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The quality of vermicast from biotransformation of different organic substrates using *Lumbricus rubellus* and *Perionyx excavates*

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Absract The study discovered that substrates from farming activities and weeds offered a different quality of vermicast produced using *Lumbricus rubellus* and *Perionyc excavatus*. All substrates in the bio-convertor bin had similar temperatures and humidity; however, the pH was significantly different, where goat substrate exhibited the highest pH during eight weeks of vermicomposting. Substrate from cattle waste, fermented Melastoma, and rice straw yielded comparable vermicast production, but that from goats had lower production. The production of vermicast using Perionyx was greater than Lumbicus. Even though the yield of vermicast from goat substrate was the lowest, it had the highest P, K, Mg, and Fe contents. The contents of N and Ca were comparable in vermicast produced from goat and Melastoma substrates. Besides, Cu and Zn were higher in vermicast from animal substrate than plant residues. The contents of organic C and Pb were similar among all substrates. Both Lumbricus and Perionyx produced similar quality of vermicast. Melastoma weed is a prospective substrate for earthworm biotransformation to produce nutrient-rich organic fertilizer. The study further reveals that epigeic species worms, Lumbricus rubellus and Perionyx excavatus are equally suitable for the biotransformation of agricultural wastes and weeds.

Keywords: Vermicast, Biotransformation, Vermicomposting, *Lumbricus rubellus*, *Perionyc excavatus*

Introduction

Abandoning solid organic wastes from livestock and agricultural activities has contributed to environmental problems. Alshehrei and Ameen (2021) projected a significant increase in global waste, where in 2050 will reach approximately 3.5 billion tons from 2 billion tons in 2016. Unwise management of solid organic wastes will negatively impact public health and

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environmental sustainability (Abubakar *et al*., 2022). Likewise, the abundance of weeds has caused a significant reduction in crop production. Crop yield lowered by up to 28% due to weed invasion (Vila *et al*., 2021), competing for nutrients and light. Environmental deterioration is also associated with invasive weed species (Devi and Khwairakpam, 2023). Leftover livestock, agricultural by-products, and weed biomass might be potential substrates for vermicomposting to recycle soil nutrients.

Vermicomposting is a biotransformation involving microorganisms and earthworms to convert organic materials into nutrient-rich-organic fertilizers. This process distinguishes from composting, in which earthworms facilitate the microbial action in the substrates during biotransformation (Sharma and Garg, 2018; Hussain and Abbasi, 2018; Vukovic *et al*., 2021). Microorganisms have a role in the biodegradation of organic matter during vermicomposting. At the same time, earthworms are significant in breaking down organic materials to increase the surface area exposed to microorganisms, consequently promoting microbial activity (Dominguez *et al*., 2003). During vermicomposting, earthworms convert a partial organic substrate into their biomass and respiration product and expel the remaining by-product, known as vermicast/vermicompost (Suthar, 2007). This organic fertilizer is rich in macro and micronutrients, minerals, vitamins, enzymes, hormones, and valuable microorganisms (Vega, 2016; Olle, 2019).

Earthworms can transform organic residue and enhance organic materials' biological and chemical characteristics. *Lumbricus rubellus* and *Perionyx excavatus* are commonly used worldwide for vermicast production. Lumbricus is an epigeic species, often found in the upper layer of soil, compost, and leaf litter, and actively gizzard with high reproduction (Ahmad *et al*., 2021). Perionyx is also epigeic species native to Asia and lives best in temperatures 25-30 ^{o}C with increased production of cocoons (Edwards *et al*., 1998). The quality of vermicast highly depends on both types of worms and substrates. Mahanta and Jha (2009) confirmed that vermicast derived from various agricultural waste and weeds using three indigenous earthworms contained significantly different quality of plant nutrients. Likewise, Vodounnou *et al*. (2016) found that vermicast from sheep waste substrate had the highest nitrogen content compared to rabbits, cows, pigs, and poultry. Getachew *et al*. (2016) tested various substrates in vermicast production and concluded that nutrient content depends on the substrates' chemical and biological composition.

The study aimed to determine vermicast quality produced from agricultural wastes and weeds substrate using epigeic earthworm *Lumbricus rubellus* and *Perionyx excavatus*.

