (Uvarov et al. 2017). Poultry manure inoculated with lactic acid bacteria was fermented with 10% molasses at 30°C and it was found that no *Salmonella* was detected after 7 days of fermentation (El-Jalil et al. 2008).

4. Conclusion

To protect the environment and produce inexpensive ruminant feed, the use of poultry manure is a good option. However, the quality and safety are the concerns before introducing it as a feed. Fermentation is a desirable method associated with processing it. In this experiment, the quality and safety of poultry manure improved after fermentation. Along with that, the addition of microorganisms and increasing the fermentation period helped in improving the organoleptic, quality and safety parameters. All parameters were found desirable in 10% IMO treated PM. Although organoleptic parameters (color, smell, and texture) were satisfactory in aerobic fermentation, quality and safety parameters were acceptable in anaerobic fermentation conditions. pH dropped significantly ($P < 0.05$) with increasing duration. Therefore, if poultry manure is fermented in an air-tight bag in an anaerobic condition with a fermentation mixture of PM (90%) and molasses (10%) inoculating 10% IMO at least for 7 days, it can be used safely for ruminant feeding. However, there are significant gaps in this study. In the future, more heavy metal analyses (Cd and Cr) and in-vivo trials in animals can be done.

Authors' contribution

Sharmeen Islam: conception and design, data collection, analysis and interpretation of results, draft manuscript preparation. A. K. M. Ahsan Kabir and Md. Rokibul Islam Khan: draft manuscript editing and review. The results were evaluated by all authors, and the final version of the manuscript was approved.

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Compliance with ethical standards

Conflict of interest: The authors declare that there are no conflicts of interest associated with this study.

Table 1. Effects of fermentation condition and time on organic matter (OM) content of treatments.

F	T	OM(%) observations				Total mean	SEM
		0 day	**3 days**	**5 days**	**7 days**		
Ae	$\mathbf{T_0}$	61.22	60.70	60.69	60.64	60.81[a]	0.136
	$\mathbf{T_1}$	61.31	60.65	60.62	60.31	60.72[a]	0.210
	$\mathbf{T_2}$	59.29	58.81	58.69	58.83	58.90[b]	0.132
	$\mathbf{T_3}$	57.28	56.86	56.29	56.33	56.69[c]	0.235
FA	$\mathbf{T_0}$	61.22	60.56	60.48	60.37	60.65[a]	0.191
	$\mathbf{T_1}$	61.31	60.61	60.53	60.20	60.66[a]	0.233
	$\mathbf{T_2}$	59.29	59.08	58.33	56.93	58.40[b]	0.533
	$\mathbf{T_3}$	57.28	57.16	56.19	56.20	56.70[c]	0.296
An	$\mathbf{T_0}$	61.22	60.76	60.59	60.56	60.78[a]	0.152
	$\mathbf{T_1}$	61.31	60.58	60.28	58.97	60.28[a]	0.488
	$\mathbf{T_2}$	59.29	58.84	57.97	57.50	58.40[b]	0.406
	$\mathbf{T_3}$	57.28	56.56	55.90	55.40	56.28[c]	0.407
Total mean		59.78[a]	59.25[ab]	58.88[b]	58.54[c]		
SEM		0.28	0.27	0.32	0.33		
Main and Interaction effects (P value)							
F		1.000	0.660	0.209	0.001		
T		0.000	0.000	0.000	0.000		
F×T		1.000	0.976	0.958	0.005		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 2. Effects of fermentation condition and time on crude fiber (CF) content of treatments.

F	T	CF(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	13.36	13.28	13.20	12.76	13.15^{ab}	0.134
	T_1	13.97	13.90	13.10	13.00	13.49^{a}	0.256
	T_2	13.36	12.17	11.63	11.36	12.13^{bc}	0.443
	T_3	13.10	11.88	11.43	11.10	11.87^{c}	0.437
FA	T_0	13.36	13.26	13.10	12.78	13.12^{ab}	0.126
	T_1	13.97	13.93	13.76	13.33	13.74^{a}	0.146
	T_2	13.36	11.90	11.40	11.55	12.05^{bc}	0.448
	T_3	13.10	11.33	11.33	11.15	11.72^{c}	0.459
An	T_0	13.36	13.30	13.06	12.66	13.09^{ab}	0.158
	T_1	13.97	13.86	13.73	13.36	13.73^{a}	0.132
	T_2	13.36	11.36	11.03	10.95	11.67^{c}	0.568
	T_3	13.10	11.00	10.76	9.54	11.10^{c}	0.739
Total mean		13.44^{a}	12.61^{b}	12.36^{b}	11.96^{c}		
SEM		0.126	0.206	0.215	0.215		
Main and Interaction effects (P value)							
F		1.000	0.272	0.385	0.133		
T		0.173	0.000	0.000	0.000		
F×T		1.000	0.926	0.985	0.303		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 3. Effects of fermentation condition and time on crude protein (CP) content of treatments.

