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Research article

Different Substrates of Animal Wastes and Their Combinations for Quality Improvement of Vermicompost Using Earthworm *Perionyx excavatus*

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Abstract

The use of organic fertilizer to prevent soil degradation has received high attention. Vermicompost is a solid organic fertilizer excreted by the earthworm; however, the content of nutrients is dependent on the substrate used for earthworm feeding. The present study aimed to determine the nutrient content and urease activity of vermicompost derived from animal wastes and their combinations. A greenhouse experiment using a completely randomized design with three substrates: dairy cattle, chicken, and goat manure, and their combinations, and utilizing *Perionyx excavatus* was undertaken, with three replications. The vermicomposting process lasted 60 days, after which the nutrient content was analyzed. The results showed that vermicompost from goat manure had the highest nitrogen (N) content, urease activity, and pH, while chicken manure produced the highest phosphorus levels. The organic carbon (C), potassium (K), calcium (Ca), magnesium (Mg), and C/N ratio were similar across all substrates. The vermicompost from all substrates complied to the quality standards released by the Indonesian and Thai governments, although the carbon content and C/N ratio were below the standard. Future research should focus on combining animal wastes with lignin-rich materials to enhance the organic carbon content and C/N ratio to meet quality standards.

Keywords: vermicompost; animal waste; earthworm; substrate; *Perionyx excavatus*

1. Introduction

Awareness of the community on environmental deterioration and costly synthetic fertilizers have led to the shift of conventional to sustainable agricultural practices. Likewise, excessive and long-term use of synthetic fertilizers has brought about the degradation of soil chemical, physical and biological properties (Fang et al., 2012; Savci, 2012; Coolon et al., 2013; Tetteh, 2015). Sustainable agricultural practice is highly dependent on the availability of organic fertilizer as a source of plant nutrients. The weaknesses of organic fertilizers are that they are low in plant nutrient content and release nutrients slowly. A study

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by Muktamar et al. (2020) noted that available P was increased after 2-3 weeks of the application of solid organic fertilizer.

Vermicompost is a solid organic fertilizer which is the by-product of earthworm vermicomposting process. Vermicomposting converts substrates from different kinds of agricultural and animal wastes into high nutrient organic fertilizers (Gajalakshmi & Abbasi; Mohite, 2024). A range of enzymes, such as urease, phosphatase, dehydrogenase, protease, lipase, chitinase, cellulase, and arylsulfatase, play a crucial role in the bioconversion of organic waste materials into vermicompost (Enebe & Erasmus, 2023). Earthworm excrete contains plant nutrients, soil microbes, and growth hormones (Adhikary, 2012). More than 3000 earthworm species are present in the ecosystem and *Perionyx excavatus* is one of the most suitable earthworms for vermicomposting (Pierre-Louis et al., 2021).

A previous study indicated that different sources produced vermicompost of different characteristics. The content of N, P, K, and C of vermicompost derived from cow dung was 3,6%, 0.23%, 0.89%, respectively (Pierre-Louis et al., 2021). Vermicompost from rice straw contained 16.67% organic-C, 1.31% N, pH 6.6, 0.81% P, 0.54% K, 2.5% Ca, and 0.69% Mg, while that from grass had 20,7% organic-C, 1.41% N, 0.95% P, 0.53% K, 2.07% Ca and 0.75% Mg, and pH 6.2 (Ramnarain et al., 2019). Another study by Bajal et al. (2019) showed that N, P and K contents of vermicompost derived from vegetable waste were 1.85%, 1.32%, and 1.64%, respectively, while those from cattle manure were 1.08%, 1.08%, and 1.04%, respectively. Additionally, vermicompost prepared from goat manure had 1.90% N, 0.90% P, and 3.05% K with a C/N ratio of 18.22 (Anandyawati et al., 2023). Thus, the quality of the fertilizer highly depends on the sources used in the process.