Materials and methods

Earthworm culture and substrate preparation

Earthworm species *Lumbricus rubellus* and *Perionyx excavatus* were collected from Closed Agricultural Production System (CAPS) Research Station in Air Duku Village, Bengkulu, Indonesia, at 1054 m above sea level. Each species was cultured in cow manure at a 4x4x0.5 m cemented block for two weeks for new habitat adaptation until calm behavior. The culture was maintained moist during the adaptation, and sufficient feed was provided. Cattle and goat wastes were gathered from a farmer-owned farm nearby the CAPS Research Station. The feces (consisting of animal excreta and discarded animal feed) were incubated for two weeks for pre-composting to avoid the death of the earthworm. Similarly, the rice straw was collected from a farmer's paddy field in Pekik Nyaring Village, Central Bengkulu, approximately 15 m above sea level. At the same time, Melastoma was mobilized from nearby the farmer's field. Both organic materials were copped and fermented in a composting bag for four weeks. The effective microorganism was homogenously dispensed to the bag to accelerate the fermentation. The cattle waste contained 36.2% total carbon (C), 1.57% nitrogen (N), 0.17% phosphorous (P), and 0.26% potassium (K) with a C/N ratio of 23.2 while the goat waste had 33.7% C, 2.84% N, 0.24% P, 0.82% K and C/N ratio of 11.9. The fermented rice straw comprised 36.4% C, 1.62% N, 0.03% P, and 0.60% K with a C/N ratio of 22.5, while those of fermented Melastoma were 39.12%, 3.60%, 0.60%, 0.95%, and 10.8, respectively.

Experimental design and procedure

The study was located in the Greenhouse, the University of Bengkulu, assigning Completely Randomized Design (CRD) with two factors and three replications. The first factor consisted of 4 different vermicomposting substrates, i.e., cattle, goat wastes, rice straw, and *Melastoma malabathricum*. In contrast, the second factor was two epigeic earthworm species, *Lumbricus rubellus*, and *Perionyx excavatus*.

Vermicomposting experimentation used plastic bins (44x32x15 cm) for earthworm culture. Three kg of substrates was put into the bin, and 25 g of earthworm was evenly placed on the media. As a starter, cow manure was incorporated with fermented Melastoma or rice straw at a ratio of 1:3. The bin was covered with a fine screen to prevent earthworms from leaving the reactor. The vermicomposting bin was randomly placed on a 150 cm high wooden rack. The vermicomposting lasted for eight weeks. 100 g of the substrate was added to the cultural bin every other day. However, unlike the first addition of substrate, the fermented Melastoma or rice straw was not mixed with cow manure until the vermicomposting end. During the vermicomposting, the media was maintained moist by watering when required. The media was monitored weekly for temperature, humidity, and pH.

At the end of vermicomposting experimentation, vermicast and fresh earthworm were collected to determine the vermicast production and the weight of the earthworm. Vermicast was analyzed for total carbon (C), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and lead (Pb). The ratio of C/N was calculated from carbon and nitrogen content in vermicast. The productivity of vermicast was the percentage of yielded vermicast from the total substrate added to the reactor bin during the vermicomposting.

Statistical analysis

Data were analyzed for variance using SAS University Edition at a probability level of 5%, and separation of treatment means using DMRT at 5%.

Results

Temperature, humidity, and pH of the vermicomposting media

Temperature, moisture content, and pH of the media are essential for earthworm life. The study resulted that media temperature varied during the biotransformation process, ranging from 25.9 to 28.8 ^{o}C. The temperature tended to increase at weeks 2-4, then decrease at week 6. Temperature was insignificantly different among substrates and earthworm species (Figures 1a and b). The humidity of media ranged from 90.4 to 97.2% during biotransformation, insignificance among different substrates and earthworm species. There was a decrease of medium humidity at week 3, but it continuously leveled off afterward. Humidity at weeks 5, 6, and 7 tended to be higher for medium with Perionyx than Lumbricus (Figures 1c and d).

The pH of the media also varied during the vermicomposting process, increasing at week 3 and leveling off from week 4 to the end of the process. There was a significant difference among vermicomposting substrates but not between the earthworm species. The substrate from goat wastes consistently exhibited the highest pH during eight weeks of vermicomposting, while Melastoma was the lowest (Figures 1e and f). The pHs of media from cattle waste, Melastoma, and rice straw were not significantly different. At week 8, the pH of the substrate from goat waste is 14.1% higher than that of rice straw.

