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**Research Article**

# The use of animal manure for improving chemical properties of degraded Ultisol, yield, and secondary metabolic of *Zingiber montanum*

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**Abstract**

Ultisols in Indonesia have the potential for agricultural development, but the soils have low pH and nutrient contents that hinder plant growth and yield. Using animal manure can be an alternative to improve soil productivity and crop yields. This study aimed to examine the effects of animal manure on the chemical properties of Ultisol, yield, and secondary metabolic of *Zingiber montanum*. The treatments tested were combinations of types of manure (cow and chicken manure) and manure application levels, namely P0 (control), P1 (cow manure 20 t/ha), P2 (cow manure 40 t/ha), P3 (cow manure 60 t/ha), P4 (chicken manure 20 t/ha), P5 (chicken manure 40 t/ha), and P6 (chicken manure 60 t/ha). The results showed that the application of chicken manure of 60 t/ha increased N and P contents of the soil, and the application of cow manure of 60 t/ha increased soil cation exchange capacity. The application of cow manure of 60 t/ha gave the highest plant height, the number of leaves, and the number of at 18 weeks after planting, while the application of chicken manure dose of 60 t/ha produced the longest plant roots. The highest fresh and dry rhizome weight was observed for the 60 t/ha cow manure treatment. The highest secondary metabolic levels in each parameter were found in dry rhizomes (phenolic, flavonoid, and tannin) and fresh rhizomes (phenolic and flavonoid), with the highest tannin compound in the treatment of 40 t chicken manure/ha. The application of chicken manure at a dose of 60 t/ha resulted in a strong antioxidant yield in fresh and dry rhizomes.

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## Introduction

*Zingiber montanum* Roxb. (WFO, 2021) that is commonly known as *banada* in Bangladesh, *phlai* in Thailand, *jangliadrak* in India, and *bangle* in Malaysia and Indonesia, is extensively planted in Thailand, Malaysia, and Indonesia (Hassan et al., 2019). This plant is commonly used in traditional medicines to treat constipation, dyspepsia, gastritis, stomach bloating, and stomach ache. Various parts of *Z. montanum* are used in Thailand as a daily diet (Lim, 2016), while the rhizome is used as a vermifuge in Malaysia and applied for abscesses, colic, diarrhea, fever, and intestinal disorders. In Northeast India, rhizome paste was reported to be used to treat dyspepsia and stomach bloating (Anasamy, 2013). In East Kalimantan, Indonesia, the productivity of this plant which is commonly cultivated in the area dominated by the Ultisol soil order, is still low due to the low fertility of the soil.

Ultisol contains 1:1 clay minerals that contribute to low cation exchange capacity (CEC) and the dominance of $Al^{3+}$ in exchangeable sites, resulting in low pH. It is well-known that soil acidity can inhibit nitrification (Shibata et al., 2017). A distinct Ultisol is found in East Kalimantan with extremely high aluminum saturation, which can cause severe toxicity to the plant. $Al^{3+}$ enhances the desorption and leaching of nutrient cations from the soil exchange complex, hampering their absorption in the plant root area (Singh et al., 2017; Jaiswal et al., 2018; Zhao and Shen, 2018). Low pH exacerbates that effect by increasing the micronutrients/trace elements availability (e.g., Fe, Mn, Cu, and Zn), which is potentially toxic for plants. This condition and other soil properties may lead to nutrient deficiency, resulting in limited plant growth and development, including poor root systems, weak stalks or stems, and declining plant productivity (Bojórquez-Quintal et al., 2017; Moore et al., 2020). Besides nutrient deficiencies, the upland Ultisols soil contains low organic matter, high soil bulk density, low total pores space, soil permeability, and available water.

Liming and fertilization are the common solutions to this problem (Purwanto et al., 2020). However, most farmers cannot afford to buy lime and fertilizer to improve soil fertility to increase the productivity of the *Z. montanum* they cultivate. Alternatively, farmers use animal manure as a source of organic matter to improve soil fertility and production of *Z. montanum*. Several research workers demonstrated the positive effects of animal manure on the physical, chemical, and biological properties of Ultisols, particularly in alleviating soil acidity and Al toxicity, as well as a valuable source of essential macro and micronutrients (Zhou et al., 2013; Ngo et al., 2014; Ch'ng et al., 2015; Peng et al., 2016; Shi et al., 2019; Ye et al., 2019). Maholtra et al. (2018) and Masmoudi et al. (2020) reported that manure increases plant productivity, soil organic matter and structure, water infiltration and holding capacity, and over time, the application of manure can reduce sediment loss and soil erosion, which has advantageous effects on plant growth and development. Therefore, manure application is necessary to reduce the problems of N, P, and K deficiency for plants and poor fertility in acid upland soils (Castellanos-Navarrete et al., 2015).

This study aimed to examine the impact of animal manure on the fertility of a degraded Ultisol of East Kalimantan on the growth and secondary metabolite content of *Z. montanum*.

## Materials and Methods

### *Place and time*

The experiment was conducted at the Experimental Farm of Mulawarman University Teluk Dalam, East Kalimantan, lasting about five months from September 2021 to March 2022. The second place was in the Soil Laboratory and Laboratory of Post-Harvest and Packaging of Agricultural Products, Faculty of Agriculture, Mulawarman University. The chemical compositions of the soil, cow manure, and chicken manure used in this study are presented in Table 1.

Table 1. Chemical composition of the soil, cow manure, and chicken manure used in this study.

| Chemical Properties | Soil | Cow Manure | Chicken Manure |
|---|---|---|---|
| pH | 5.62 | 6.50 | 6.40 |
| Organic C (%) | 0.18 | 29.55 | 40.51 |
| N (%) | 0.14 | 2.08 | 1.37 |
| C/N | 1.29 | 14.21 | 29.57 |
| Available P (ppm) | 10.52 | 0.17 | 0.67 |
| CEC (me/100 g) | 14.33 | - | - |
| Water (%) | - | 72.90 | 60.63 |

### *Research design*

The dosages of manure applied were $P_0$ (control; without fertilizer), $P_1$ (cow manure 20 t/ha), $P_2$ (cow manure 40 t/ha), $P_3$ (cow manure 60 t/ha), $P_4$ (chicken manure 20 t/ha), $P_5$ (chicken manure 40 t/ha), and $P_6$ (chicken manure 60 t/ha). The seven treatments were arranged in a completely randomized block design with three replications with one treatment factor using organic fertilizers (cow manure and chicken manure).

