Effects of Probiotic Supplementation on Eisenia Fetida Growth and Tissue Protein Composition: A Comparative Study

***Komal**

Abstract:

Eisenia fetida earthworms were cultivated over a period of 60 days using garden leaf litter as the control group, waterhyacinth (Eichornia sps) as the experimental group (E1), and waterhyacinth supplemented with probiotics for the second experimental group (E2). Tissue protein content was determined following the method described by Lowry et al. (1951). The findings revealed significant enhancements in the growth parameters of earthworms raised on Eichornia sps supplemented with probiotics compared to the control group. Specifically, the worms exhibited an 88.60% increase in length, a 128.00% increase in weight, and an 84.90% higher tissue protein content. These results underscore the potential of earthworms as a valuable protein source for animal feed formulation. Moreover, vermicomposting of probiotic-enriched earthworms utilizing aquatic weeds presents a cost-effective strategy to obtain a high-quality protein source, bio-fertilizer, and an efficient means to remediate aquatic habitats.

Keywords: - Probiotics, growth, aquatic weeds, earthworms, and tissue protein.

INTRODUCTION

It has been shown that aquatic weeds may serve as a source of protein for diets (Mukherjee et al., 2010). Aquatic weeds are a rich supply of nutrients for vermiculture, and earthworms are thought to be a strong source of protein and a possible constituent for feed formulation. The free-floating, invasive aquatic macrophyte known to seriously harm aquatic environments is the water hyacinth.

According to published research, water hyacinth and other noxious weeds are resistant to all physical, chemical, biological, and hybrid approaches used to eliminate them (Abbasi and Ramaswamy, 1999).

Earthworms' function in the bioconversion of various waste products has previously been shown in the literature (Sangwan, et al 2008). Furthermore, vermicompost may be produced by the bioconversion of water hyacinth, as shown by technology (Gajalakshmi, et al 2002). According to Ngyuon and Yang (2007), a meal based on earthworm powder has 6–11% fat, 5–12% carbohydrate, and 2-3% minerals and vitamins of different kinds. When compared to meat or fish meal, earthworms have 60–70% more protein and higher levels of key amino acids (lysine and methionine) (Sigh et al., 1978).

According to Park et al. (2014), probiotic bacteria are live microorganisms that, when consumed in appropriate amounts, have positive impacts on human health. These benefits include enhanced gut microbial balance, immunomodulation, and longer life spans. The concept of employing yeast as an animal probiotic originated from the fact that it contains numerous vital enzymes, including zymase and protease.

Because protease works to break down the protein in animal feed into simpler molecules that animal cells may more readily absorb, it can be utilised to enhance the quality of animal feed. Earthworms have been used to treat inflammatory, haematological, oxidative, and neurological diseases because they contain a variety of chemicals that may have therapeutic benefits (Chen, et al 2010). Earthworm powder that has been ground up has been used orally to treat blood disorders and promote circulatory health (Pan, et al 2010). In light of this context, the current study set out to determine the protein content of earthworms cultivated on water hyacinth and earthworms enhanced with probiotics.

Materials and methods

Substrates and earthworms

Eisenia fetida earthworms were acquired from a culture bank kept at the Agricultural College and Research Centre in Killikulam. Eichornia sps, an aquatic weed, was taken from the Thamirabarani River close to Eral. Leaf litter was gathered from the campus of the institution. We bought cow manure from a nearby farm yard.

Experimental design

Earthworm fed with Lactobacillis sporogens

Lacto Bacillus sporogens were procured from a commercially available Sporlac sachet, which contained 150 million spores in a single gramme of powder. A solution of 100 mg Sporlac powder and 10 ml deionized water was prepared. Deionized water was used to cleanse the earthworms to prevent contamination from their mucous coats. Ten earthworms, moistened with deionized water and placed in a 1 kilogramme plastic box with filter paper, were permitted for the probiotic enrichment of Eiseneia fetida. Put the box's perforated cover back on. 100 mg of chopped tissue sheets with a 1 mm size were added. A 10 millilitre sporlac solution containing 15 million spores was used to moisten the pieces of tissue paper. In 24-36 hours, the earthworms began to consume the tissue paper.

Vermiculture

The aquatic weed water hyacinth was used in the vermiculture of Eisenia fetida in a dark, laboratory setting with an average temperature of 25 oC ± 2 oC and a substrate moisture content of 70–75 percentages. The dry weight ratio of cow dung to weeds was 70:30. Eisenia fetida earthworms, numbering fifty, were thrown into the mixture. The experiment lasted for sixty days after the earthworms' discharge.

A three-foot-wide by two-foot-tall wooden box was used for the culture. For around ten days, the cut and piled weed biomass was exposed to the sun. The bottom of the box was filled with a little layer of dirt. The soil layer was covered with cow manure that had partially decomposed. The layer of cow manure was covered with chopped vegetation.