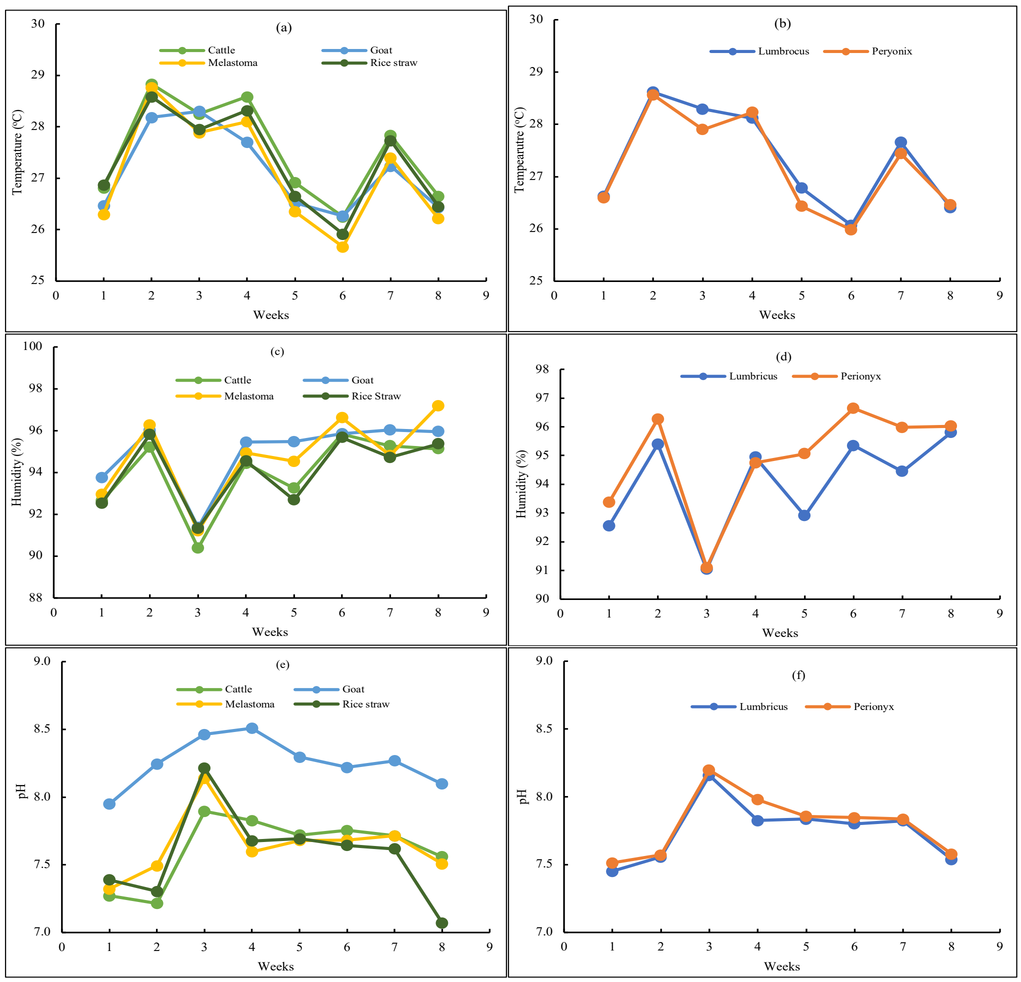

Figure 1. Temperature, humidity, and pH during the biotransformation period

Vermicast production

Bioconversion and vermicast production are strongly associated with substrate quality, mainly the chemical and biological composition. This study resulted that vermicast production depends on its sources. Substrate from cattle waste reached the highest vermicast production using Lumbricus or Perionyx earthworm. Still, it did not differ from Melastoma and rice straw, while the lowest was from goat waste. Generally, vermicast production by Perionyx was higher than that of Lumbricus (Figure 2a). The weight of fresh worms after eight weeks of bioconversion was most significant in the substrate from cattle and Melastoma, while the lowest was when cultured in goat waste. The weight of fresh worms for both substrates was higher than that of the original weight. In contrast, the weight of fresh worms from goats decreased significantly, almost 2.5 folds lower than the original weight. The weight of Lumbricus was slightly higher than Perionyx (Figure 2b).

The productivity of worms was highest in the substrate from cattle waste, but it was not significantly different from rice straw. On the other hand, the lowest productivity was when the worm was cultured in goat substrate. In general, Lumbricus had similar productivity with Perionyx, even though using substrate from goat and Melastoma, the productivity of Perionyx was slightly higher (Figure 2c). The moisture content of vermicast was similar among substrates and types of earthworms; however, moisture content tended to be higher in goat substrate using Perionyx (Figure 2d).