F	T	CP(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	18.4	18.5	18.9	19.0	18.7 [c]	0.16
	T_1	18.5	18.7	18.9	19.2	18.8 [c]	0.14
	T_2	22.5	23.5	23.6	23.7	23.3 [b]	0.28
	T_3	26.5	28.3	28.8	29.1	28.1 [a]	0.60
FA	T_0	18.4	18.4	18.7	18.7	18.5 [c]	0.09
	T_1	18.5	18.8	18.9	19.0	18.8 [a]	0.11
	T_2	22.5	23.5	23.9	24.3	23.6 [b]	0.39
	T_3	26.4	28.6	29.8	30.4	28.8 [a]	0.90
An	T_0	18.4	18.4	18.5	18.9	18.5 [c]	0.12
	T_1	18.5	18.9	19.0	19.6	18.9 [c]	0.14
	T_2	22.5	23.6	24.3	25.7	24.1 [b]	0.67
	T_3	26.3	29.5	30.8	31.8	29.6 [a]	1.18
Total mean		22.1[d]	22.8[c]	23.2[b]	23.4[a]		
SEM		0.49	0.66	0.72	0.77		
Main and Interaction effects (P value)							
F		1.000	0.003	0.000	0.000		
T		0.000	0.000	0.000	0.000		
F×T		1.000	0.047	0.006	0.000		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 4. Effects of fermentation condition and time on ether extract (EE) of treatments.

F	T	EE(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	2.15	2.81	2.88	2.87	2.70 ^{b}	0.187
	T_1	2.22	2.44	2.61	2.92	2.54 ^{b}	0.147
	T_2	2.69	4.42	5.06	6.06	4.54 ^{ab}	0.708
	T_3	2.72	5.36	6.07	6.66	5.20 ^{a}	0.869
FA	T_0	2.14	2.81	2.82	2.92	2.67 ^{b}	0.179
	T_1	2.22	3.94	4.20	4.43	3.69 ^{ab}	0.502
	T_2	2.69	4.48	5.43	5.96	4.64 ^{ab}	0.718
	T_3	2.72	5.81	6.26	7.05	5.46 ^{a}	0.948
An	T_0	2.14	2.80	2.80	2.71	2.66 ^{b}	0.176
	T_1	2.22	4.09	4.20	4.43	3.73 ^{ab}	0.509
	T_2	2.69	5.40	5.93	5.99	5.00 ^{a}	0.782
	T_3	2.72	5.86	6.48	7.17	5.55 ^{a}	0.982
Total mean		2.44^{c}	4.18^{b}	4.56^{b}	4.93^{a}		
SEM		0.045	0.206	0.246	0.285		
Main and Interaction effects (P value)							
F		1.000	0.000	0.000	0.000		
T		0.000	0.000	0.000	0.000		
F×T		1.000	0.000	0.000	0.000		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 5. Effects of fermentation condition and time on nitrogen-free extract (NFE) of treatments.

F	T	NFE(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	27.65	26.45	26.04	25.35	26.37 [a]	0.482
	T_1	24.20	23.26	23.05	22.42	23.23 [ab]	0.368
	T_2	20.53	18.54	18.25	18.03	18.83 [cd]	0.573
	T_3	18.13	11.30	10.59	10.01	12.50 [e]	1.892
FA	T_0	27.65	26.29	26.05	25.45	26.36 [a]	0.464
	T_1	24.20	22.82	21.54	21.05	22.40 [abc]	0.705
	T_2	20.53	19.18	17.54	15.08	18.08 [cd]	1.172
	T_3	18.13	11.41	8.78	7.56	11.47 [e]	2.360
An	T_0	27.65	26.12	26.00	25.87	26.41 [a]	0.416
	T_1	24.20	22.64	21.02	19.53	21.84 [bc]	1.000
	T_2	20.53	18.46	16.64	14.83	17.61 [d]	1.221
	T_3	18.13	10.22	7.84	6.89	10.77 [e]	2.551
Total mean		21.88[a]	19.56[ab]	18.79[b]	18.28[b]		
SEM		0.784	0.955	1.096	1.186		
Main and Interaction effects (P value)							
F		1.000	0.000	0.000	0.000		
T		0.000	0.000	0.000	0.000		
F×T		1.000	0.000	0.000	0.000		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 6. Effects of fermentation condition and time to change In-vitro Organic matter digestibility (IVOMD) of treatments.