A non-factorial completely randomized design is as follows:

$$Yij = \mu + \tau i + \varepsilon ij.$$

where:

| | | |
|---|---|---|
| Yij | = | The observed value in the i-th treatment and j-th repetition |
| μ | = | General mean value |
| τi | = | Effect of i-th treatment |
| εij | = | Effect of error (error) in the i-th treatment experiment and j-th repetition |

The manure was applied evenly according to the treatment and then mixed with soil at 15-20 cm depth using hand hoes. The plot had a length of 6 m and a width of 1 m. Planting one crop per planting hole resulted in a spacing of approximately 50 x 100 cm. To prevent waterlogging and seedling rot, seedlings were planted in ditches with good drainage. In order to facilitate landfilling later, planting was done in the trench. The parameters measured in this study were chemical properties of soil, cow manure, and chicken manure, i.e., pH using the electrometric method, organic C using the Walkley and Black method, total N using the Kjeldahl method, available P using the Bray II method, and cation exchange capacity (CEC) using the leaching method with 1N ammonium acetate at pH 7. Plant height, number of leaves, and number of tillers were measured at 3, 6, 9, 12, 15, and 18 WAP (weeks after planting). At harvest, fresh weight, dry weight, root length, secondary metabolic, and antioxidant levels of the plants were measured.

### *Laboratory measurement*

Soil analyses were carried out in the Soil Science Laboratory, Faculty of Agriculture, Mulawarman University. The initial examination of soil reaction was determined using soil to water mixture of 1:1. Organic carbon was extracted using the Walkley and Black method. Total N was extracted using the Kjeldahl method. Exchangeable aluminum was extracted using KCl 1 N. Base cations and CEC were extracted using an ammonium acetate ($NH_4OAc$) solution pH 7.0. Clay to CEC ratio (CCR) was calculated by dividing CEC by clay percentage. Since the observed soil pH was acidic, the effective cation exchange capacity (ECEC) was applied as the CEC reference for calculating base saturation.

### *Data analysis*

Soil data were analyzed descriptively, while the crop data were analyzed using the SAS Systems for Linear Models, v.6.12 for Windows (Ramon et al., 1992). The data obtained were subjected to the analysis of variance (ANOVA) with α 5% confidence level, followed by DMRT (Duncan Multiple Range Test) at α 5% level to detect significant differences among treatments. The results of the secondary metabolic level identification and antioxidant activity tests were analyzed qualitatively using the descriptive method. The comparison of phytochemical compound levels in each treatment was based on secondary metabolism levels. Antioxidant activity was assessed by comparing the IC50 values in each treatment. A spectrophotometry method was used for all of the parameters of the secondary metabolic level test.

## Results

### *Manure quality*

The results of manure analysis (Table 1) showed that the organic C content was relatively high (29.55%) for cow manure and 40.51% for chicken manure, and the water contents were characterized at a moderate level of 72,9% and 60.63%, respectively. The total N contents were 2.08% for cow manure and 1.37% for chicken manure, with C/N ratios of 14.21 and 29.57, respectively. The manure contained some microelements required by the plant. Therefore, the quality of manure used in this study was high. Manure can improve soil chemical and physical properties effectively, and plant growth is affected by the maturity of manure (Cai et al., 2019). Animal manure contains nitrogen as well as other minerals like magnesium, potassium, and calcium. The primary benefit of manure is that it preserves the physical structure of the soil, allowing roots to grow properly, as well as supporting the biological and chemical properties of the soil (Melsasail et al., 2019). Therefore, fertilization aims to replenish lost nutrients and increase the amount of nutrients available to plants, thereby increasing plant quality and quantity by gradually releasing nutrients into the soil solution and maintaining nutrient balance for the healthy growth of crop plants. They also act as an effective energy source for soil microbes, improving soil structure and crop growth (Shaji et al., 2021).

As a function of soil chemistry, organic manure can increase soil CEC which is important to hold a given inorganic fertilizers and soil buffering capacity so that the crops can avoid the negative effect of soil acidity (Shi et al., 2019). The use of animal manure increased the availability of some nutrients and improved the efficiency of P absorption by crops because in the process of organic matters decomposition produces humic acid and fulvic acid (polyelectrolyte) that can bind Al and Fe in the soil (Al-Juthery et al., 2021). To eliminate P fixation in the soil, the active anion of organic manure forms a chelate bond with Si-Al-OOCR (allophane). The higher the carboxyl and phenolic compounds in organic matter, the higher the ability of organic matter to release $AlHPO_4$ bonds so the P nutrient becomes more available for plants (Ch'ng et al., 2016; Asap et al., 2018; Maru et al., 2020). By increasing organic matter contents in the soil, the total N and N mineralization, soluble P, exchangeable K, and N uptake by plants and soil water content can increase (Kafeel et al., 2023).

### *Soil chemical properties*

The effect of manure on the soil chemical properties presented in Table 2 showed that the initial soil pH (before planting) was 5.62 and increased to 4.21 after manure applications. A significant decrease in soil acidity was obtained due to the addition of chicken manure at 60 t/ha. The addition of manure reduced soil organic C at all levels of addition of manure. A significant decrease in organic C occurred with the addition of chicken manure at 20 t/ha (Table 3). In soil ecosystems, organic C is a critical component that influences soil properties to support plant growth, namely as a source of energy for soil organisms and a trigger for nutrient availability for plants (Qiu et al., 2018).

Application of all levels of manure increased the N content in the soil (Table 4). The highest increase in N content in the soil was obtained due to the addition of chicken manure at 60 t/ha. The C/N ratio of organic matter is the ratio between the elemental carbon (C) and elemental nitrogen (N) presented in an organic matter. Good manure must have a C/N ratio of <20 (Macias-Corral et al., 2019). This study showed that the C/N ranged from 2.00 to 11.91 and decreased to 2.05 after 18 weeks of incubation (Table 5). Manure input at all application levels increased the P content in the soil along with the incubation time. The highest increase in P in the soil was obtained due to the addition of chicken manure at 60 t/ha (Table 6). The results of this study indicated that the addition of all levels of manure provided an increase in cation exchange capacity. The highest increase in cation exchange capacity was obtained due to the addition of cow manure at 60 t/ha, as shown in Table 7.

Table 2. Soil pH after manure treatment.

| **Manure Treatments** | **pH** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Week of Incubation** | | | | | | |
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** |
| Control | 5.62 | 5.62 | 5.62 | 5.61 | 5.62 | 5.62 | 5.62 |
| Cow manure 20 t/ha | 6.40 | 6.20 | 6.00 | 5.80 | 5.70 | 5.70 | 5.60 |
| Cow manure 40 t/ha | 6.50 | 6.40 | 6.30 | 5.90 | 5.70 | 5.50 | 5.40 |
| Cow manure 60 t/ha | 6.80 | 6.60 | 6.40 | 6.60 | 6.20 | 6.00 | 5.80 |
| Chicken manure 20 t/ha | 5.83 | 5.60 | 5.40 | 5.00 | 4.83 | 4.70 | 4.51 |
| Chicken manure 40 t/ha | 6.04 | 6.00 | 5.80 | 5.04 | 4.74 | 4.90 | 4.80 |
| Chicken manure 60 t/ha | 6.08 | 5.43 | 5.08 | 4.76 | 4.77 | 4.08 | 4.21 |

Table 3. Soil organic C after manure treatment.

