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EFFECT OF *AZOLLA* AND BEEF BONE MEAL ADDITION ON THE NUTRIENT CONTENT OF LOF BASED ON BEEF CATTLE MANURE AND LAYING HENS EXCRETA

Rania Zabina Safitry[1, a], Roni Ridwan[2], Ellin Harlia[3]
[1] *Post Graduate Animal Husbandry Program, Padjadjaran University, Sumedang 45363, West Java, Indonesia.*
[2] *Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong, West Java, 16911, Indonesia*
[3] *Department of Microbiology and Livestock Waste. Faculty of Animal Husbandry, Padjadjaran University, Sumedang 45363, West Java, Indonesia*
[a]*email:* rania20003@unpad.ac.id

Abstract
In Liquid Organic Fertilizer (LOF), the problem that is often found is that the nutrients contained do not reach quality standards. Azolla and beef bone meal are known to contain macronutrients needed by plants, in addition to maximizing the existing content, functional bacteria are also added. This study aims to analyze the effect of the addition of azolla and beef bone meal on macro nutrient content, C-organic, coliform, and viability. The research used a completely randomized design (CRD) with 6 treatments and 3 replicates, namely (P1) 25% Azolla + 15% beef bone meal, (P2) 25% Azolla + 20% beef bone meal, (P3) 25% Azolla + 25% beef bone meal, (P4) 25% azolla + 15% beef bone meal + 10% functional bacteria, (P5) 25% azolla + 20% beef bone meal + 10% functional bacteria, (P6) 25% azolla + 25% beef bone meal + 10% functional bacteria. The research data were analyzed statistically using variance analysis and Duncan's multiple range test with the IBM SPSS Statistics 25 program. The results showed that the addition of 25% azolla + 25% beef bone meal (P3) had a significant effect ($P \leq 0.05$) on increasing macro nutrient content and functional bacteria viability, C-organic, and coliform values according to standards.
Keywords: Azolla, Beef Bone Meal, Fuctional Bacteria, Liquid Organic Fertilizer

PENGARUH PENAMBAHAN AZOLLA DAN TEPUNG TULANG SAPI TERHADAP KANDUNGAN NUTRISI PUPUK ORGANIK CAIR

Abstrak
Pada Pupuk Organik Cair (POC), permasalahan yang sering ditemukan adalah unsur hara yang terkandung tidak mencapai standar mutu. Azolla dan tepung tulang sapi diketahui mengandung unsur hara makro yang dibutuhkan oleh tanaman, selain itu untuk memaksimalkan kandungan yang ada juga ditambahkan bakteri fungsional. Penelitian ini bertujuan untuk menganalisis pengaruh penambahan azolla dan tepung tulang sapi terhadap kandungan unsur hara makro, C-organik, coliform, dan viabilitas. Metode penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 6 perlakuan dan 3 kali ulangan, yaitu (P1) 25% Azolla + 15% tepung tulang sapi, (P2) 25% Azolla + 20% tepung tulang sapi, (P3) 25% Azolla + 25% tepung tulang sapi, (P4) 25% azolla + 15% tepung tulang sapi + 10% bakteri fungsional, (P5) 25% azolla + 20% tepung tulang sapi + 10% bakteri fungsional, (P6) 25% azolla + 25% tepung tulang sapi + 10% bakteri fungsional. Data hasil penelitian dianalisis secara statistik dengan menggunakan analisis ragam dan uji jarak berganda Duncan dengan program IBM SPSS Statistics 25. Hasil penelitian menunjukkan bahwa penambahan 25% azolla + 25% tepung tulang sapi (P3) berpengaruh nyata ($P \leq 0{,}05$) terhadap peningkatan kandungan unsur hara makro dan viabilitas bakteri fungsional, nilai C-organik, dan coliform sesuai standar.
Kata Kunci: Azolla, Bakteri Fungsional, Pupuk Organik Cair, Tepung Tulang Sapi