The nutrient content of vermicompost is also dependent on the stage of the vermicomposting process. A study by Aslam et al. (2019) indicated that nutrient content increased as vermicomposting process went on. The content of N, P, and K of vermicompost derived from paper waste increased by 33%, 31% and 70%, respectively, at 180 days of vermicomposting as compared to that of 90 days. The objective of the present study was to characterize the nutrient content and urease activity of vermicompost derived from animal waste using the earthworm *Perionyx excavatus.*

2. Materials and Methods

2.1 Substrate collection and earthworm preparation

Three substrates used in this study included dairy cattle, goat and chicken wastes. Dairy cattle waste was collected from the Closed Agricultural Production System (CAPS) Research Station in Air Duku Village, Rejang Lebong Bengkulu at 1054 m above sea level, while chicken and goat wastes were obtained from Commercial Zone of Animal Laboratory, Faculty of Agriculture, located in Bengkulu at 15 m above sea level. The substrates were air-dried for a day in indirect sunlight. Earthworm *Perionyx excavatus* were obtained from the CAPS Research Station. The dairy cattle waste contained 0.57% N, 0.17% P, 0.26% K, and 46.2% C. The goat waste contained 2.84% N, 0.24% P, 0.82% K, and 33.6% C. Additionally, the chicken waste had 1.13% N, 0.78% P, 1.04% K, and 25.74% C. The worms were pretreated using dairy cattle manure as initial habitat for adaptation from high altitude with lower air temperature to low altitude. The pre-composting period was to avoid the exposure of the earthworms to unfavorable conditions and to provide appropriate conditions for earthworm life. In this study, the pre-composting period was 2 weeks when the worms had adapted to the new environment, which was indicated by their calm behavior.

2.2 Experimental design and treatments

The vermicomposting study was carried out at the Greenhouse of the Department of Soil Science, Faculty of Agriculture, the University of Bengkulu from October to December 2021. The experimental site was in the City of Bengkulu at 25 m above sea level. The experiment used completely randomized design with 7 treatments and 3 replications. The treatments were the different kinds of substrates: dairy cattle (T1), chicken (T2) and goat wastes (T3), the incorporation of dairy cattle and chicken wastes at 2:1 ratio (w/w, T4) and 1:1 ratio (w/w, T5), and the incorporation of dairy cattle and goat wastes at 2:1 ratio (w/w, T6) and 1:1 ratio (w/w, T7).

2.3 Vermicomposting process

The experiment was conducted in a plastic tray (20x40x10 cm) covered with a net to prevent the worms getting out of the tray and to provide good oxygen circulation. Two kg of substrate was gently incorporated with 25 g of *Perionyx excavatus* earthworms. Every other day, 50 g of substrate was added to the tray. The temperature and moisture of the media were monitored every day, i.e. temperature of approximately 25^{o}C and moisture content of 80% using soil thermometer and hygrometer, respectively. Likewise, pH of the media at 1: 5 ratio (w/v) was measured every 2 weeks. After 60 days of incubation, vermicompost was harvested by separating the vermicompost and earthworms using a spatula. A sample of vermicompost was collected for its characterization.

2.4 Characterization of vermicompost

Total organic carbon was analyzed using the Walky and Black Method, nitrogen using Kjeldahl Method, phosphorous detected using spectrophotometer, K, Ca and Mg using atomic absorption spectrophotometer. Urease was also analyzed using a colorimetric method developed by Schinner et al. (1996).

2.5 Statistical analysis

Statistical analysis was carried out using SAS OnDemand for Academic for MacIos. Data were assigned for analysis of variance at 95% confidence level. Treatment means were separated using Duncan Multiple Range Test (DMRT) at 5%.

3. Results and Discussion

3.1 pH and color of vermicompost

The study used cattle, chicken and goat wastes and the combination of cattle waste with either chicken or goat wastes. The experiment results showed that the color of the vermicompost turned darker from week 2 to the end of the vermicomposting process (week 8) as indicated by lower value seen in Table 1. At the 2nd week of the vermicomposting, the color ranged from 7.5 YR; 5/4 to 7.5 YR; 2.5/2 (brown to very dark brown) while at the 8th week, it was 10 Y 2.5/1 to 2.5/N (greenish black to black) (Table 1). At the early process, vermicompost derived from chicken waste (T2) and its combination with dairy cattle waste had brighter color than those from cattle and goat wastes and their combinations. The lighter color of chicken waste dictated the color of its combinations with other animal wastes. Likewise, at the end of the vermicomposting process, the vermicompost color from chicken waste and its combination had brighter color (greenish brown) than those from dairy cattle and goat waste (black). The vermicompost color turning darker as vermicomposting process was indicated by a higher value. This result was associated with the formation of humic substances during the process (Nogales et al., 2005). A study by Hernández-Rodríguez et al. (2012) also confirmed that vermicompost harvested after 16 weeks of decomposition process were darker and odorless. Our study also indicates that each source has slightly different color which might have been due to the differences in the initial substance of each substrate.