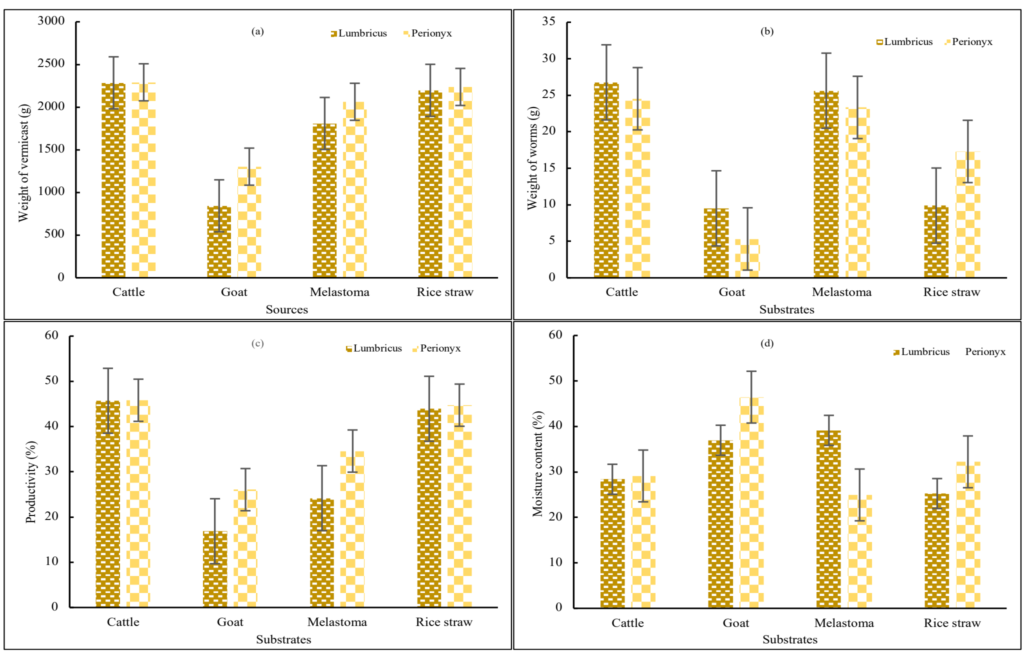

Figure 2. Vermicast production after biotransformation of substrates using Lumbricus and Perionyx

Nutrient content of vermicast

The chemical composition of the vermicast was assessed after eight weeks of vermicomposting. Organic carbon of the vermicast did not differ among substrates, ranging from 194.7 to 214.5 g kg^{-1}, and between types of earthworms, ranging from 196.2 g kg^{-1} for Perionyx to 214.7 g kg^{-1} for Lumbricus (Figure 3a). However, N content was the highest in vermicast derived from Melastoma and the lowest in that from cattle. The nitrogen content of vermicast from Melastoma is approximately 1.2 folds more elevated than that from rice straw. Both earthworm types produced comparable N content (Figure 3b). The ratio of C/N is closely related to the decaying resistance. Vermicast from rice straw had the highest C/N ratio, while that from Melastoma was the most minor (Figure 3c).

Phosphorous content was significantly different among substrates but comparable between the types of earthworms. Vermicast from goat substrate had the highest P content compared to other substrates, being produced by Perionyx had higher P content than Lumbricus. Meanwhile, P content was comparable among substrates from cattle, Melastoma, and rice straw (Figure 3d). Vermicast using goat substrate exhibits two folds with greater P content than rice straw. A similar trend to P content, vermicast from goat substrate provided the highest K, followed by Melastoma substrate, while the lowest was that from rice straw. There were no substantial differences in K content in vermicast using both earthworms. However, the K content of vermicast from goat substrate using Perionyx tended to be higher than Lumbricus (Figur 3e). Vermicast from goat substrate had the highest Ca content, which did not significantly differ from Melastoma, while the smallest Ca content was from rice straw. Also, the Ca content of vermicast produced by both earthworms was comparable (Figure 3f).

Among the substrates tested, vermicast produced from goat substrate possessed the greatest Mg content, but the other three substrates were not significantly different. The magnesium content in vermicast of goat substrate is approximately two folds higher than those of cattle, Melastoma, and rice straw substrates. However, the content of Mg in vermicast did not differ between those produced by Lumbricus and Perionyx (Figure 4a).