INTRODUCTION

The continuously increasing livestock waste requires the right solution for handling it. One of the waste processing methods that can be done is to make organic fertilizer. Currently, many Indonesian farmers still depend on chemical fertilizers. The use of chemical fertilizers at high rates continues to cause soil damage and environmental pollution (Ammurabi et al., 2020). Chemical fertilizers are considered more practical than organic fertilizers because they require less dose for the same nutrient value, so the use of organic fertilizers began to be abandoned. However, the increase in production is sloping and continues to decline (Anas, 2016). The application of NPK fertilizer without organic matter can only significantly increase maize production in the first 12 years (Li, et al., 2017). The continuous use of high doses of chemical fertilizers causes soil, water, and air pollution, as well as damage to the soil, including the destruction of soil structure, the destruction of soil biota biodiversity, excessive nutrient leaching, and water pollution (Savci, 2012; Anas, 2016). Therefore, there is a need for organic fertilizers in restoring sustainable environmental stability.

Organic fertilizers are fertilizers whose basic materials come from living things, such as plants and animals (Hartatik et al., 2015). Solid and liquid organic fertilizers are highly beneficial for enhancing agricultural production in both quality and quantity, reducing environmental pollution, and improving land quality continuously. LOF (liquid organic fertilizer) is a solution from the decomposition of organic matter derived from crop residues, agro-industrial waste, and animal manure that contains more than one nutrient.

Azolla pinnata is a type of small fern that lives in aquatic habitats. *Azolla pinnata* plants are generally used as organic fertilizers that improve the physical, chemical, and biological properties of the soil. Azolla has a high content of nutrients, namely N 1.96-5.30%, P 0.16-1.59%, Si 0.16-3.35%, Ca 0.31-5.97%, Fe 0.04-0.59%, Mg 0.22-0.66%, Zn 26-989 ppm, Mn 66-2944 ppm C/N: 10 (Batan, 2014). According to the research by Sari et al. (2021), Azolla-based LOF has a total N content of 1.65%, total P of 0.07%, total K of 2.36%, and Mg of 0.09%. Besides azolla, beef bone meal is also known to have a high protein content of 25.54% (Lestari, 2015). Beef bone meal is a by-product of the meat industry and is an important pathway for recycling N and P. Beef bone meal contains about 8% N, 5% P, and 10% Ca (Jeng et al., 2004). Beef bone meal is a nutrient-rich organic fertilizer that supports healthy plant growth. Jeng et al. (2004) stated that beef bone meal works just as effectively as urea.

Besides the need for additional nutrients in LOF, the role of microorganisms is also one of the important things for plant sustainability. The main role of this group of bacteria is as a provider of nutrients such as N_2 fixers from the air, P solvents, and other nutrients. These soil microorganisms have their respective roles in the ecosystem related to energy flow and nutrient cycling as a result of the main activities of microorganisms (growth and development) (Pati et al., 2016). Therefore, this research needs to be carried out in order to get the best composition of nutrient sources and bacteria.

MATERIALS AND METHODS

The research was conducted for 2 months, in December 2024 - January 2025, at the Laboratory of Microbiology and Livestock Waste, Faculty of Animal Husbandry, Padjadjaran University. Sample testing was carried out at the Plant Fertility and Nutrition Laboratory, Faculty of Agriculture, Universitas Padjadjaran.

Liquid Organic Fertilizer Preparation

The basic ingredients of LOF in this study consisted of 95% beef cattle feces and 5% laying hen excreta, which were decomposed together with fiber sources of cocopeat and straw (1:1 ratio). The ratio of fiber sources and manure was 3:2. Decomposition was carried out for 14 days using an anaerobic method. The decomposition indicator was if the decomposition temperature reached 50 °C on the 7^{th} day and returned to room temperature on the 14^{th} day. The substrate from the decomposition results was dissolved with water in a 1:2 ratio of water to substrate, then the mixture was filtered because LOF production only requires liquid without pulp. In the first stage of the research, the macro nutrient sources added to the LOF were azolla and cow bone meal.