| **Manure Treatments** | **Organic C (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Week of Incubation** | | | | | | |
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** |
| Control | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.17 |
| Cow manure 20 t/ha | 0.83 | 0.80 | 0.78 | 0.64 | 0.56 | 0.46 | 0.40 |
| Cow manure 40 t/ha | 1.31 | 1.27 | 1.20 | 1.19 | 1.08 | 1.07 | 1.01 |
| Cow manure 60 t/ha | 1.40 | 1.37 | 1.35 | 1.32 | 1.31 | 1.31 | 1.30 |
| Chicken manure 20 t/ha | 0.36 | 0.33 | 0.32 | 0.30 | 0.27 | 0.21 | 0.18 |
| Chicken manure 40 t/ha | 0.42 | 0.42 | 0.41 | 0.38 | 0.35 | 0.31 | 0.26 |
| Chicken manure 60 t/ha | 0.98 | 0.98 | 0.95 | 0.92 | 0.87 | 0.80 | 0.78 |

Table 4. Soil N after manure treatment.

| **Manure Treatments** | **N (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Week of Incubation** | | | | | | |
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** |
| Control | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.15 | 0.14 |
| Cow manure 20 t/ha | 0.08 | 0.10 | 0.15 | 0.16 | 0.18 | 0.19 | 0.19 |
| Cow manure 40 t/ha | 0.11 | 0.11 | 0.12 | 0.17 | 0.19 | 0.19 | 0.21 |
| Cow manure 60 t/ha | 0.13 | 0.15 | 0.16 | 0.16 | 0.17 | 0.19 | 0.23 |
| Chicken manure 20 t/ha | 0.18 | 0.18 | 0.19 | 0.19 | 0.20 | 0.21 | 0.21 |
| Chicken manure 40 t/ha | 0.20 | 0.21 | 0.22 | 0.30 | 0.32 | 0.33 | 0.35 |
| Chicken manure 60 t/ha | 0.20 | 0.22 | 0.32 | 0.35 | 0.36 | 0.37 | 0.38 |

Table 5. Soil C/N ratio after manure treatment.

| **Manure Treatments** | **C/N ratio** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Week of Incubation** | | | | | | |
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** |
| Control | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.20 | 1.21 |
| Cow manure 20 t/ha | 10.38 | 8.00 | 5.20 | 4.00 | 3.11 | 2.42 | 2.11 |
| Cow manure 40 t/ha | 11.91 | 11.55 | 10.00 | 7.00 | 5.68 | 5.63 | 4.81 |
| Cow manure 60 t/ha | 10.77 | 9.13 | 8.44 | 8.25 | 7.71 | 6.89 | 5.65 |
| Chicken manure 20 t/ha | 2.00 | 1.83 | 1.68 | 1.58 | 1.35 | 1.00 | 0.86 |
| Chicken manure 40 t/ha | 2.10 | 2.00 | 1.86 | 1.27 | 1.09 | 0.94 | 0.74 |
| Chicken manure 60 t/ha | 4.90 | 4.45 | 2.97 | 2.63 | 2.42 | 2.16 | 2.05 |

Cation exchange in the soil occurs due to a negative charge from the soil colloid, which adsorbs cations in an exchangeable form (Meetei et al., 2020). Soils with high CEC can absorb and provide nutrients better than soils with low CEC. Because these elements are in the soil adsorption complex, water does not quickly lose or wash away these nutrients. Mineralization of soil organic fractions provides limited supplies of N, S, and micronutrients; during mineral dissolution and surface exchange reactions, re-supply P, K, Ca, Mg, and micronutrients. Nutrient mobility in soil influences ion transport to plant roots, evaluation of nutrient availability to plants, and ultimately nutrient management decisions (Havlin, 2020). The nutrient added to the soil with low CEC can not be held and is easily lost. This condition was reflected in the increasing soil organic C contents in the treatment with manure application. It did not increase significantly in the treatment without manure application, and vice versa. This study showed that the application of chicken and cow manure gave significant differences in plant height, number of tillers, number of leaves, root length, fresh weight of rhizomes, and dry weight of rhizomes. Only root length was not affected significantly by the treatments. For growing zingiber, N, P, and K play as essential nutrients.

Table 6. Soil available P after manure treatment.

| Manure Treatments | P (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week of Incubation | | | | | | |
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Control | 10.52 | 10.52 | 10.52 | 10.52 | 10.51 | 10.52 | 10.52 |
| Cow manure 20 t/ha | 0.35 | 0.36 | 0.36 | 0.37 | 0.37 | 0.38 | 0.38 |
| Cow manure 40 t/ha | 0.40 | 0.40 | 0.42 | 0.43 | 0.44 | 0.45 | 0.45 |
| Cow manure 60 t/ha | 0.44 | 0.44 | 0.45 | 0.47 | 0.48 | 0.48 | 0.49 |
| Chicken manure 20 t/ha | 12.78 | 12.78 | 12.8 | 12.81 | 12.90 | 12.92 | 12.93 |
| Chicken manure 40 t/ha | 13.42 | 13.44 | 13.47 | 13.48 | 13.49 | 13.49 | 13.5 |
| Chicken manure 60 t/ha | 15.68 | 15.68 | 15.68 | 15.69 | 15.70 | 15.72 | 15.73 |

Table 7. Soil CEC after manure treatment.

| Manure Treatments | CEC (me/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Week of Incubation | | | | | | |
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Control | 14.33 | 14.33 | 14.32 | 14.33 | 14.34 | 14.33 | 14.33 |
| Cow manure 20 t/ha | 19.15 | 19.15 | 19.17 | 19.17 | 19.18 | 19.18 | 19.19 |
| Cow manure 40 t/ha | 19.35 | 19.36 | 19.36 | 19.37 | 19.37 | 19.38 | 19.38 |
| Cow manure 60 t/ha | 20.45 | 20.45 | 20.46 | 20.50 | 20.51 | 20.55 | 20.56 |
| Chicken manure 20 t/ha | 18.32 | 18.32 | 18.33 | 18.34 | 18.37 | 18.38 | 18.40 |
| Chicken manure 40 t/ha | 18.38 | 18.38 | 18.40 | 18.41 | 18.43 | 18.44 | 18.45 |
| Chicken manure 60 t/ha | 19.00 | 19.12 | 19.14 | 19.15 | 19.18 | 19.20 | 19.22 |

### *Plant growth*

#### *Plant height*

Table 8 shows that when the plant was about six weeks old, the application of cow manure at a dose of 60 t/ha produced the best plant growth (26.16 cm), followed by chicken manure at a dose of 60 t/ha, chicken manure of 40 t/ha, and cow manure of 40 t/ha. Compared to the previous week, the plant age of nine weeks after planting showed a rapid increase in plant height. At nine weeks after planting, the best dose was 40 t/ha of chicken manure with an average plant height addition of about 32.45 cm. The best results were obtained at week 12, with cow manure at a dose of 60 t/ha that yielded an average plant height of 37.24 cm. The application of 20 t/ha manure did not produce significantly different plant heights from the control. The best dose at 15 weeks after planting was 60 t cow manure/ha, with an average plant height of 40.47 cm and 38.38 cm of chicken manure. The highest dose of cow manure yielded an average plant height of 42.78 cm at 18 weeks after planting.