Besides plant macronutrients, vermicast contains micronutrients essential for plant growth. Figure 4b shows that the highest content of Fe was achieved in vermicast resulting from goat substrate, while the least was by Melastoma. Iron content in vermicast from goat substrate tended to be higher when produced by Lumbricus than Perionyx, but that from rice straw was the reversal. Figure 4c indicates that Zn content in vermicast from animal waste substrates was higher than from plant residues. Zinc content in vermicast from cattle is comparable to goat substrate, as does from Melastoma and rice straw. In goat substrate, Lumbricus produced vermicast with slightly higher Zn content than Perionyx.

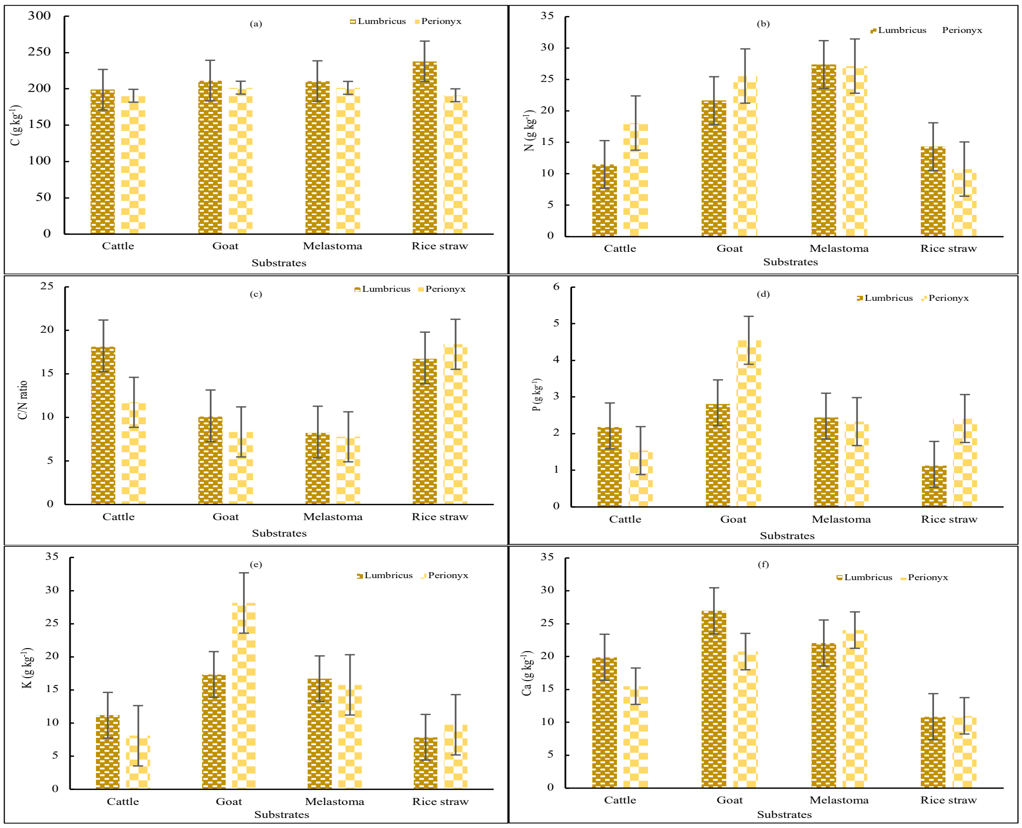

Figure 3. Carbon, nitrogen, phosphorous, potassium, calcium content, and C/N ratio of vermicast

Moreover, Mn content was the utmost in vermicast developing from Melastoma substrate, followed by cattle, rice straw, and goat substrates. This element increases by more two folds in Melastoma compared to goat substrates. The content of Mn in vermicast produced by Lumbricus was comparable to that by Perionyx (Figure 4d).

The study also indicates that Cu content was significantly different among substrates. Vermicast resulting from cattle substrate had the greatest Cu, followed by goat, and the lowest was that of rice straw. On average, the copper content of vermicast from cattle substrate reached 76.5 mg kg^{-1}, merely 1.75

folds higher than rice straw's. However, there were no differences in Cu content between the types of earthworms (Figure 4e). The lead content of vermicast did not significantly vary among substrates or between Lumbricus and Perionyx, even though Pb content in vermicast from goat substrate produced by Lumbricus was higher than Perionyx (Figure 4f).