The research added nutrient sources and functional bacteria with 6 treatments with 3 replications, namely (P1) 25% azolla + 15% beef bone meal; (P2) 25% azolla + 20% beef bone meal; (P3) 25% azolla + 25% beef bone meal; (P4) 25% azolla + 15% beef bone meal + 10% functional bacteria; (P5) 25% azolla + 20% beef bone meal + 10% functional bacteria; (P6) 25% azolla + 25% beef bone meal + 10% functional bacteria. In this treatment, the functional bacteria added were a liquid consortium of *Pseudomonas sp.* (K4N.J6) as nitrogen-fixing bacteria (NFB), Herbaspirillum sp. (K4N.PSV1) as phosphate-solubilizing bacteria (PSB), and *Streptomyces sp.* as plant growth-promoting bacteria (PGPB). A study by Bhaskoro et al. (2020) found that adding bone meal at concentrations of 25% LOF increased nitrogen (N), phosphorus (P), and potassium (K) content. Fermentation was carried out for 14 days at room temperature and aeration was carried out by shaking the fermentation bottle and opening the lid. In addition, 3% molasses was added to each treatment before fermentation as an energy source for the functional bacteria.

Chemical Measurements

In this study, the pH level of each Liquid Organic Fertilizer was measured using a pH meter. The C-organic content was determined using the Walkley and Black method (Omposunggu et al., 2015), while the total nitrogen (N-total) content was analyzed using the Kjeldahl method (Hermawati et al., 2021). The phosphorus content was measured using the Olsen method (Nursyamsi & Setyorini, 2009; Dhasa & Mutiara, 2019), and the potassium content was measured flamephotometrically based on the intensity of the emission rays (Horwitz, 2000; SNI 02-3776-2005).

Bacterial Measurements

Total coliform in LOF was tested in 2 stages using the MPN (Most Probable Number) method (Janie and Fardiaz 1989). While the viability test will be seen from the Total Plate Count (TPC) every week for 4 weeks.

Statistical Analysis

The research method used was experimental with a complete randomized design. The test was continued, using further tests with Duncan's multiple range test.

RESULTS AND DISCUSSION

The average results of the C, N, P, and K tests on the following LOF are presented in Table 1.

pH Value

The pH of the LOF was measured using a pH meter at the beginning and end of the fermentation, as presented in Illustration 1 and Illustration 2.

Based on Illustration 1, there was a significant increase in pH in the P4, P5, and P6 treatments. P4, P5, and P6 treatments each had 10% functional bacteria added, so there was an increase in pH in the three treatments. The increase in pH means that the composting process of liquid organic fertilizer occurs faster, along with the increasing levels of beef bone meal and azolla added. Beef bone meal is rich in calcium phosphate and proteins, which can contribute to alkalinity and further support ammonia production when decomposed. Besides that, an increase in pH during the composting or fermentation process of liquid organic fertilizer (LOF), especially in treatments with added functional bacteria, like in P4, P5, and P6, is typically caused by the production of ammonia (NH_3) (Caceres et al., 2018). This is in accordance with the opinion of Suwahyono (2014) that the higher the pH level in composting, the faster the decomposition of organic matter occurs. The pH range in LOF, which ranges from 6.82 to 7.81, already reaches the quality standards in KEPMENTAN Number 261/KPTS/SR.310/M/ 4/2019 which states that the pH of LOF must range from 4-9.