#### *Number of tillers*

The highest number of tillers was found at a dose of 60 t/ha of chicken manure, with an average of 1.65 tillers at 6 weeks after planting (Table 9). At 9 weeks after planting, a control with an average of about 2.55 tillers produced tillers that were not significantly different from chicken manure of 20 t/ha. The best application of cow manure was 60 t/ha, which yielded an average number of 4.15 tillers. At 12 weeks after planting, the effect of manure treatment revealed that the best dose was 40 t/ha chicken manure, with an average of 6.65 tillers. This best dose did not differ significantly between chicken and cow manure at

60 t/ha or chicken manure at 20 t/ha. Cow and chicken manure at a dose of 60 t/ha at 15 weeks after planting produced the highest yields, with cow manure yielding 9.50 tillers and chicken yielding 9.00 tillers, respectively. At 18 weeks, the control did not differ significantly from the cow manure doses of 20 and 40 t/ha. The highest number of tillers was observed for the treatment of cow manure at 60 t/ha, with an average of 14.45 tillers, and chicken manure at 60 t/ha, with an average of 13.37 tillers.

Table 8. Effect of cow and chicken manure on plant height (cm).

| Manure Treatments | Week After Planting (WAP) | | | | |
|---|---|---|---|---|---|
| | 6 | 9 | 12 | 15 | 18 |
| Control | 11.38 a | 18.61 a | 24.68 a | 20.41 a | 18.82 a |
| Cow manure 20 t/ha | 17.46 b | 20.26 ab | 25.44 a | 24.02 ab | 28.51 b |
| Cow manure 40 t/ha | 20.51 c | 27.95 b | 29.81 ab | 35.57 cd | 37.19 c |
| Cow manure 60 t/ha | 26.16 e | 31.18 b | 37.24 c | 40.47 d | 42.78 c |
| Chicken manure 20 t/ha | 17.36 b | 27.21 b | 30.16 abc | 29.15 bc | 28.82 b |
| Chicken manure 40 t/ha | 22.84 cd | 32.45 b | 34.83 bc | 37.61 cd | 36.37 c |
| Chicken manure 60 t/ha | 25.68 de | 32.17 b | 36.84 bc | 38.38 d | 39.22 c |

Notes: Based on the Duncan Multiple Range Test at a 5% significance level, values in the same columns followed by the same letter do not differ significantly.

Table 9. Effect of cow and chicken manure on the number of rhizome tillers.

| Manure Treatments | Week After Planting (WAP) | | | | |
|---|---|---|---|---|---|
| | 6 | 9 | 12 | 15 | 18 |
| Control | 0.80 a | 2.55 a | 3.95 a | 5.80 a | 8.60 a |
| Cow manure 20 t/ha | 0.70 b | 3.45 b | 5.20 b | 6.60 a | 10.40 ab |
| Cow manure 40 t/ha | 1.70 ac | 3.40 b | 5.05 ab | 7.35 ab | 11.55 b |
| Cow manure 60 t/ha | 1.60 c | 4.15 b | 6.10 bc | 9.50 c | 14.45 d |
| Chicken manure 20 t/ha | 1.30 bc | 3.35 ab | 5.70 bc | 7.40 ab | 11.05 b |
| Chicken manure 40 t/ha | 1.50 c | 4.00 b | 6.65 c | 8.45 bc | 12.15 bc |
| Chicken manure 60 t/ha | 1.65 c | 3.75 b | 6.05 bc | 9.00 c | 13.70 cd |

Notes: Based on the Duncan Multiple Range Test at a 5% significance level, values in the same columns followed by the same letter do not differ significantly.

*Number of leaves*

Table 10 shows that after 6 weeks of treatment with a dose of 20 t chicken manure/ha, the number of leaves was not significantly different from the control but significantly different from other treatments. The best dose of 60 t/ha cow manure produced an average increase of 37.95 leaves at 9 weeks. At 12 weeks, the number of leaves in the control treatment showed no significant difference from those in the treatments of cow and chicken manure of 20 and 40 t/ha. With an average of 52.80 leaves, cow manure of 60 t/ha was the best dose at 12 weeks after planting. The highest number of leaves (84.55) was found in cow manure treatment at a rate of 60 t/ha at week 15. The application of 60 t cow manure/ha produced the largest number of leaves, 116.65 in the 18th week. The application of 60 t chicken manure/ha increased the number of leaves by 105.80.

***Rhizome root length, fresh weight, and dry weight***

Table 11 shows that the lowest root length of 24.36 cm was obtained by the application of 20 t/ha cow manure. The control yielded root length that was not statistically different from the application of 20 t/ha cow and chicken manure. This result indicates that the application of different doses of manure did not affect the root length of the *bangle* plant. The chicken manure dosage of 20 t/ha yielded the lowest rhizome fresh weight of 392.35 g. In comparison, the control yielded fresh weight that was not significantly different from 20 t/ha and 40 t/ha cow and chicken manure. The fresh weight of the *bangle* rhizome yielded from the application of 40 t/ha chicken and cow manure also did not differ significantly from those yielded from the application of 60 t/ha chicken manure. The treatment of 60 t/ha cow manure yielded the highest rhizome fresh weight of 822.00 g, which was significant for all treatments. The lowest dry weight of *bangle* rhizome was shown by the cow and chicken manure treatment at a dose of 20 t/ha, respectively 86.00 g for cow manure treatment and 78.25 g for chicken manure treatment. These values were lower than that of the control, with an average of 93.75 g. The control yielded insignificantly different rhizome dry weight with chicken manure at 20 and 40 t/ha, cow manure at 20 and 40 t/ha, and chicken manure at 60 t/ha but yielded significantly different rhizome dry weight with the best dose of 60 t/ha cow manure with an average rhizome dry weight of 179.75 g, followed by 60 t chicken manure/ha with an average rhizome dry weight of 135.75 g.

Table 10. Effect of cow and chicken manure on the number of rhizome leaves.

| Manure Treatments | Week After Planting (WAP) | | | | |
|---|---|---|---|---|---|
| | 6 | 9 | 12 | 15 | 18 |
| Control | 4.70 a | 19.60 a | 35.80 a | 52.25 a | 77.55 a |
| Cow manure 20 t/ha | 5.55 a | 25.00 ab | 41.05 ab | 55.65 a | 83.80 a |
| Cow manure 40 t/ha | 9.65 b | 28.25 abc | 42.70 abc | 66.00 ab | 95.35 ab |
| Cow manure 60 t/ha | 10.05 b | 37.95 c | 52.80 c | 84.55 c | 116.65 c |
| Chicken manure 20 t/ha | 9.20 b | 26.00 ab | 40.25 ab | 56.85 a | 81.75 a |
| Chicken manure 40 t/ha | 9.65 b | 30.90 bc | 43.25 abc | 67.05 bc | 91.35 a |
| Chicken manure 60 t/ha | 11.05 b | 30.45 abc | 50.75 bc | 79.55 bc | 105.80 bc |

Notes: Based on the Duncan Multiple Range Test at a 5% significance level, values in the same columns followed by the same letter do not differ significantly.