Table 1. The color of vermicompost during the vermicomposting process

Treatment	Color			
	2nd week	4th week	6th week	8th week
T1	7.5YR;5/4 (brown)	7.5YR;2.5/1 (black)	10Y2.5/1 (greenish black)	2.5/N (black)
T2	7.5YR;4/6 (strong brown)	7.5YR;2.5/3 (very dark brown)	7.5YR;2.5/1 (black)	10Y2.5/1 (greenish black)
T3	3/N (very dark gray)	5GY;2.5/1 (greenish black)	10 Y 2.5/1 (greenish black)	2.5/N (black)
T4	7.5YR;4/6 (strong brown)	7.5YR;3/2 (dark brown)	7.5YR;2.5/1 (black)	10Y2.5/1 (greenish black)
T5	7.5YR;3/4 (dark brown)	7.5YR;2.5/3 (very dark brown)	7.5YR;2.5/1 (black)	10Y2.5/1 (greenish black)
T6	7.5YR;2.5/3 (very dark brown)	7.5YR;2.5/1 (black)	10Y2.5/1 (greenish black)	2,5/N (black)
T7	7.5YR;2.5/2 (very dark brown)	7.5YR;2.5/1 (black)	10Y;2.5/1 (greenish black)	2,5/N (black)

Analysis of variances shows that vermicompost from dairy cattle waste and its combination significantly differed in pH at 2, 4, 6, and 8 weeks of the incubation, and in content of N, P, and Ca. However, there were no differences in K, Mg, C, and C/N ratio across all substrates (Table 2). The lowest coefficient of variation (CV) was observed in the pH data at the end of the incubation, while the highest CV was found in the magnesium content.

Table 3 indicates that pH for all treatments fluctuated during the vermicomposting process, increased from week 2 to week 4 or 6, but media from the combination of dairy cattle and chicken wastes at the ratio of 2:1 had pH that continuously decreased to week 8. At the end of the process, pH of all treatments consistently decreased. Vermicompost from goat waste (T3) always had highest pH followed by its combination with dairy cattle waste (T6 and T7). At week 8, pH of vermicompost from goat waste (T3) was approximately 20% higher pH than that from dairy cattle waste (T1). Lower pH of vermicompost from the combination of dairy cattle and other animal wastes was related to the pH of dairy cattle waste.

The increase in pH might have been related to the microbial activity as reported by Das et al. (2012) and the decrease might have been associated with the continuously increasing release of organic acid and the production of nitrate at the end of vermicomposting (Ndegwa & Thompson, 2000). The pH range of this study was close to neutral. This result was different from that reported by Ramnarain et al. (2019) where pH vermicompost from rice straw and grass using *Eisenia foetida* decreased to week 4 and then continuously increased to week 8. The difference might have been due to the different vermicompost substrates used and the type of earthworm. But, in another study, Hance & Vasak (2015) noted that pH during the vermicomposting of straw continuously lowered for 5 months. Yadav et al. (2010) reported that a pH increase was obtained in the first week, then continuously decreased to the 6th week of the incubation. Our study also revealed that the pH of vermicompost was dependent on the substrate and that the vermicompost derived from goat waste had greatest pH (7.36) at week 8. Previous studies also confirmed that vermicompost cattle and goat manures had different pHs, in which the pH was higher than that derived from cattle manure (Loh et al., 2005).

Table 2. Analysis of variance for variables observed in the experiment

Variable	Prob (5%)>F	CV (%)
pH2	0.0455	2.45
pH4	0.0247	2.67
pH6	< 0.0001	2.28
pH8	< 0.0001	1.62
N	< 0.0001	7.22
P	0.0349	12.13
K	0.170	18.60
Ca	0.0361	13.71
Mg	0.161	50.92
C	0.583	14.23
C/N	0.594	37.85

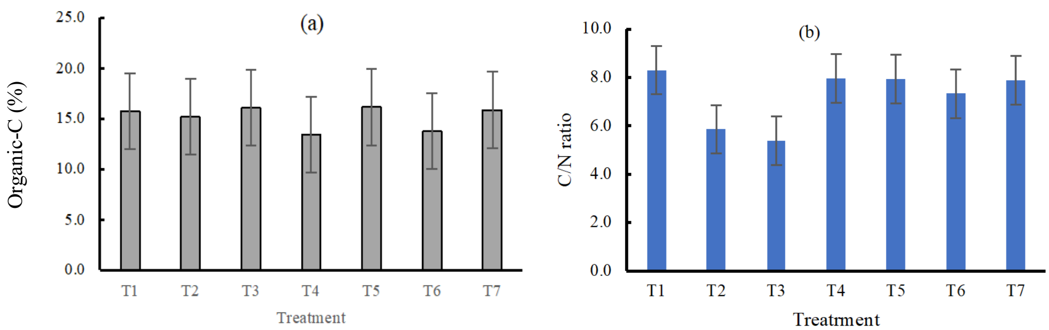
Table 3. pH during the vermicomposting process