Table 1. Average Result of C, N, P, and K on Liquid Organic Fertilizer (LOF)

Variables	Treatments						
	P1	P2	P3	P4	P5	P6	Stdv
	..................%						
C-Organik	17,5^{c}	17,8^{cd}	18,7^{d}	9,7^{ab}	8,8^{a}	10^{b}	0,6
N-Total	0,28^{ab}	0,42^{bc}	0,51^{c}	0,22^{a}	0,30^{ab}	0,27^{ab}	0,1
P-Total	0,54^{abc}	1,13^{c}	1,23^{c}	0,16^{ab}	0,05^{a}	0,37^{abc}	0,2
K-Total	0,24^{b}	0,21^{b}	0,26^{b}	0,10^{a}	0,10^{a}	0,09^{a}	0,02

Notes: Data are presented as mean values (n=3). Means with different superscripts in the same row indicate significant differences ($P \leq 0.05$). The treatments are as follows (P1) 25% azolla + 15% beef bone meal; (P2) 25% azolla + 20% beef bone meal; (P3) 25% azolla + 25% beef bone meal; (P4) 25% azolla + 15% beef bone meal + 10% functional bacteria; (P5) 25% azolla + 20% beef bone meal + 10% functional bacteria; (P6) 25% azolla + 25% beef bone meal + 10% functional

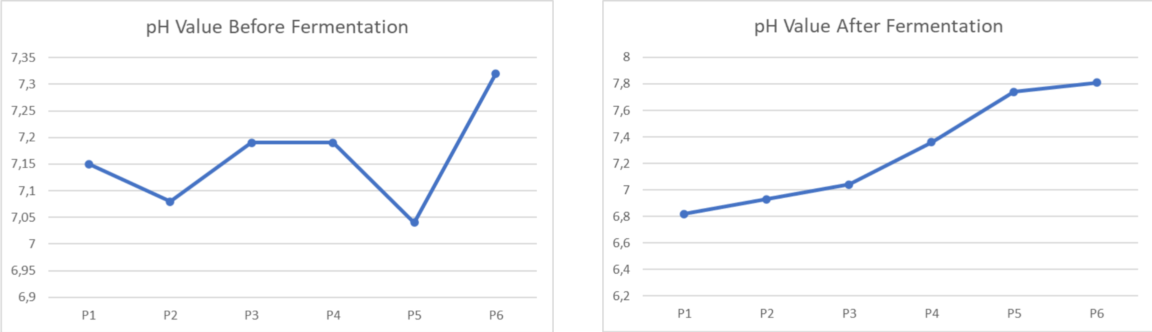

Figure 1. pH Value Before and After Fermentation

C-Organic Contents

Based on Table 1, the average C-Organic content in LOF made from beef cattle feces and laying hen excreta, with the addition of azolla and bone meal, ranged from 8.8 to 18.7. According to the results of Duncan's multiple range test in Table 1, the P3 treatment (18.7) was significantly different from the P1, P2, P4, P5, and P6 treatments. The high C-Organic levels in the P3, P2, and P1 treatments were likely due to the addition of 3% molasses. Molasses is commonly used as an energy source and an alternative to sugar (Gumilar et al., 2023).

The high C-Organic levels may also have been influenced by microorganisms, as their decomposition contributes to increased C-Organic content (Marlinda, 2020). In contrast, despite the addition of 3% molasses, P4, P5, and P6 treatments also added 10% functional bacteria, leading to different C-Organic levels. The lower C-Organic levels in the P4, P5, and P6 treatments are thought to be due to the breakdown of carbon compounds, which serve as an energy source for microorganisms (Pandi et al., 2023).

Only treatments P1, P2, P3, and P6 meet the LOF quality standards in the Decree of the KEPMENTAN Number 261/KPTS/SR.310/M/ 4/2019, which states that the minimum C-Organic content in LOF is 10%. This is in line with research by Siregar (2017), which states that the higher the total C-Organic content, the better the mineral soil quality.

Nitrogen Content (N)

Referring to Table 1, the addition of azolla and bone meal at different levels in LOF made from beef cattle feces and laying hen excreta resulted in Nitrogen (N-Total) values ranging from 0.22% to 0.51%. This range was higher than that of LOF made solely from livestock manure waste. In the study conducted by Wirne et al. (2022), LOF made from beef cattle and chicken feces produced Nitrogen (N) values of only 0.06% and 0.03%, respectively.