Table 11. Effect of cow and chicken manure on root length and rhizome weight.

| Manure Treatments | Root length (cm) | Rhizome fresh weight (g) | Rhizome dry weight (g) |
|---|---|---|---|
| Control | 31.90 ab | 415.25 ab | 93.75 ab |
| Cow manure 20 t/ha | 24.36 a | 444.75 ab | 86.00 a |
| Cow manure 40 t/ha | 35.36 b | 530.00 bc | 119.00 ab |
| Cow manure 60 t/ha | 36.50 b | 822.00 d | 179.75 c |
| Chicken manure 20 t/ha | 33.20 ab | 393.25 a | 78.25 a |
| Chicken manure 40 t/ha | 38.95 b | 487.50 abc | 99.75 ab |
| Chicken manure 60 t/ha | 41.03 b | 618.75 c | 134.75 bc |

Notes: Based on the Duncan Multiple Range Test at a 5% significance level, values in the same columns followed by the same letter do not differ significantly.

***Secondary metabolic level***

Data presented in Table 12 show that the *bangle* rhizome contained phenolic, flavonoid, and tannin-containing active substances, and also steroids, alkaloids, and terpenoids. Different levels of the compounds in this secondary show that, despite using the same amount of fertilizer, the active compound content of the *bangle* rhizomes was highest when the rhizome was dry (low moisture content) as opposed to when the rhizome was still fresh. The amount of chicken manure that produced the highest phenolic content in the rhizome under fresh and dry conditions, correspondingly around 178.56 mg/L and 202.79 mg/L, was 60 t/ha. A dose of 60 t/ha of chicken manure also yielded higher levels of active flavonoids and tannins than other doses. In comparison with the fertilizer treatment, the control had the lowest active compound content. The plant yields of the cow manure treatments were lower than those of the chicken manure treatments.

Table 12. Secondary metabolic levels in *bangle* rhizome in each treatment (mg/L).

| Condition | Manure Treatments | Level mg/L | | |
|---|---|---|---|---|
| | | Phenolic | Flavonoid | Tannin |
| Fresh | Control | 31.64 | 23.08 | 2,034.83 |
| | Cow manure 20 t/ha | 43.18 | 41.55 | 2,991.50 |
| | Cow manure 40 t/ha | 77.03 | 64.30 | 3,151.50 |
| | Cow manure 60 t/ha | 86.90 | 71.14 | 3,198.17 |
| | Chicken manure 20 t/ha | 97.67 | 74.79 | 4,086.50 |
| | Chicken manure 40 t/ha | 107.54 | 78.71 | 4,144.83 |
| | Chicken manure 60 t/ha | 178.56 | 104.39 | 3,861.33 |
| Dry | Control | 124.97 | 38.44 | 1,932.33 |
| | Cow manure 20 t/ha | 133.56 | 67.23 | 2,504.00 |
| | Cow manure 40 t/ha | 148.44 | 68.76 | 3,034.83 |
| | Cow manure 60 t/ha | 181.38 | 99.43 | 4,734.67 |
| | Chicken manure 20 t/ha | 175.23 | 120.06 | 4,903.00 |
| | Chicken manure 40 t/ha | 198.69 | 156.10 | 5,088.00 |
| | Chicken manure 60 t/ha | 202.79 | 181.91 | 5,406.33 |

Note: The number followed by yellow denotes the best outcome in each observation variable for the flavonoid, tannin, and phenolic contents.

*Antioxidant*

Results of antioxidant analysis of *bangle* rhizomes based on fresh and dry rhizomes are shown in Table 13. $IC_{50}$ value obtained described how well the sample captured free radicals. The dose of chicken manure of 60 t/ha was found to give the lowest $IC_{50}$ value of 9.52 ppm in the fresh sample, while the control gave the highest $IC_{50}$ value of 53.58 ppm in the fresh rhizomes. The same quality was obtained for the dry rhizomes. The application of chicken manure of 60 t/ha produced the lowest $IC_{50}$ value, and the largest was in control. The results of the ANOVA at a 5% level showed that the treatments of giving organic fertilizers in the form of chicken and cow manure gave significant differences in plant height, number of tillers, number of leaves, root length, fresh weight of rhizomes, and dry weight of rhizomes. The treatments did not significantly affect root length.

Table 13. Antioxidant based on IC50 value.

| Antioxidant (IC50) (mg/L) | Control | Manure Treatments (t/ha) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cow Manure | | | Chicken Manure | | |
| | | 20 | 40 | 60 | 20 | 40 | 60 |
| Fresh rhizomes | 53.58 | 48.55 | 47.36 | 47.14 | 32.73 | 27.42 | 9.52 |
| Dry rhizomes | 40.91 | 38.30 | 34.78 | 34.46 | 24.41 | 14.05 | 8.06 |

Note: The smaller the IC50 value, the stronger the antioxidant.

## Discussion

### *Effect of manure addition on soil chemical properties*

The changes in several soil chemical parameters during the incubation of three types of animal manure are presented in Tables 2, 3, 4, 5, and 6. According to the analysis of variance, almost all of the observed soil chemical characteristics were strongly affected by the differences in incubation time and manure rate. There were significant differences between the incubation time on pH $H_2O$, pH KCl, total N, exchangeable acid and base cations, CEC, base saturation ($p<0.001$), and organic C ($p<0.01$). Some parameters (e.g., pH $H_2O$, pH KCl, organic C, exchangeable bases, and base saturation) showed statistically high values and concentrations in the first two weeks, which decreased in the next four and six weeks. The increment in incubation time seemingly exhibited an inconsistent effect on the soil. Adekiya et al. (2020) reported that utilizing organic manure to meet crop nutrient requirements would be an unavoidable practice to enhance sustainable agriculture. This is because the physical, chemical, and biological properties of soil are generally improved by the addition of organic manure, which in turn enhances crop productivity and maintains the quality of crop production. Poultry litter treatments were positively correlated with greater soil fertility levels, as well as higher crop yield and soil biodiversity. These results underscore linkages between manure additions and cropping sequences within the nutrient cycling, soil health, and crop production continuum (Asworth et al., 2018).