Remaining garden leaves were used as a control for cultivating earthworms. For the first series of experiments, waterhyacinth was used as feed (E1). In order to break down waterhyacinth (E2), earthworms were given Lactobacillus sporogens for the second set of experiments.To do a statistical analysis of the data, three replicates were put up. The experimental setup was maintained in the shade, wrapped with jute fabric, and given two daily mists. Extreme caution was used to monitor the physical parameters, such as pH, temperature, and moisture content. After 60 days, the earthworm's tissue protein was examined.

Protein approximation:

A ten-gram powdered sample was homogenised using two millilitres of 10% TCA. The homogenate was centrifuged for 30 minutes at 3000 rpm. After the residue had been dissolved in 0.1N NaOH, it was centrifuged once more for ten minutes at 3000 rpm. This was then combined with 5 millilitres of biurette reagent and 1 millilitre of supernatant. Next, 0.5 millilitres of recently diluted folin phenol reagent were added. A colorimeter was used to measure the color's intensity at 650 nm after 30 minutes.

The BSA is used as the standard and distilled water is introduced in corresponding amounts to the reagents mentioned above in place of the supernatant to act as a control. Protein content was stated in milligrammes per gramme.

Results

Table 1 : Length, Weight and Protein content of Earthworm Eisenia fetida

Earthworm Feed	Initial Body Length (cm)	Final Body Length (cm)	Body Length Gain (cm)	Initial Body Weight (mg)	Final Body Weight (mg)	Body Weight Gain (mg)	Initial Tissue Protein (mg)	Final Tissue Protein (mg)	Tissue Protein Gain (mg)
Garden Leaf litter	7.2 ± 0.6	10.5 ± 0.4	3.3 ± 0.8	340 ± 25	460 ± 20	120 ± 2	460 ± 40	595 ± 30	135 ± 35
Eichornia Sps	7.5 ± 0.4	12.2 ± 1.4	4.7 ± 1.2	350 ± 30	580 ± 40	230 ± 4	465 ± 35	645 ± 35	180 ± 20
Eichornia & Earthworm with Probiotics	7.4 ± 1.3	13.8 ± 0.4	6.4 ± 1.4	360 ± 40	650 ± 60	390 ± 6	470 ± 50	725 ± 50	255 ± 55

Table: 2. Length, Weight and Protein gain of earthworm in %

S. No	Sample	Length (cm)	Weight (mg)	Protein (mg/g)
1	Garden Leaf litter	45.83	35.29	29.34
2	Eichornia Sps	36.72	73.16	31.90
3	Eichornia & Earthworm with Probiotics	88.60	128	84.90

Results and Discussion

The current study's results (Table-1) made it abundantly evident that the protein content of worms grown on aquatic weed water hyacinth and probiotic-enriched earthworm was much higher than that of worms grown on garden leaf residue and water hyacinth without probiotic-enriched bacteria. Eisenia fetida vermiculture utilising garden leaf wastes increased length by 45.83%, weight by 35.29%, and tissue protein by 29.34% compared to baseline values (Table 2).

After 60 days of experimentation, earthworms raised on Eichornia sps grew in length by 36.72%, weight by 73.16%, and tissue protein content by 31.90% relative to the control values. Lee (1985) asserts that as organic materials go through earthworms' digestive tracts, phosphorus that isn't readily accessible to plants is changed into forms that are. According to Manyuchi et al. (2019), vermicomposting water hyacinth offers a chance to manage lignocellulose trash and produce value-added bioproducts like vermicompost. The current research came to the conclusion that feeding earthworms with aquatic weeds significantly increased the worm biomass and that the worms may be used as food to increase the amount of protein required.

After 60 days of experimentation, earthworms fed with probiotic bacteria Lactobacillus sporogens cultivated on Eichornia sps grew in length by 88.60%, weight by 128%, and tissue protein by 84.90% compared to the control values.

According to Van Gestal et al. (2015), microbial biofilms are three-dimensionally organised communities of adhering microorganisms enclosed in an extracellular matrix that they manufacture on their own. These biofilms have networks of channels for long-distance cell-to-cell communication and nutrition delivery. According to Vidhyalaxmi et al. (2014), Bacillus subtilis is a model beneficial bacteria that may exhibit a variety of unique cell types under developmental control, including the capacity to construct intricate and durable biofilms. Remarkably, a recent research revealed that, apart from its function as a food source, planktonic B. subtilis affects the lifetime of the model organism Caenorhabditis elegans and the bacteriovorus nematode. When given planktonic B. subtilis cells to eat, worms develop 15% longer on average than when treated with E. coli or various alternative food sources (Kim, et al 2013). These data support the probiotic's ability to raise Eisenia fetida tissue protein in the experiment's E2 group.

Earthworms are rich in protein, and this protein is particularly rich in amino acids, which are thought to be necessary for humans and other domesticated animals to eat. The stated protein values vary from 48 to 71% (dry weight basis), although the protein content fluctuates depending on the type of earthworm and the experimental diet used for the earthworms. According to Serna-Cock et al. (2017), E. fetida is a species with a comparatively high protein content of 58–71% dry weight, or around 9.7% of its live weight. Enzymes readily break down earthworm protein into free amino acids. Growing large-bodied earthworms seems to be a potential way to replenish protein for human consumption as well as animal feed.

