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Utilization of *Hydrilla* Through Earthworm Cultivation to Enhance Amino Acid Content and Promote Waste Recycling

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ABSTRACT

This study examines the valorization of the invasive aquatic weed *Hydrilla verticillata* through earthworm (*Eisenia fetida*) cultivation, highlighting improvements in amino acid profiles and sustainable waste recycling. Earthworms cultured on Aquatic weed *Hydrilla verticillata* displayed significantly elevated concentrations of several essential amino acids compared to control worms. Notably, lysine increased by 40% (from 4.5 ± 0.17 to 6.3 ± 0.66 g/100 g), leucine by 57.14% (from 4.2 ± 0.12 to 6.6 ± 0.46 g/100 g), and isoleucine by 70.37% (from 2.7 ± 0.70 to 4.6 ± 0.32 g/100 g). Among non-essential amino acids, glutamic acid showed a 54.5% rise (from 4.4 ± 1.00 to 6.8 ± 0.78 g/100 g), and alanine increased by 32.1% (from 2.8 ± 0.34 to 3.7 ± 0.36 g/100 g). The dominant essential and non-essential amino acids were leucine and glutamic acid, respectively. These augmentations are credited to the protein-rich nature of Hydrilla, suggesting that worms cultured on this weed can serve as high-quality protein sources for livestock feed. Vermicomposting of Hydrilla aids in managing its ecological overgrowth, turning a challenging weed into a value-added contribution for organic waste recycling and sustainable animal nutrition.

Keywords: *H.verticellata*, *Eisenia fetida*, Vermicompost, Amino acid Profile.

INTRODUCTION

In earthworms, the highest essential amino acid content is often dominated by histidine (0.63% on a dry matter basis), while earthworm meal typically shows the highest levels of isoleucine (1.98% on a dry matter basis) (Padmavathi et al., 2017). Tram et al. (2005) reported that the highest essential amino acid in *Perionyx excavatus* was leucine (3.47% and 0.76% of dry matter basis, respectively). Regarding non-essential amino acids, glutamic acid dominated the composition in both earthworms and earthworm meal (1.52% and 3.60% on a dry matter basis, respectively).

Julendra (2003) demonstrated that *Lumbricus rubellus* meal contains 65.63% crude protein (Damayanti et al., 2008), while *Lumbricus terrestris* meal contains 32.60% protein, and *Perionyx excavatus* meal contains 57.2% crude protein along with a complete amino acid profile (Tram et al., 2005). Additionally, *L. rubellus* produces "lumbricin I," an antimicrobial peptide composed of 62 amino acids, contributing to its medicinal properties (Salzet et al., 2006).

Earthworms can serve as an excellent source of essential amino acids, particularly lysine, which is often a limiting amino acid in many staple foodstuffs (Albarran, 1996). The lysine content in earthworm flour meets the daily requirement for children aged between 2 and 5 years. The amino acid composition of earthworm meal closely resembles that of fishmeal and is potentially superior to meat meal. It contains all essential amino acids, including phenylalanine, leucine, lysine, methionine, and valine (Gabriel and Dedeke, 2010).

Hydrilla infestations can alter the physical and chemical characteristics of water bodies. Due to its multiple competitive strategies, *Hydrilla* has been called the “perfect aquatic weed.” Once established, its turions (specialized vegetative propagules) are extremely difficult to eradicate (Franck and Michael, 2005). Abdelhamid & Gabr (1991) reported that Hydrilla can alter dissolved oxygen levels, especially at night when respiration exceeds photosynthesis, leading to hypoxic conditions and fish kills. Sakthika et al. (2014) noted in a study on Tamil Nadu’s inland waters that dense Hydrilla mats reduced oxygen levels, affecting fish like *Mystus montanus*.

Vermicomposting has emerged as a sustainable method for managing *Hydrilla* biomass. This biological process not only aids in decomposing the invasive plant but also results in the production of vermicompost. The study aimed to analyze amino acid enrichment in earthworms by feeding with hydrilla. The study proposed an integrated solution that addresses organic waste utilization and the ecological threat posed by Hydrilla infestations.

MATERIALS AND METHODS

Earthworms and Substrates: Earthworms (*Eisenia fetida*) were obtained from a culture bank maintained at DCW Ltd., Arumuganeri, Thoothukudi District. Aquatic weed (*Hydrilla verticillata*) was collected from the Thamirabarani River near Eral. Garden soil was used as the control substrate. Cow dung was obtained from a local farmyard.

Experimental Design: The aquatic weed (*Hydrilla verticillata*) was air-dried, cut into small pieces, and mixed with cow dung (nutrient mixture) on a dry weight basis in a ratio of 5:1 for the experiment. This mixture was pre-decomposed for 15 days to make it palatable for the earthworms. The compost was prepared in a wooden box measuring 3 feet in breadth and 2 feet in height. A thin layer (1.5 cm thick) of sterilized soil was filled at the bottom as supporting material for vermicomposting. Partially decomposed cow dung was placed over the soil layer.