### *Plant height*

According to Table 8, the average increase in plant height starting at 6, 9, 12, 15, and 18 WAP tended to fluctuate. At the age of 6 weeks, the treatment without fertilizer (control) continued to grow until week 12 when it reached its peak with an average height increase of about 24.68 cm before the delining height in the following weeks. In order for a plant to grow, nutrients must be obtained from the soil by the roots through their root hairs (Wang et al., 2021). Organic matter affects plant growth by influencing the physical, chemical, and biological properties of the soil (Delgado and Gómez, 2016). The more organic matter is provided, the faster the plant will grow. Compared to chicken manure, cow manure typically produced better plant growth. The application of chicken manure at all levels significantly increased plant height at 9 weeks after planting, but at 12 weeks, the plant height slightly decreased. In the 20 t/ha chicken manure treatment, the plant height was an average of 30.16 cm at 12 weeks after planting and decreased in the following weeks. A high nitrogen content was found in chicken manure (0.38%) although the amount of nitrogen required by plants is always higher than other nutrients, a deficiency or excess of nitrogen can hinder and disrupt plant growth (Jiang et al., 2017). After planting, the growth rate of the *bangle* plant accelerated between 2 and 5 months. As plants get older, their growth rate for height starts to slow down (Rademacher, 2015).

The application of chicken manure always resulted in the best plant response in the first growing season. This is because chicken manure decomposes relatively quickly and has enough nutrients compared to other manure of the same weight. Table 1 shows that the application of chicken manure tended to increase plant height rapidly at 6, 9, and 12 weeks of age, then declined as plant age increased. Large-scale application of chicken manure is thought to be less effective because the nutrients will exhaust quickly. The same result was also shown by the application of chicken manure at a dose of 40 t/ha, which decreased the plant height; the maximum height increase at this dose was at 15 weeks, with an average height increase of 37.61 cm. When compared to the other two doses of chicken manure, the plant height for the 60 t/ha chicken manure was different.

The plant height increase over 18 weeks demonstrates this, but the increase in plant height was typically not too different from the previous weeks. Even though the application of cow manure at a dose of 40 t/ha initially produced fewer yields than that produced by the application of chicken manure at a dose of 40 t/ha, at the end of the observation at 18 weeks, the increase in height was more apparent and might even have exceeded that of the chicken manure at a dose of 40 t/ha, which caused a decrease in plant height.

The addition of cow manure improves soil permeability, total pore space, aggregate stability, bulk density, texture, color, and temperature (Shrinet et al., 2021). A dose of 60 t/ha of chicken and cow manure had the tendency to produce steady, dependable results. The application of cow manure at a dose of 60 t/ha tended to yield less than chicken manure at doses of 40 and 60 t/ha from the start of planting until the plant was 9 weeks old, but the yields increased in the following weeks. The plant's need for nutrients grows as it ages. If the nutrient requirements are not met, and the nutrients are not readily available, plants may get nutrient deficiency at specific times (Bindraban et al., 2015).

*Bangle* plants absorb N (0.06-3.07 g), P (0.01-0.53 g), and K (0.10- 2.25 g) at 2 to 10 months after planting in the canopy. N is the nutrient that is most required in the plant canopy itself. The primary nutrient for plants, nitrogen, is typically essential for the development and expansion of vegetative parts of plants, such as leaves, stems, and roots (Tegeder and Masclaux-Daubresse, 2018). A sufficient supply of plant N is indicated by high photosynthetic activity, good vegetative growth, and dark green plant colors (Wei et al., 2015). Due to the individual characteristics of each animal, which are influenced by the type of feed and the animal's age, each manure contains a different mix of nutrients (Asworth et al., 2020). Because each treatment dose of fertilizer has a different nutrient content, they all produce different yields and have different recommended doses. Due to their movement with crop yields, surface runoff, erosion, or evaporation, nutrients in the soil will gradually decrease over time (Liao et al., 2021).

### *Number of tillers*

The total number of tillers from each level reveals increased with plant age and are influenced by the quantity of fertilizer applied (Table 9). Plants without fertilizer developed more tillers every week, but the growth was typically modest. This slight increase resulted from the fact that during the initial stages of planting, the products of photosynthesis were utilized for the vegetative development of plants. In comparison to manure application, treatment without fertilizer produced the lowest yield. Data presented in Table 9 show that there was a noticeable increase in the number of tillers at 18 WAP of age. A plant needs nutrients for its physiological processes during growth and development. Plant growth and production will be subpar due to a lack of nutrients (Reich et al., 2014). Organic matter functions as a biological buffer so that the soil can supply plants with balanced nutrients (Nair, 2019). Loosening the topsoil, increasing water absorption and storage, and boosting soil fertility are all important functions of manure (Murphy, 2015). A sudden rise in the number of tillers can result from the ease with which new shoots can emerge from loose, moist soil. At the start of planting, there were typically fewer and nearly identical numbers of tillers in each treatment. The nutrients in this fertilizer are not readily available to plants, which is the cause of the slow plant growth at the start of the planting period. The extent of mineralization or decomposition of these materials has a significant impact on nutrient availability. The low nutrient availability of manure is partially caused by the presence of N, P, and other elements in complex compounds that are challenging to decompose (Cui et al., 2022). At 6 weeks after planting, all treatments tended to be similar, and the differences between the tillers in each treatment tended to be minimal. Although cow manure at a dose of 60 t/ha was the best dose, with an average number of tillers of 14.45 tillers, chicken manure typically produced better results than cow manure at the same dose. In comparison to other manure doses, chicken manure 40 t/ha at 12 weeks after planting produced the best results with 6.65 tillers.

### *Number of leaves*

An increasing number of leaves was observed each week as a result of the weekly application of cow and chicken manure. Data presented in Table 10 show that the number of leaves increased significantly at about 12 weeks of age. Every week, the increase varied depending on the treatment. The number of leaves significantly increased with a dose of 60 t/ha of cow manure, averaging 15-37 leaves every three weeks. Similarly, the number of leaves tended to increase when manure was applied in the same amount. Both cow and chicken manure at a dose of 20 t/ha and 40 t/ha produced a nearly identical number of leaves during plant growth.

Manure also contains humic acids, fulvic acids, growth hormones, and other substances that promote plant growth and increase plant nutrient uptake (Canellas et al., 2015). The number of leaves present influences the amount of photosynthesis, and plants with more leaves may produce heavier and bigger rhizomes. The number of leaves is also affected by the number of shoots and plant height. The number of leaves increases as the plant ages and grows taller, produces more leaves on a single stem, and produces more tillers. The nutrients required for plant growth are present in sufficient amounts in manure. The related observation variables aare affected by the nutrients and planting age.

In relation to the addition of the number of leaves, the most influential element is N. In comparison to other nutrients, nitrogen is required in sufficient amounts for plant growth. N makes up 40-50% of the dry weight of protoplasm, the living component of plants (Kathpalia and Bhatla, 2018). Since protein is the source of all plant enzymes, nitrogen participates in all enzymatic processes in plants. Additionally, nitrogen is one of the constituent elements of chlorophyll, the primary component of chloroplasts, and it contributes to improving the quality and quantity of the dry matter produced (Wen et al., 2020). Fertilizer use and the amount of nutrients in the soil have a significant impact on how plants grow and develop. Nutrient uptake is restricted by nutrients in a minimum state (Purba et al., 2021). In terms of the addition of leaves, the treatment of plants without fertilizer differed significantly enough for each observation. In comparison to other treatments, plants without fertilizer produced the lowest yield.