Conclusion

Further study into sustainable animal protein replacers is needed due to problems related to the sustainability of protein feed component sources, including cost, nutritional value, and resource constraints.

Earthworms are becoming a popular alternative protein source due to their nutritional content, which is comparable to fish meal. Enriching earthworms with probiotic microorganisms enhances their protein biomass. Vermiculture combines probiotic-enriched earthworms with aquatic weed waterhyacinth to reduce pollution, produce organic manure, and provide adequate protein.

***Research Scholar**
Department of Zoology
MLSU, Udaipur (Raj.)

BIBLIOGRAPHY

1. Abbasi S. A., E. V.Ramaswamy. (1999). Biotechnological Methods of Pollution Control. Orient Longman (Universities press India Ltd), Hyderabad. 1999, p. 168.

2. Chen, C T., J.G. Lin, T.W. Lu et al., (2010). "Earthworm extracts facilitate pc12 cell differentiation and promote axonal sprouting in peripheral nerve injury," The American Journal of Chinese Medicine, vol. 38, no. 3, pp. 547–560.

3. Gajalakshmi S., E. V. Ramaswamy and S. A. Abbasi (2002). "High rate compostingvermicomposting of water hyacinth (Eichhornia crassipes, Mart. Solms)", Bioresour. Technol., 2002; 83(3):235-239.

4. Kim, D. H. Bacteria and the aging and longevity of Caenorhabditis elegans. Annu. Rev. Genet. 47, 233–246 (2013).

5. Lee, K E., (1985). Earthworms: their Ecology and Relationships with Soils and Land use", Academic Press Inc.

6. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., R.J. Randall, (1951). "Protein measurement with the Folin phenol reagent". Journal of Biochemistry, 193, pp 265-275.

7. Manyuchi Mercy M, Mbohwa, Muzenda, N. Mutusva (2019). Degradation of Water Hyacinth (Eichhornia Crassipes) to Vermicompost through Application of the Vermicomposting Technology, Proceedings of the International Conference on Industrial Engineering and Operations Management Bangkok, Thailand, March 5-7, 2019 © IEOM Society International.

8. Mukherjee, A.K., P. Kalita., B.G. Unnj., S.B.Wann., D.Sajkia and P.K.Mukhopadhyay, (2010). Fatty acid composition of four potential aquatic weeds and their possible use as fish feed neutraceuticals. Food Chemistry 123: 1252-1254.

9. Nguyen Phu Hoa and Yang Yi, (2007). Prey ingestion and live food selectivity of the fish marble goby (Oxyeleotris marmorata) using rice field prawn (Macrobrachium lanchesteri) as prey. Aquaculture, volume 273, issue 4, pages 443-448.

10. Pan, R., Z. J. Zhang, and R.Q.He, (2010). "Earthwormprotease," Applied Environmental Soil Science, vol. 2010, Article ID 294258, 13 pages.

11. Park, M., Yun, H., Son, S., Oh, S. & Y. Kim (2014). Short communication: Development of a direct in vivo screening model to identify potential probiotic bacteria using Caenorhabditis elegans. J. Dairy Sci. 97, 6828–6834.

12. Sangwan P., C. P. Kaushik, and V. K. Garg. (2008). "Vermiconversion of industrial sludge for recycling the nutrients", Bioresour. Technol., 99: 8699-8704. http://dx.doi.org/10.1016/j.biortech.2008.04.022.

13. Serna-Cock L, C.A.Rengifo-Guerrero., M.A.Rojas-Restrepo, (2017). Use of earthworm (Eisenia foetida) flour and hydrolyzed chicken feathers as sources of nitrogen and minerals for ethanol

production. Waste & Biomass Valorization,1:1-10.

14. Sigh, B.N., V.R. Sinha and D.P. Chakraborty, (1978). Effect of protein quality and temperature on the growth of fingerlings of Rohulabeo rohita. Paper Presented at the World Symposium of Fish Nutrition and Fish Feed Technology. Hambour Germany.

15. Sinha, R.K., S. Heart., S. Agarwal., R. Asadi and E. Carretero, (2002). Vermiculture and waste management: Study of action of earthworms Elsenia foetida, Eudrilus euginae and Perionyx excavatus on biodegradation of some community wastes in India and Australia. The Environmentalist 22, 261268.

16. Van Gestel, J., Vlamakis, H. & R. Kolter, (2015). Division of labor in biofilms: the ecology of cell differentiation. Microbiol. Spectrum 3, MB-0002–MB-2014.

17. VidyaLaxme, B., Rovetto, A., Grau, R. & R. Agrawal, (2014). Synergistic effects of probiotic Leuconostoc mesenteroides and Bacillus subtilis in malted ragi (Eleucine corocana) food for antagonistic activity against V. cholerae and other beneficial properties. J. Food Sci. Technol 51,3072–3082