The experiment was set up by taking 2 kg of the nutrient mixture (on a dry weight basis) in each wooden box. No additional feed was provided during the study. Fifty earthworms (*Eisenia fetida*) were released onto the nutrient mixture. The compost mixture was then covered with paddy straw. Two vermibeds were prepared: one for the control (garden soil) and one for the aquatic weed mixture. Three samples were used for statistical analysis.

Vermicomposting was conducted under laboratory conditions, maintained in darkness at an average temperature of 25 °C and a substrate moisture content of 70–75%. The experiment was continued for 60 days after releasing the earthworms. The amino acid content of the earthworms was analyzed after 60 days.

Preparation of Earthworm Powder: The harvested earthworms were washed under running tap water to remove any dirt on the body surface and then submerged in warm water (40°C) for 2 hours to allow gut contents to be excreted. Subsequently, the earthworms were thoroughly washed with distilled water and then kept in a hot air oven at 45 °C for 5 days to achieve complete drying.

Assay of Total Amino Acids: The total amino acid content of protein-free supernatants was estimated by the modified dinitrophenyl (DNP) derivatization method (Varley et al., 1980). The methanol extract (100 µl) of biological fluid or the reference standard (an equimolar mixture of glutamate:glycine) was made up to 250 µl with 80% (v/v) methanol. An equal volume (250 µl) of borate buffer was added to each tube, followed by 0.5 ml of DNFB reagent. Tubes were incubated at 45 °C for 30 minutes and then allowed to cool to room temperature. After adding 1 ml of 0.25 M HCl to each tube and mixing, the absorbance was measured at 420 nm. Apart from reagent blanks and reference standards, some of the samples were spiked with a known amount of the glutamate:glycine mixture to assess the recovery of total amino acids through the different steps of the assay procedure.

Statistical Analysis: The differences in amino acid contents between control earthworms and earthworms cultured on *Hydrilla verticillata* were analyzed using one-way Analysis of Variance (ANOVA). The F-value indicates the ratio of variance between the groups to the variance within the groups. The corresponding P-value determines the statistical significance of the observed differences. A P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The essential amino acid composition of earthworm tissues is presented in Table 1. Earthworms cultured on *Hydrilla verticillata* exhibited significantly higher levels of most essential amino acids compared to the control worms.

Table 1: Essential amino acid contents (g per 100g) of earthworm tissue

Essential Aminoacid	Control worm	Worms cultured on *H. verticillata*	F Value	P Value
Lysine	4.5±0.1.7	6.3±0.66	52.17	0.0019
Histidine	2.3±0.25	3.6±0.22	16.82	0.14
Arginine	3.1±0.98	5.2±0.76	37.50	0.003
Threonine	3.7±0.55	4.5±0.43	8.37	0.44
Valine	3.2±0.94	5.0±0.56	41.77	0.0029
Methionine	1.8±0.45	2.2±0.20	8.13	0.046
Leucine	4.2±0.1.2	6.6±0.46	47.18	0.0023
Isoleucine	2.7±0.70	4.6±0.32	34.46	0.0042
Phenylalanine	2.5±0.70	3.3±0.32	12.46	0.2423

Lysine content increased markedly from 4.5 ± 0.17 g/100 g in control worms to 6.3 ± 0.66 g/100 g in worms cultured on *Hydrilla verticillata* (F = 52.17, P = 0.0019). Similarly, arginine levels rose from 3.1 ± 0.98 g/100 g to 5.2 ± 0.76 g/100 g (F = 37.50, P = 0.003), and valine content increased from 3.2 ± 0.94 g/100 g to 5.0 ± 0.56 g/100 g (F = 41.77, P = 0.0029).

Kumar, et al (2019), found significant increases in amino acid content (including lysine and arginine) when earthworms were fed organic waste such as kitchen waste, compared to a control group. The increase in lysine content was especially noted to be a result of the earthworms' enhanced ability to synthesize proteins from organic materials.

Leucine and isoleucine contents were significantly elevated in worms fed *Hydrilla* spp., than in controls (P < 0.005). Methionine content also showed a significant increase (P = 0.046). Although histidine, threonine, and phenylalanine levels were higher in worms cultured on *Hydrilla verticillata*, these differences were not statistically significant (P > 0.05).

The high essential amino acid content in *Hydrilla* fed worms can be attributed to the rich protein profile of *Hydrilla verticillata*. Notably, leucine was the highest recorded essential amino acid in experimental worms (8.6 g/100 g), followed by lysine (7.3 g/100 g). Percentage increases compared to control worms were observed as follows: leucine (57.14%), lysine (40.00%), isoleucine (70.37%), valine (56.25%), methionine (22.22%), histidine (56.52%), arginine (51.21%), threonine (21.62%), and phenylalanine (32.00%).