In Duncan's multiple range test result, treatment P3 shows a significant difference when compared with treatments P1, P3, P4, P5 and P6. One of the elements that can affect the high value of Nitrogen (N-Total) is the addition of macro nutrient sources, such as azolla and beef bone meal. *Azolla pinnata* has a high content of nutrients, i.e. N of 1.96-5.30% (Batan, 2014). In a research conducted by Sari et al. (2021), LOF azolla contains Nitrogen (N) of 1.645%, and beef bone meal contains a Nitrogen (N) value of 8%. Therefore, the two sources of macronutrients can contribute the Nitrogen they contain.

In the treatment with the addition of 10% functional bacteria (P4, P5, P6), the Nitrogen (N) value tends to be lower than in the treatment without the addition of functional bacteria (P1, P2, P3). This is consistent with the findings of Wirne et al. (2022), who stated that the amount of liquid used in making liquid organic fertilizer (LOF) allows the nitrogen produced by microbes to bind with water (H_2O), forming ammonia (NH_3). Microbial populations increase rapidly at the beginning of fermentation, resulting in ammonia formation, while the microorganisms have not yet fully broken down the substrate. Out of the six treatments, only the P3 treatment reaches LOF requirements in accordance with the Decree of the KEPMENTAN Number 261/KPTS/SR.310 /M/4/2019 which states that the minimum N-total value is 0.5%.

P_2O_5 Content

Based on the addition of azolla and beef bone meal at different levels in LOF based on beef cattle feces and laying hen excreta, the P-Total value ranges from 0.05-1.23%. This range is higher when compared to the Total P_2O_5 content in chicken excreta waste LOF alone with a value of 0.085% (Nofrianil and Ibnusina, 2021). Based on Duncan's multiple tests, the P3 treatment was significantly different from the P4 and P5 treatments. Although the P3 treatment did not show a significant difference with the P1, P2, and P6 treatments when compared, the highest P-Total value remained in the P3 treatment.

The high value of P_2O_5 can be influenced by several factors, one of which is the value of Nitrogen (N-Total). In accordance with the opinion of Hidayati et al. (2011) and Situmorang (2018) that the high phosphorus content is influenced by the high nitrogen content, where the higher the nitrogen content, the activity of microorganisms that break down phosphorus will increase, causing the phosphorus content in liquid organic fertilizer to increase. The macro nutrient sources used are also thought to contribute Phosphorus to LOF, especially beef bone meal. It is known that beef bone meal contains 18.5% phosphorus (Carter and Spengler, 1978).

K_2O Content

In Table 1, the addition of azolla and beef bone meal at different levels, as well as the addition of functional bacteria, resulted in a range of Potassium levels, ranging from 0.09% to 0.24%. The range is below the value of Potassium (K_2O) research results, as reported by Wirne et al. (2022), which are 0.37% for beef cattle feces and 0.48% for chicken excreta. However, the value of Potassium (K_2O) range in this study is above the value of Potassium (K-Total) in the research of Durubanua et al. (2024), namely LOF from chicken excreta with a value of 0.07%.

According to Duncan's multiple tests, treatments P1, P2, and P3 were significantly different from P3, P4 and P5. The significant difference was thought to be caused by the addition of functional bacteria in treatments P3, P4, and P5. The low potassium value in each treatment is likely due to the possibility that the fermentation process is not perfect, resulting in slow material decomposition (Sulfianti et al., 2021). Potassium in LOF will increase if the fermentation process goes well. This is also in line with the statement of Rahmawati et al. (2021), which states that potassium acts as a catalyst for microorganisms in the process of accelerating fermentation.

Total NPK

The average total NPK in each LOF treatment was examined to compare it with the existing standard, namely KEPMENTAN No. 261/KPTS/SR.310/M/4/2019, as presented in Table 2.