### *Root length*

The lowest root length was observed for the 20 t/ha cow manure treatment, with an average root length of about 24.36 cm, which was lower than the control. With an average root length of 41.03 cm, the application of chicken manure yielded the longest roots. When plants respond to water shortages by reducing the rate of transpiration to conserve water, the roots play a crucial role (Sourour et al., 2017). Plant roots have a significant impact on overall plant growth and development. The failure of the root function will result in a complete change in the plant for the top. Manure can bind water in the soil. Because the soil around the roots in the deeper layers is still moist, the roots will continue to grow. Maximizing exposure to groundwater will encourage the growth of the root. Plant roots directly respond to the physical characteristics of the soil (Jiang et al., 2017).

Data presented in Table 11 show that the application of chicken manure yielded better root length than cow manure. The use of organic fertilizers can loosen the soil, increase aeration, and increase the soil water holding capacity, all of which can improve the physical properties of the soil (Shaji et al., 2021). Additionally, organic matter has the ability to control soil temperature, slow down phosphorus fixation, increase soil cation exchange capacity, and lessen the leaching of nutrients like potassium, calcium, and magnesium (Baghbani-Arani et al., 2021).

Another environmental factor affecting the nitrate absorption process is the temperature around the roots (Le Deunff, 2019). The initial analysis of the soil revealed that the pH ranged from 3.86 to 4.86. Al is commonly excessive in acidic soils, and it can poison plants and bind phosphorus (P). Low soil pH can hinder plant growth by preventing the roots from properly absorbing nutrients. As Mažeika et al. (2021) demonstrated, giving chicken manure can maintain stable nutrient content in soil and minimize mineral fertilizer influx into the environment. This can raise the pH of the soil. By raising pH, Al in the exchangeable form will be reduced, and nutrients will become more available to plants.

According to Rosita et al. (2005), nutrient uptake on the roots of *bangle* plants at 2-10 months after planting is as follows: N (0.01-0.52 g), P (0.002-0.15 g), and K (0.02- 0.82 g). It is discovered that the roots of the *bangle* plant have more K buildup than N and P. K is primarily used to aid in the synthesis of proteins and carbohydrates. In the face of drought, illness, and pests, potassium gives plants strength (Hasanuzzaman et al., 2018). Organic fertilizers can help the soil's physical and chemical composition, which will facilitate root development. Up until the soil reaches its critical water potential, plant roots expand into moist soil and draw water (Sisouvanh et al., 2021). The looseness of the soil can promote root development. Strong roots will make it simpler for plants to absorb nutrients and water.

### *Rhizome fresh weight*

Plant biomass is a common parameter used to study plant growth. When plant nutrient requirements are met, yields will be optimal (Timsina, 2018). The rhizome of the *bangle* plant is the most advantageous part of cultivation. One could also argue that this rhizome's fresh weight is a crucial factor in determining how well *bangle* plant cultivation is going. The cultivation method is better and more productive the more weight of the wet rhizome can be obtained. According to the results of the application of the organic fertilizers at the age of 18 weeks, cow manure at a dose of 60 t/ha produced the highest fresh weight of rhizomes, averaging 822 g/plant, and chicken manure at a dose of 20 t/ha produced the lowest fresh weight of rhizomes, averaging 393.25 g/plant. Manure increases crop yield and quality while also enhancing the chemistry, physical characteristics, and biological properties of the soil (Ma et al., 2021).

Data presented in Table 11 demonstrate that the yield of fresh weight of rhizomes increased with increasing manure dosage. The physical condition of the soil must support plant growth in addition to a supply of adequate and balanced nutrients (El-Ramady et al., 2014). These soil aggregates will keep the soil in a loose condition (Murphy, 2015). Cow manure will enhance the physical characteristics of the soil. Improved soil physical characteristics include things like increased permeability, total pore space, aggregate stability, bulk density, texture, color, and temperature (Agbede, 2021). Intensive tillage affects the physical properties of the soil. Low organic matter soils will have more severe damage to the soil's structure (Murphy, 2015). When the soil does not receive enough water and becomes dense and hard, soil damage is evident. Plant rhizomes will not be able to grow or spread out in compacted or hard soil.

The ability to maintain loose soil conditions that are difficult to harden or compact increases with the amount of organic matter added. Additionally, manure helps to improve soil structure, cation exchange capacity, and water resistance. Giving manure has the indirect effect of making it simpler to keep water in the soil (Zhang et al., 2016). Since water availability plays a significant role in plant growth, water frequently restricts the growth and development of cultivated plants. The plants will experience drought conditions if there is not enough water in the soil. Due to decreased primary metabolism, reduced leaf area, and decreased photosynthetic activity, drought stress can lower plant productivity (biomass). Smaller leaves grow due to a lack of water during the vegetative stage, which can reduce light absorption.

Lack of water also inhibits the synthesis of chlorophyll and some enzymes, such as nitrate reductase, from working (Altuntaş et al., 2020). Organic substances in the soil may have physiological effects on plant growth that are direct or indirect (Basilio et al., 2013). Compared to other types of manure, chicken manure contains a fair amount of P. This is due to the fact that chicken manure contains feed (Agbede, 2021). Phosphorus aids in the growth of plant roots, photosynthesis, transfer respiration, cell division, and growth (Malhotra et al., 2018). The number of cells increases more quickly when they divide quickly, which causes the rhizomes to grow larger.

### *Rhizome dry weight*

The dry weight reflects a plant's nutritional status because it is affected by the rate of photosynthesis and respiration in each treatment. Based on the collected data, it was found that applying chicken and cow manure at a dose of 20 t/ha resulted in lower dry-weight yields than the control. Additionally, the results from the application of 40 t/ha manure dose were not significantly different from the control. This is the active vegetative formation stage. The application of cow manure at a dose of 60 t/ha demonstrated different results and produced significantly better outcomes than other doses of chicken manure. The amount and timing of fertilizer applied can impact crop yields, among other things. Organic matter plays a crucial role in soil health because it can create stable soil aggregates, increase soil fertility, and serve as a source of energy for organisms (Magdoff, 2018).

The application of manure enhances the chemical, physical, and biological properties of the soil, increases crop yield, and improves crop quality (Du et al., 2022). High organic matter soils have beneficial microorganisms that encourage the breakdown of organic matter and release inorganic nutrients that are then available for plant uptake. Organic fertilizers can help to create ideal conditions in the soil for microorganisms that are beneficial to plants (Du et al., 2022). Chicken manure is an organic fertilizer with high nitrogen content, despite not being the best dose for *bangle* rhizome weight yield. As they ensure the best nutrient management for plants, such fertilizers should be used promptly to partially replace chemical fertilizers (de Araujo Guimaraes et al., 2019).