The amino acid analysis of *Eisenia fetida* and *Eudrilus eugeniae* by Sonntag, E., et al. (2023) revealed that the earthworm biomass was rich in essential amino acids, including lysine, methionine, and threonine, cultured on spent mushroom substrate making it a promising alternative protein source for animal feed and possibly human consumption. Nair et al. (2005) reported that *Perionyx excavatus* cultured on pig manure showed elevated essential amino acids, with leucine at 3.47%, isoleucine at 1.98%, and lysine at 2.62% (dry matter basis). Similarly, Istiqomah et al. (2009) analyzed *Lumbricus rubellus* and found notable levels of leucine (3.15%), lysine (2.75%), valine (2.04%), and methionine (1.21%). However, the Hydrilla-fed *Eisenia fetida* in the current study demonstrated significantly higher concentrations: leucine (8.6 g/100 g), lysine (7.3 g/100 g), isoleucine (4.6 g/100 g), and valine (5.0 g/100 g), indicating a considerable enhancement in protein quality. These comparisons clearly demonstrate that culturing *E. fetida* on *Hydrilla verticillata* not only boosts essential and non-essential amino acid levels but may also surpass traditional organic feeds in nutritive value, establishing Hydrilla as a promising substrate for sustainable protein production.

As shown in Table 2, worms cultured on *Hydrilla verticillata* also exhibited increased concentrations of several non-essential amino acids. Glutamic acid content significantly increased from 4.40 ± 1.00 g/100 g in control worms to 6.80 ± 0.78 g/100 g (F = 57.76, P = 0.001). Aspartic acid levels rose from 4.30 ± 0.90 g/100 g to 5.68 ± 1.45 g/100 g (P = 0.019), and alanine increased from 2.80 ± 0.34 g/100 g to 3.70 ± 0.36 g/100 g (P = 0.033). Although serine, proline, glycine, cystine, and tyrosine contents were slightly higher in worms fed *Hydrilla*, these increases were not statistically significant (P > 0.05).

Table 2: Non Essential amino acid contents (g per 100g) of earthworm tissue

Non-Essential Aminoacid	Control Worm	Worms Cultured on *H.verticillata*	F Value	P Value
Aspartic acid	4.30 ±0.90	5.68 ±1.45	14.11	0.019
Serine	2.40 ±0.60	3.03 ±0.54	7.49	0.519
Glutamic acid	4.40 ±1.00	6.80 ±0.78	57.76	0.001
Proline	2.55± 0.32	2.76 ±0.34	4.37	0.127
Glycine	0.64 ±0.23	0.75 ±0.12	2.55	0.185
Alanine	2.80± 0.34	3.70 ±0.36	10.089	0.033
Cystine	0.61 ±0.45	0.70± 0.12	6.627	0.061
Tyrosine	2.30 ±0.27	2.48 ±0.56	3.43	0.137

These findings align with earlier studies where glutamic acid dominated non-essential amino acid profiles in species such as *Perionyx excavatus* and *Lumbricus rubellus* (Istiqomah et al., 2009).

Glutamic acid plays a critical role in protein synthesis, supplies energy to intestinal cells, enhances immune function, and exhibits anti-inflammatory properties (Sakthika et al., 2014).
The high glutamic acid levels observed reflect the active metabolism and high nutritive value of earthworms grown on enriched *Hydrilla* substrates. Earthworm meal is highly digestible (~98%) due to its balanced composition of amino acids and vitamins, making it an excellent protein source for livestock including cows, goats, sheep, and poultry (Gabriel and Dedeke, 2010).

The enhanced amino acid profiles in worms cultured on *Hydrilla* indicate that such feeding strategies could produce superior earthworm meal for animal nutrition compared to conventional methods. Moreover, non-essential amino acids (NEAAs), once considered less important, are now recognized for their regulatory roles in gene expression, immune function, oxidative stress mitigation, and gut health (Wang et al., 2012). For instance, dietary glutamine reduces intestinal oxidative stress and NEAAs like arginine and proline enhance immune responses and pathogen resistance (Tan et al., 2009).

Additionally, *Eisenia fetida* efficiently degrades organic matter, and faster decomposition rates have been reported compared to *Lampito mauritii* (Tripathi and Bhardwaj, 2004). Substrate quality greatly influences vermicomposting efficiency; cow dung degrades faster than aquatic weeds like water hyacinth. Thus, utilizing *Hydrilla* not only improves earthworm biomass quality but also offers an effective organic waste management strategy, contributing to sustainable agriculture and environmental conservation.

CONCLUSION

The present study clearly demonstrates that culturing *Eisenia fetida* on *Hydrilla verticillata* significantly enhances both essential and non-essential amino acid profiles compared to control worms. Notably, essential amino acids such as lysine, leucine, isoleucine, and valine, critical for protein synthesis, energy metabolism, and immune function, were significantly higher in worms fed with *Hydrilla* spp. Similarly, non-essential amino acids like glutamic acid and aspartic acid, important for intestinal health and immune regulation, also showed substantial increases. These findings highlight that *Hydrilla* fed earthworms can serve as a superior protein source rich in high-quality amino acids, making them an excellent ingredient for animal feed formulations. Vermicomposting *Hydrilla* not only provides a method for managing aquatic weed waste but also enhances the nutritive value of earthworms, contributing to sustainable animal nutrition and environmental management.

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