According to the KEPMENTAN No. 261/KPTS/SR.310/M/4/2019, the quality standard for macro nutrients ($N + P_2O_5 + K_2O$) must be equal to 2, with a minimum nitrogen (N) content of 0.5. Referring to Table 2, only treatment P3 meets this quality standard. In P3, the total NPK was 2, and the total nitrogen (N-Total) value was 0.51, whereas the other treatments did not meet the standard. Thus, increasing the addition of Azolla and bone meal can enhance the macro nutrient content in liquid organic fertilizer (LOF). In this study, the optimal composition was found to be 25% Azolla + 25% beef bone meal.

Table 2. Total NPK in LOF

Treatment	N-Total	P-Total	K-Total	Total
		...................................%....................................		
P1	0,28	0,54	0,243	1,063
P2	0,42	1,13	0,217	1,767
P3	0,51	1,23	0,26	2
P4	0,22	0,16	0,103	0,483
P5	0,3	0,05	0,107	0,457
P6	0,27	0,37	0,09	0,73

Table 3. Total Coliform in Liquid Organic Fertilizer (LOF)

Treatments	Average (MPN/mL)	Significance	Stdv
P1	1,66	ab	0,5
P2	2,33	b	0,6
P3	1,86	ab	0,8
P4	1,46	ab	0,8
P5	1,13	a	0,3
P6	1,2	a	0,5

Note: Data are presented as mean values (n=3). Means with different superscripts in the same row indicate significant differences (P≤0.05).

In the P6 treatment, although 25% Azolla and 25% beef bone meal were added—just like in the P3 treatment—an additional 10% of functional bacteria was also introduced. As a result, the macro nutrient content differed from that of the P3 treatment. This discrepancy was likely because nitrogen, phosphorus, and potassium were utilized by the functional bacteria during their metabolic processes for cell proliferation. A study by Yustinah et al. (2016) showed that bacteria prefer organic nitrogen sources, which promote better cell growth. Additionally, phosphate sources are also utilized by bacteria in ATP synthesis. ATP, along with the IAA hormone produced, plays a role in cellular processes related to energy transfer and accelerates cell division (Wijaya & Prabaningtyas, 2022).

Total Coliform

The total coliform of the Most Probable Number (MPN) method in LOF made from beef cattle feces and laying hen excreta with the addition of azolla and beef bone meal is presented in Table 3.

Based on the table, the average total coliform in this study ranged from 1.13 to 2.23 MPN/mL. Based on the results of Duncan's multiple range test in Table 3, the P2 treatment was significantly different from P5 and P6, where P2 had the highest total coliform value (2.23 MPN/mL). Temperature and pH are two of the factors that can affect bacterial growth (Fifendy, 2017). The pH of LOF made from beef cattle feces and laying hen excreta, with the addition of azolla and beef bone meal, ranged from 6.82 to 7.81. Naillah et al. (2021) mentioned that coliform bacteria only grow optimally at a pH of 7 and at 37ºC.

According to the quality standards listed in the Decree of the KEPMENTAN Number 261/KPTS/SR.310/M/4/2019, the maximum allowable limit of coliform bacteria, such as *E. coli* and *Salmonella sp.*, is less than 1 x 10^2 MPN/mL. The result of this study (Table 3) shows that the total number of coliforms was still below the minimum quality standards that exist. The coliform limit in liquid organic fertilizer is set to maintain the safety and quality of the product to prevent harm to human health, plants, or the environment. The presence of coliform indicates microbial contamination by pathogens, suggesting that the processing or fermentation was not optimal. Setting this limit helps ensure the fertilizer is safe for use.

LOF Storage Viability Test

Viability testing of functional bacteria in LOF was carried out at the incubator temperature. The incubator temperature used in this study was 37°C.

a. NFB (Nitrogen Fixing Bacteria)

Total Plate Count (TPC) of NFB in LOF had the highest value in P3 and P2 treatments, respectively, although the addition of N-fixing bacteria was only given in treatments P4, P5, and P6. This is thought to be because the added NFB must compete with the indigenous bacteria in the LOF, so that the bacteria lack energy and reduce their activity. Besides that, NFB still maintains the total population until week 4.

b. ***PSB (Phospat Solubizing Bacteria)***

Total Plate Count (TPC) PSB in LOF has the highest value in P3 treatment. Similar to NFB, the treatment that added functional bacteria had a smaller population when compared to the treatment without the addition of functional bacteria. In addition, the PSB population was still present until week 4.

c. ***Actinomycetes***

The highest Total Plate Count (TPC) of Actinobacteria in LOF at the end of the test was observed in the P3 treatment. The bacteria in P3 are suspected to be indigenous *Actinomycetes* naturally present in the LOF. Furthermore, the *Actinomycetes* population remained present until the 4th week.