Treatment	pH of vermicompost			
	2nd week	4th week	6th week	8th weeks
T1	6.98^{ab}	7.01^{ab}	6.41^{e}	6.15^{e}
T2	6.72^{c}	6.78^{b}	6.81^{cd}	6.56^{cd}
T3	7.12^{a}	7.16^{a}	7.59^{a}	7.36^{a}
T4	7.05^{ab}	6.83^{b}	6.67^{ce}	6.31^{e}
T5	6.79^{bc}	6.99^{ab}	7.00^{bd}	6.51^{c}
T6	6.99^{ab}	7.07^{ab}	7.16^{b}	6.73^{bd}
T7	7.21^{a}	6.83^{ab}	7.15^{b}	6.80^{b}

T1: dairy cattle waste; T2: chicken waste; T3: goat waste; T4: dairy cattle and chicken wastes at 2:1; T5: dairy cattle and chicken wastes at 1:1; T6: dairy cattle and chicken wastes at 2:1; T7: dairy cattle and chicken wastes at 1:1.
Numbers followed by the same letter between rows at the same column are not significant using DMRT 5%.

3.2 Nutrient content of vermicompost

The sources of vermicompost did not significantly affect the organic-C content and C/N ratio of the vermicompost (Figure 1a & b). The content of organic-C ranged from 13.44% for vermicompost derived from the combination of dairy cattle and chicken wastes at 2:1 ratio to 16.10% for that from goat waste. Only vermicompost from the combination of dairy cattle waste with both chicken and goat wastes at the ratio of 2:1 had organic-C lower than 15%. Also, there was no significant difference of C/N ratio among the treatments. Lowest C/N ratio was reached by the treatment of goat waste (T3) while the highest was achieved by dairy cattle waste (T1). C/N ratio of vermicompost from the animal waste combination was apparently related to the that of dairy cattle. C/N ratio of the vermicompost from all sources was lower than 12, indicating that is likely ready for soil application.



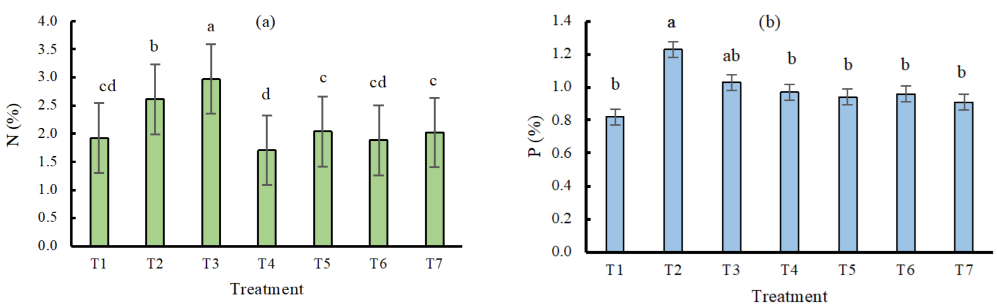
Note: T1: dairy cattle waste; T2: chicken waste; T3: goat waste; T4: dairy cattle and chicken wastes at 2:1; T5: dairy cattle and chicken wastes at 1:1; T6: dairy cattle and goat wastes at 2:1; T7: dairy cattle and goat wastes at 1:1.

Figure 1. Effect of animal waste sources on organic-C (a) and C/N ratio (b) of vermicompost

The nitrogen and phosphorous contents of the vermicompost were significantly different among sources of animal wastes and their combinations. Figure 2a presents the nitrogen content of the vermicompost in this study. Vermicompost from goat waste (T3) had the highest N content (2.98%) while the lowest was attained for the combination of dairy cattle and chicken wastes at the ratio of 2:1 (T4, 1.71%). The increase in N content between both treatments was more than 74%. There were no significant differences among other treatments. Nonetheless, the addition of chicken and goat wastes to dairy cattle waste did not stimulate an increase in N of the vermicompost. The higher nitrogen content in vermicompost derived from goat waste was attributed to the initially higher nutrient level in the substrate. In general, the nitrogen content in vermicompost derived from animal wastes increased when compared to its initial levels.