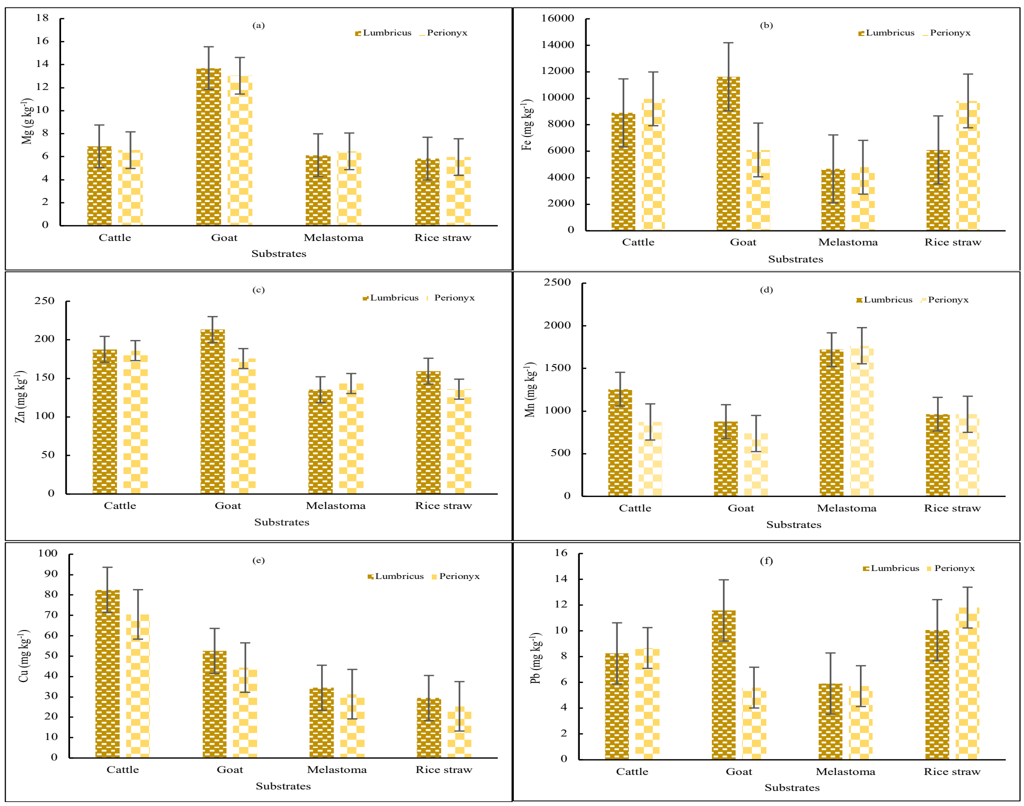

Figure 4. Magnesium, iron, zinc, manganese, copper, and lead content of vermicast

Biological properties of vermicast

Biological reaction during the vermicomposting process is essential to produce good quality vermicast. The study showed that humic acid in vermicast was not significantly different among substrates and between earthworms, ranging from 0.73% to 0.86%. Vermicast from goats using Perionyx had higher humic acid than Lumbricus (Figure 5a). Figure 5b presents the fulvic acid content in vermicast from various substrates using Lumbricus and Perionyx worms. The results indicate that fulvic acid was not prominently different among substrates tested in this study. Fulvic acid ranged from 0.99% to 1.27%.

An enzymatic reaction in vermicomposting also involves the activity of urease, acid phosphomonoesterase (acid PMease), and alkaline phosphomonoesterase (alkaline PMease). Alkaline PMease activity was higher than acid PMease. The activity of acid PMease was highest in the Melastoma substrate, reaching 5.08 uP g^{-1} h^{-1}, but it did not differ from that in rice straw substrates. This enzyme activity in vermicast developed by Lumbricus was not different from Perionyx (Figure 5c). A similar trend was observed in the activity of alkaline PMease, where Melastoma and rice straw substrates provided a similar environment for this enzyme but better than cattle and goat substrates. Both earthworms also offered a similar milieu for the activity of this enzyme (Figure 5d). Urease activity differed among substrates, where goat substrate provided the lowest activity compared to other substrates. The urease activity in vermicast resulting from Lumbricus was higher than Perionyx, as indicated in Figure 5e.