F	T	IVOMD(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	61.7	63.8	64.6	64.9	63.7 [b]	0.73
	T_1	61.0	64.8	65.3	65.6	64.2 [b]	1.08
	T_2	61.5	66.4	68.9	69.6	66.6 [a]	1.83
	T_3	62.2	66.8	69.8	70.2	67.7 [a]	1.84
FA	T_0	61.7	63.7	64.5	65.9	63.9 [b]	0.89
	T_1	61.0	65.0	65.4	65.4	64.2 [b]	1.07
	T_2	61.5	65.5	67.7	69.6	66.1 [a]	1.72
	T_3	62.2	67.4	68.5	69.7	67.0 [a]	1.66
An	T_0	61.7	63.1	63.7	64.8	63.3 [b]	0.66
	T_1	61.0	65.1	65.7	65.9	64.4 [b]	1.16
	T_2	61.5	67.2	68.5	69.5	66.7 [a]	1.78
	T_3	62.2	67.9	70.7	71.8	68.1 [a]	2.14
Total mean		61.4[c]	65.6[b]	66.9[a]	67.5[a]		
SEM		0.11	0.25	1.39	0.48		
Main and Interaction effects (P value)							
F		1.000	0.060	0.079	0.401		
T		0.011	0.000	0.000	0.000		
F×T		1.000	0.212	0.063	0.773		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 7. Effects of fermentation condition and time on metabolizable energy (ME) of treatments.

F	T	ME(Kcal/kg DM) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	2570.43	2569.73	2564.63	2560.56	2566.34 ^{b}	0.319
	T_1	2558.20	2555.81	2546.36	2550.03	2552.60 ^{b}	0.836
	T_2	2657.43	2605.42	2566.53	2537.86	2591.81 ^{ab}	0.550
	T_3	2588.40	2555.76	2542.83	2531.36	2554.59 ^{b}	0.800
FA	T_0	2570.43	2567.53	2564.96	2559.99	2565.73 ^{b}	0.756
	T_1	2558.20	2631.87	2619.43	2597.24	2601.69 ^{a}	0.860
	T_2	2657.43	2604.80	2588.90	2565.73	2604.22 ^{a}	0.600
	T_3	2588.40	2662.17	2575.90	2548.12	2593.65 ^{ab}	0.630
An	T_0	2570.43	2584.93	2563.96	2561.61	2570.23 ^{b}	0.490
	T_1	2558.20	2641.40	2627.36	2537.98	2591.24 ^{ab}	0.600
	T_2	2657.43	2645.50	2624.67	2507.60	2608.80 ^{a}	0.260
	T_3	2588.40	2560.76	2558.50	2528.32	2559.00 ^{b}	0.830
Total mean		2593.62^{a}	2598.80^{a}	2578.67^{b}	2548.87^{c}		
SEM		0.716	0.889	0.653	0.746		
Main and Interaction effects (P value)							
F		1.000	0.558	0.607	0.149		
T		0.000	0.000	0.000	0.000		
F×T		1.000	0.780	0.790	0.892		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 8. Effects of fermentation condition and time on phosphorus content in treatments.