Vermicomposting involves enzymatic and microorganism activity in the gastric of the earthworm, which releases nutrients and CO_2 and leads to the reduction of organic carbon (Parthasarathi et al., 2016). This study showed that there were no significant differences among the animal waste treatments and their combinations in terms of organic-C. Using water hyacinth substrate, Manyuchi et al. (2019) also found that organic-C decreased during the 40 days of vermicomposting. However, N content was greatest for vermicompost derived from goat waste. Nitrogen is the result of the mineralization process of organic matter including the ammonification and nitrification process (Benbi & Richer, 2002). The breakdown of substrates by microorganisms releases simple compound but only 5-10% is absorbed by the tissues of the worms and the rest is extracted as vermicompost (Kaur, 2020). The different content in chemical composition of vermicompost is dependent on different substrates since each substrate has different characteristics and can differently affect the performance of worms (Manaig, 2016). This result was in agreement with that presented by Nath et al. (2009) where goat dung produced vermicompost with highest N compared to cattle and buffalo dungs. Another result by Raza et al. (2019) also indicates that highest N content was vermicompost from pig manure, followed by cow manure. In our study, the significant difference of N content in vermicompost was not reflected in the C/N ratio. This might have been related to the content of organic-C rather than the N content in the vermicompost. Other studies showed that C/N ratio of vermicompost did not differ among substrates (Parthasarathi et al., 2016; Ro et al., 2022).

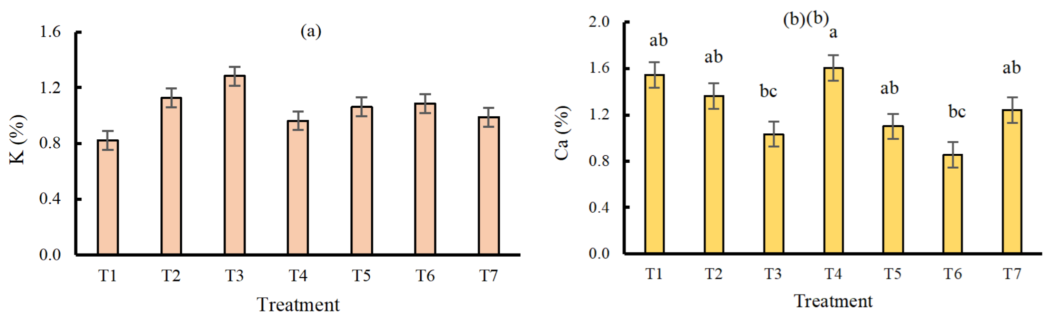
Unlike the N content, the highest P in vermicompost was attained from chicken waste (T2), followed by that from goat waste (T3). The increase in P between both treatments was more than 50% compared to the lowest P content, which was from cattle waste (T1) (Figure 2b). Also, the P content in vermicompost from chicken waste did not differ from goat waste. The study also showed that the combination of dairy cattle waste with other animal wastes offered significant increase in P content of the vermicompost. The phosphorous content in vermicompost derived from animal waste was significantly higher than the initial amount, with an average increase exceeding 150%. Previous studies indicated that chicken manure had a higher content of P than goat and cattle manures (Almeida et al., 2019; Situmeang et al., 2019). Consequently, the P content of vermicompost from the former manure was higher than the latter.



Note: T1: dairy cattle waste; T2: chicken waste; T3: goat waste; T4: dairy cattle and chicken wastes at 2:1; T5: dairy cattle and chicken wastes at 1:1; T6: dairy cattle and goat wastes at 2:1; T7: dairy cattle and goat wastes at 1:1. Histograms followed by the same letter are not significantly different at 5% level.

Figure 2. Nitrogen (a) and phosphorous (b) contents of vermicompost from animal wastes

The study also showed that the K content of vermicompost was comparable among the sources of animal wastes and their combinations, as indicated in Figure 3a. Potassium content ranged from 0.18% in vermicompost from dairy cattle waste (T1) to 1.28% in that from goat waste (T3). A similar result was observed for the Ca content of vermicompost (Figure 3b). On average, the K content in vermicompost prepared from animal wastes was 52% higher than the initial content. There were significant effects of



Note: T1: dairy cattle waste; T2: chicken waste; T3: goat waste; T4: dairy cattle and chicken wastes at 2:1; T5: dairy cattle and chicken wastes at 1:1; T6: dairy cattle and goat wastes at 2:1; T7: dairy cattle and goat wastes at 1:1. Histograms followed by the same letter are not significantly different at 5% level.