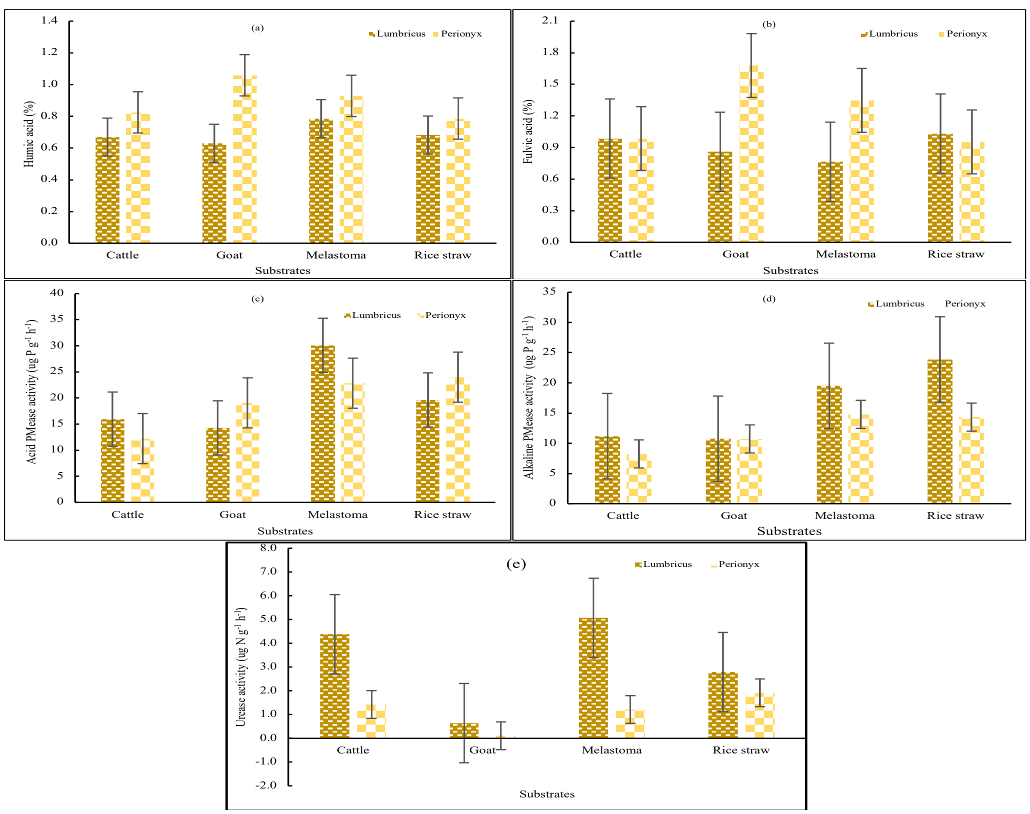

Figure 5. Humic and fulvic acids content of vermicast and enzymatic activities

Discussion

During eight weeks of vermicomposting, the temperature and humidity of the media in the bio-convertor bin varied but did not differ among all substrates. The temperature and humidity are suitable for earthworm living, as Edwards *et al.* (1998) suggested, where Perionyx excavatus has good growth performance at 20-30 ºC. In this study, the temperature of the media ranged from 25.9-28.8 ºC. Another study also indicated that temperature media for vermicomposting ranges from 27.37-27.48 ℃ (Darmawan *et al*., 2023). According to Thejesh (2020), a body weight of an earthworm comprises 75-90% of water; therefore, its living requires sufficient moisture for growth and development. In this study, the humidity of the media was suitable for earthworm living.

The pH of the media increased in the 3rd week and leveled off after 4th week. The increase in pH at an early stage of vermicomposting might be attributed to microbes participating in aerobic metabolism, forming basic hydroxides, and increasing substrate pH (Singh *et al*., 2005). The decrease in pH after 3rd week might be due to the formation of organic acids, which might be dominant compared to the formation of hydroxides. A similar result was observed by Atiyeh *et al.* (2000), where the pH of cow manure substrate significantly increases during the first two weeks of vermicomposting and decreases in 4th week. The pH of goat substrate was constantly highest during eight weeks of vermicomposting, while those of Melastoma and rice straw were the lowest. This result might be associated with the feed of the goat. High pH in the goat substrate, in some ways, will affect the growth of the earthworm.