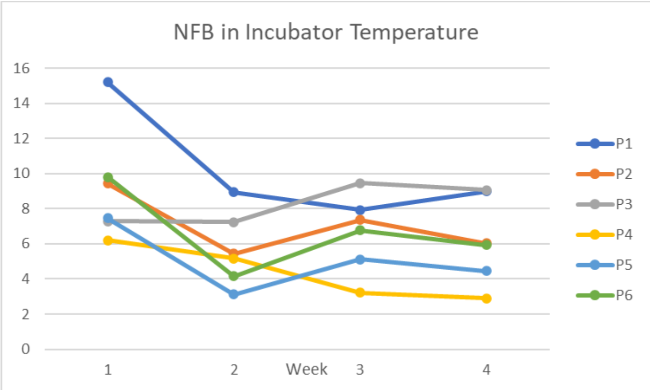

Figure 2. NFB population at Incubator Temperature

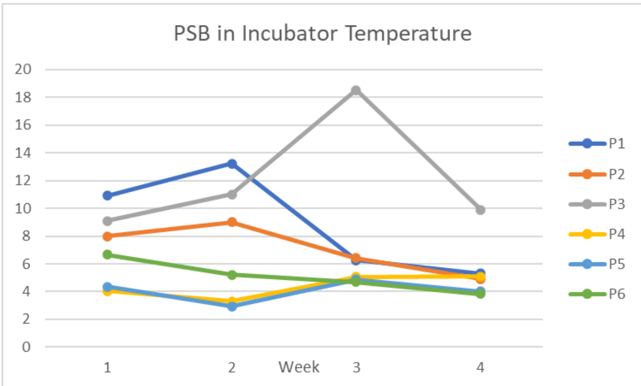

Figure 3. PSB population at Incubator Temperature

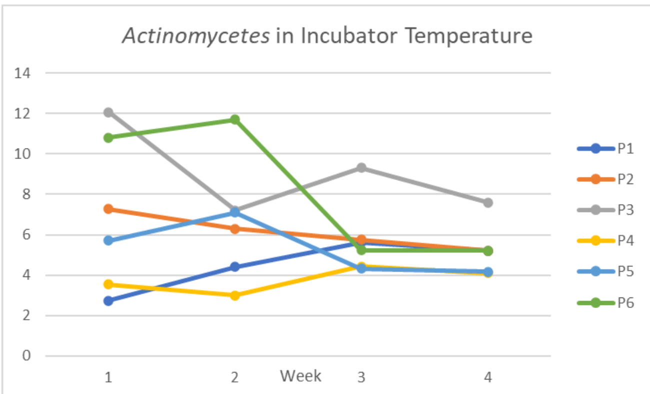

Figure 4. *Actinomycetes* population at Incubator Temperature

CONCLUSIONS

Liquid organic fertilizer (LOF) made from beef cattle feces and laying hen excreta, with the addition of 25% azolla and 25% beef bone meal in the P3 treatment, exhibited the highest C, N, P, and K contents compared to other treatments, with values of 18.7% C, 0.51% N, 1.23% P, and 0.26% K, respectively. The total macronutrient (NPK) content reached 2%, fulfilling the requirements set by KEPMENTAN No. 261/KPTS/SR.310 /M/4/2019. Additionally, in the storage stability test conducted at incubator temperature, the viability of functional bacteria in the LOF was maintained up to the fourth week of testing.

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