F	T	Phosphorus(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	0.440	0.436	0.433	0.420	0.432 ^{abc}	0.004
	T_1	0.443	0.440	0.433	0.431	0.436 ^{a}	0.002
	T_2	0.447	0.410	0.403	0.396	0.414 ^{abcd}	0.011
	T_3	0.443	0.377	0.366	0.363	0.387 ^{bcd}	0.018
FA	T_0	0.440	0.437	0.433	0.430	0.435 ^{ab}	0.002
	T_1	0.443	0.400	0.394	0.386	0.415 ^{abcd}	0.014
	T_2	0.447	0.393	0.380	0.373	0.398 ^{abcd}	0.016
	T_3	0.443	0.390	0.380	0.367	0.395 ^{abcd}	0.016
An	T_0	0.440	0.417	0.403	0.400	0.415 ^{abcd}	0.009
	T_1	0.443	0.393	0.386	0.376	0.399 ^{abcd}	0.014
	T_2	0.447	0.376	0.367	0.353	0.385 ^{cd}	0.020
	T_3	0.443	0.363	0.353	0.346	0.376 ^{d}	0.022
Total mean		0.440^{a}	0.403^{b}	0.394^{b}	0.386^{b}		
SEM		0.003	0.005	0.005	0.005		
Main and Interaction effects (P value)							
F		1.000	0.007	0.011	0.012		
T		0.762	0.000	0.000	0.000		
F×T		1.000	0.399	0.580	0.589		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 9. Effects of fermentation condition and time on potassium content in treatments.

F	T	Potassium(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	1.010	0.983	0.976	0.973	0.985 [a]	0.008
	T_1	1.013	0.973	0.969	0.960	0.978 [a]	0.011
	T_2	1.003	0.963	0.953	0.946	0.966 [ab]	0.012
	T_3	1.010	0.920	0.906	0.900	0.934 [ab]	0.025
FA	T_0	1.010	0.980	0.9700	0.967	0.981 [a]	0.009
	T_1	1.013	0.990	0.9800	0.966	0.987 [a]	0.009
	T_2	1.003	0.960	0.9433	0.938	0.961 [ab]	0.014
	T_3	1.010	0.930	0.9100	0.900	0.937 [ab]	0.024
An	T_0	1.010	0.976	0.966	0.963	0.978 [a]	0.010
	T_1	1.013	0.970	0.943	0.914	0.960 [ab]	0.021
	T_2	1.003	0.946	0.943	0.926	0.954 [ab]	0.016
	T_3	1.010	0.903	0.890	0.870	0.918 [b]	0.031
Total mean		1.009[a]	0.9564[b]	0.946[b]	0.935[b]		
SEM		0.001	0.005	0.006	0.006		
Main and Interaction effects (P value)							
F		1.000	0.337	0.179	0.009		
T		0.432	0.000	0.000	0.000		
F×T		1.000	0.787	0.872	0.517		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

Table 10. Effects of fermentation condition and time on sulfur in treatments.

F	T	Sulfur(%) observations				Total mean	SEM
		0 day	3 days	5 days	7 days		
Ae	T_0	0.976	0.950	0.936	0.930	0.948 ^{ab}	0.010
	T_1	0.933	0.903	0.900	0.896	0.908 ^{abc}	0.008
	T_2	0.955	0.893	0.873	0.863	0.896 ^{abc}	0.020
	T_3	0.967	0.876	0.856	0.850	0.887 ^{c}	0.027
FA	T_0	0.976	0.953	0.943	0.936	0.952 ^{a}	0.008
	T_1	0.933	0.900	0.896	0.886	0.903 ^{abc}	0.010
	T_2	0.955	0.893	0.883	0.873	0.901 ^{abc}	0.018
	T_3	0.967	0.876	0.850	0.845	0.884 ^{c}	0.028
An	T_0	0.976	0.940	0.933	0.930	0.944 ^{abc}	0.010
	T_1	0.933	0.900	0.900	0.893	0.906 ^{abc}	0.008
	T_2	0.955	0.880	0.870	0.860	0.891 ^{abc}	0.021
	T_3	0.967	0.870	0.853	0.842	0.883 ^{bc}	0.0285
Total mean		0.956^{a}	0.903^{b}	0.891^{c}	0.885^{c}		
SEM		0.006	0.005	0.005	0.006		
Main and Interaction effects (P value)							
F		1.000	0.321	0.850	0.673		
T		0.167	0.000	0.000	0.000		
F×T		1.000	0.989	0.966	0.959		

**Means with different superscripts within row and column are significantly different ($P < 0.05$), where, T_0 = Only poultry manure (100%), T_1 = Poultry manure (90%) + molasses (10%), T_2 = Poultry manure (90%) + molasses (10%), addition of Saccharomyces cerevisiae (10%), T_3 = Poultry manure (90%) + molasses (10%), addition of IMO (10%). F = Fermentation; T = Treatment; Ae = Aerobic; FA = Facultative anaerobic; An = An-aerobic*

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