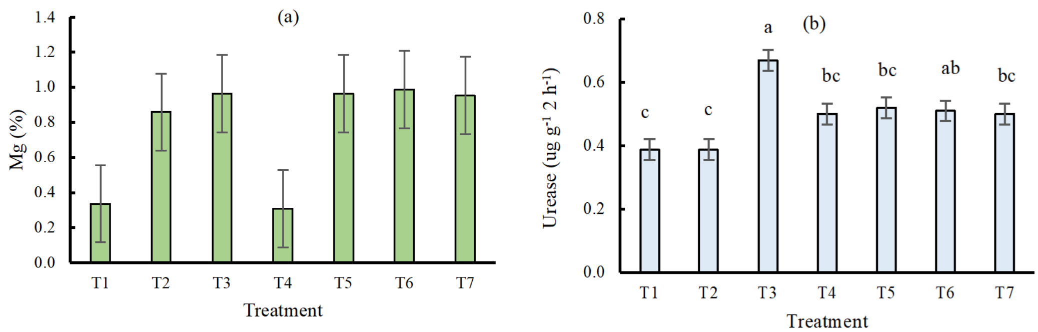
Figure 3. The content of potassium (a) and calcium (b) of vermicompost from animal wastes

vermicompost sources on Ca content. The percentage of Ca ranged from 0.85% attained by the combination of dairy cattle and goat wastes at the ratio of 2:1 (T6) to 1.61% for the combination of dairy cattle and chicken wastes at the ratio of 2:1 (T4).

Figure 4a shows the insignificant effect of animal sources and their combinations on the Mg content of vermicompost. The magnesium content of vermicompost ranged from 0.12% to 1.81%. Vermicompost resulting from the combination of dairy cattle and chicken wastes at the ratio of 2:1(T4) tended to have lower Mg content than the combination of dairy cattle and goat wastes at the ratio of 1:1 (T7). The results of the study also indicated that the greatest urea activity in vermicompost originated from goat waste (Figure 4b). The urease activity in vermicompost from goat waste was 72% higher than those from chicken or dairy cattle wastes. The combination of dairy cattle and other animal wastes did not provide significant effect on the urease activity.

The increase in urease activity in vermicompost might have been associated with the continuation of the humic substance supply, leading to stabilization and resistance to microbial biodegradation (Pramanik et al., 2007). Even though organic-C content was not different among the substrates, the N mineralization of chicken manure was faster than cattle manure, as reported by Li & Li (2014), indicating a higher activity of urease in the treatment.

The nutrient contents of the vermicompost from all animal wastes and their combinations were not significantly different, but there were significant differences in the N, P, Ca contents and pHs of the vermicompost. The N, P, K content, and the pH and color of all vermicompost complied with the quality standard of solid organic fertilizer released by Ministry of Agriculture, Republic of Indonesia (2011) as well as by Ministry of Agriculture and Cooperatives (2005). Nonetheless, C/N ratio and organic-C content of the vermicompost did not comply with the quality standards and were lower than standard. The combination of the animal wastes with substrates containing higher lignin such as weeds or agriculture wastes is suggested to increase the content of organic-C in order to meet the quality standards.



Note: T1: dairy cattle waste; T2: chicken waste; T3: goat waste; T4: dairy cattle and chicken wastes at 2:1; T5: dairy cattle and chicken wastes at 1:1; T6: dairy cattle and goat wastes at 2:1; T7: dairy cattle and goat wastes at 1:1. Histograms followed by the same letter are not significantly different at 5% level.

Figure 4. The content of Mg (a) and urease activity (b) in vermicompost from animal wastes

4. Conclusions

Vermicompost derived from goat waste exhibited the highest pH, N content, and urease activity, while chicken manure-derived vermicompost had the most P content. Vermicompost from various animal wastes showed similar levels of K, Ca, and Mg. The quality of vermicompost from all animal wastes and their combinations complied with the solid organic fertilizer standards set by the Indonesian government and Thailand, though the organic-C content and C/N ratio were below the required levels. The nutrient content of vermicompost produced from different ratios of substrate combinations was comparable. The quality of vermicompost was highly dependent on the sources of substrates supplied for *Perionyx excavatus*. Future research should explore using a combination of animal and agricultural wastes or lignin-rich weeds as substrates to enhance the C content to meet quality standards.

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6. Conflicts of Interest

The authors declare that they have no conflict of interest regarding the research, authorship or publication of this article.

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