The yield and productivity of vermicast from goat substrate were the smallest compared to the other substrates. This result is related to the pH substrate. Earthworm prefers substrate with a pH of approximately neutral, decreasing its development at a pH over 8.0 (Hou *et al*. 2005). In this study, the pH substrate from goat waste had a pH of over 8.0 during 8 weeks of vermicomposting. A pH influences environmental conditions for microbial growth and survival and can inhibit microbial metabolism. Environmental pH can influence the energies of microbial redox reactions (Jin and Kirk, 2018). Besides pH, the texture of the substrate may also affect the productivity of earthworms. In this study, goat substance has a coarse texture and firm structure. Earthworms may not like this substrate due to its hardness, causing restricted growth. Our analysis indicates that the productivity of earthworms in goat substrate is approximately two folds lower than other tested substrates. Also, the fresh worm in this substrate is 2.8 times lower than the original.

Our study shows that C-organic, after eight weeks of vermicomposting, is drastically lower than the original content of the substrate. Biodegradation of organic material by microorganisms causes the transformation of the complex organic compound into an inorganic one, indicated by a decline in the C/N ratio. The availability of carbon in the substrate leads to rapid decomposition during the bioconversion process, releasing CO_2 into the atmosphere (Devi and Khwairakpam, 2023). Our study revealed that the reduction of C-organic of Melastoma, cattle, rice straw, and goat substrates is 89.9%, 86.1%, 69.7%, and 63.1%, respectively. Likewise, the C/N ratio of substrate decreased significantly after eight weeks of vermicomposting. On average, the reduction of the C/N ratio in cattle substrate is 55.3%, followed by Melastoma at 34.9%, goat at 29.1%, and rice straw at 28.0%.

The nutrient composition of vermicast is significantly different among substrates tested in this study. The yield of vermicast from goat substrate is the lowest, but it has the highest P, K, Mg, and Fe content. Moreover, N and Ca in vermicast resulting from goat substrate are similar to Melastoma substrate. The contents of Cu and Zn are higher in vermicast produced from the animal substrate than in plant residue. However, Mn and Pb contents are similar among substrates used in this study. The different content of nutrients might be attributed to the variation of the initial nutrient content of substrates. Melastom substrate has the highest initial content of N, and the vermicast from this substrate also has the highest content. Some studies also concluded similar results where the concentration of nutrients in vermicast varies among tested substrates, environmental pH, temperature, and moisture content (Lim *et al.,* 2012; Zhou *et al*., 2021; Banupriya *et al*., 2022; Kumar *et al.,* 2023). In this study, the quality of nutrients in vermicast produced by Lumbricus is comparable to Perionyx except for Zn content where Lumbricus developed vermicast contains higher one. Purwanto *et al*. (2020) observed that vermicast developed using *Lumbricus rubellus*, *Eisenia fetida*, and *Eudrilus eugeniae* has comparable C, N, and C/N ratios. These results imply that nutrient content in vermicast differs among substrates used for vermicomposting.

Digestion of organic matter in earthworm gut with microorganism produces stabilized compounds such as humic and fulvic acids. In this study, humic and fulvic acids are similar among vermicast from all substrates; however, vermicast has higher fulvic than humic acids. Wani *et al*. (2013)

concluded a similar result that humus content in vermicast produced from chicken waste does not differ from that of cow dung. In contrast, Pramanik *et al*. (2007) reported that cow dung-based vermicast has higher humic acid than those grass, aquatic weed, and municipal solid waste.

Microorganism activity plays a crucial role in converting organic matter during vermicomposting. In this study, microbial activity is designated by enzyme activity, primarily urease, acid, and alkaline PMease. The most significant urease, acid, and alkaline PMease activity was reached in vermicast from the Melastoma substrate, and the lowest was from the goat substrate. The types of substrates significantly affect microbial activity during the decomposition of organic material, consequently, the performance of earthworms (Sanchez *et al.,* 2017). In our study, urease, acid, and alkaline PMease activities in vermicast from goat substrate were the lowest, leading to the lowest productivity of earthworms.

In summary, this study confirmed that vermicast quality differs among substrates from cattle, goat, Melastoma, and rice straw substrates. The greatest yield of vermicast was achieved in Melastoma substrate, but it was not different from cattle and rice straw, while the lowest was that from goat. However, the vermicast from goat substrate had the highest P, K, Mg, and Fe. In addition, N and Ca contents did not differ between those resulting from goat and Melastoma substrates. The quality of vermicast using Lumbricus was comparable to Perionyx. Instinctive biochemical characteristics of vermicast may be used to stimulate sustainable agriculture and to manage agricultural residues and weeds.

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