### *Secondary metabolic levels (phenolic compounds, flavonoids and tannins)*

The laboratory analysis results showed that the positive *bangle* rhizome contained compounds in the form of phenolics, tannins, and flavonoids. These compounds responded differently to the concentration of organic fertilizer applied in the form of cow manure and chicken manure. According to Table 12, the amount of tannin compounds increased as manure dosage rose. The application of chicken manure or cow manure with three doses of 20, 40, and 60 t/ha increased the tannin concentration in the rhizome as the dose increased. When compared to cow manure, applying chicken manure produced significantly better results. It is evident that the application of 20 t chicken manure/ha resulted in a higher tannin concentration than that of 60 t cow manure/ha.

Dry rhizomes produced a higher concentration of tannins than fresh rhizomes, which produced different results regarding tannin concentration. The concentration of tannins in fresh rhizomes increased non-significantly with the addition of cow manure, whereas the concentration of tannin compounds in fresh rhizomes decreased with the increase in the dose of cow manure. In comparison to the use of manure that increased fresh and dry rhizomes, the control provided the lowest tannin concentration. Tannins are chemical substances that have an astringent and bitter flavor. These substances act as controlling substances in plant metabolism as well as important defenses against herbivores and pests that prey on plants (Tiku, 2018). Tannins are metabolically active substances with multiple uses, including as astringents, antibacterial agents for treating diarrhea, and antioxidants. Leather tanning is another industrial application for tannins. Tannic acid is the type of tannin substance present in *bangle* plants. Diarrhea can be effectively treated with tannic acid. Additionally, tannic acid exhibits antimicrobial, anti-enzymatic, antioxidant, and antimutagenic properties (Dabbaghi et al., 2019).

In addition to increasing the fertilizer dose, applying organic fertilizer in the form of cow and chicken manure resulted in an increase in phenolic compounds and flavonoids. When compared to when the rhizomes were fresh, the dry rhizomes had a higher concentration of phenolic and flavonoid compounds. The content of active compounds in the simplicial is impacted by the drying process. Antioxidant activity is influenced by the total phenolic and flavonoid content (Rajkumari and Sanatombi, 2020). The highest phenolic and flavonoid concentrations were found in fresh and dry rhizomes when chicken manure was applied at 60 t/ha. Applying cow manure of 60 t/ha resulted in phenolic compound concentrations of 86.90 mg/L in the fresh rhizome and 181.38 mg/L in the dry rhizomes, while flavonoids were 71.14 mg/L in the fresh rhizomes and 99.43 mg/L in the dry rhizomes. Compared to chicken manure at the same dose, this result was smaller. A dose of 60 t chicken manure/ha resulted in a phenolic compound concentration of 178.56 mg/L in the fresh rhizomes and 202.79 mg/L in the dry rhizomes. The concentration of flavonoid in fresh rhizomes was 104.39 mg/L, and that in dry rhizomes was 181.91 mg/L. In plants, flavonoids serve as pigments for the flowers, fruits, and roots and occasionally as growth regulators and disease resistance (Kumar et al., 2018). Catechins are one class of flavonoid compounds present in *bangle* rhizomes (*catechins*). Catechins have antioxidant properties, and because they can stop the growth of viruses, bacteria, tumors, and fungi, they can also get rid of rotten and rancid odors (Isemura, 2019). Phenolic compounds are compounds that plants make in response to environmental stress. Phenolic compounds protect DNA from dimerization and damage by blocking UV-B rays and cell death (Takshak and Agrawa, 2019). Gallic acid is the type of phenolic compound found in *bangle* rhizomes. Gallic acid serves as an antibacterial, antiviral, analgesic, and antioxidant in medicine (Shrinet et al., 2021).

The application of chicken manure resulted in a higher concentration of secondary metabolism because it had a relatively higher P nutrient content than other manure (Alinejad et al., 2020). Phosphorus can be found in DNA, RNA, and the parts of nucleotides that provide metabolic energy (like ATP). The process of photosynthesis depends heavily on phosphorus. Stunted growth is one of the symptoms of phosphorus deficiency signs (de Bang, 2012). The concentration of secondary metabolism in the form of tannins, phenolic compounds, and flavonoids is influenced by the difference in the P nutrient content between these two types of manure. Environmental factors affect the levels of flavonoids and other phenolic compounds in plants, which vary among parts, tissues, and ages of plants. These include air temperature, nutrient availability, water availability, and atmospheric $CO_2$ concentrations (Ashraf et al., 2018).

### *Antioxidants*

The antioxidant activity analysis produced different $IC_{50}$ values depending on the type of organic fertilizer used (Table 13). The $IC_{50}$ decreased as the fertilizer dose increased. A concentration known as $IC_{50}$ is capable of reducing 50% of DPPH free radicals. The greater the antioxidant activity, the lower the $IC_{50}$ value (Ali et al., 2018). Antioxidants are compounds that can absorb or neutralize free radicals, thereby preventing certain diseases caused by free radicals (Poprac et al., 2017). The treatments of cow manure, as shown in Table 13, resulted in lower yields than chicken manure. The antioxidant activity of the dried rhizome samples was higher than that of the fresh rhizomes. The highest antioxidant activity was produced by cow manure at a dose of 60 t/ha, with an $IC_{50}$ value of 9.52 ppm for fresh rhizomes and 8.6 ppm for dry rhizomes. At a dose of 20 t/ha in both fresh and dry rhizome conditions, cow manure had a lower $IC_{50}$ value than chicken manure. For fresh rhizomes, the treatment without fertilizer produced an $IC_{50}$ value of around 53.58 ppm, and for dry rhizomes, it was around 40.91 ppm. Ali et al. (2018) claimed that the antioxidant activity in *bangle* rhizomes is incredibly powerful. Except for the treatment without fertilizer in fresh rhizome conditions, the $IC_{50}$ value in *bangle* rhizomes in all treatments gave a value of 50 and included a very potent antioxidant. The high secondary metabolic compounds found in the *bangle* rhizomes are inextricably linked to the high antioxidant activity. Secondary plant metabolites like flavonoids and phenolics play a part in antioxidant activity. More phenolic compounds will have a higher level of antioxidant activity (Tohma et al., 2017).

## Conclusion

A cow manure dose of 60 t/ha was the best dose for *Z. montanum* growth, with an average height increase of 42.78 cm, an increase in the number of leaves of 116.65 pieces, and an increase in the number of tillers of 14.45. A chicken manure dose of 60 t/ha produced the best root length of 41.03 cm. The weight of the rhizomes revealed that the application of cow manure at a dose of 60 t/ha resulted in the highest yields of dry weight and wet weight, which were about 179.75 g and 822 g, respectively. The application of chicken manure at a dose of 60 t/ha resulted in the highest secondary metabolic content in each parameter, including dry rhizomes (phenolic of 202.79 mg/L, flavonoid of 181.91 mg/L, and tannin of 5406.33 mg/L) and fresh rhizomes (phenolic of 178.56 mg/L, flavonoid of 104.39 mg/L). The application of chicken manure at a 40 t/ha dose resulted in the highest tannin. The application of chicken manure at a dose of 60 t/ha yielded a very antioxidant content of 9.52 ppm in the fresh rhizomes and 8.06 ppm in the dry rhizomes.

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