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PROBIOTIC EFFECT ON GROWTH AND TISSUE PROTEIN OF *EISENIA FETIDA* FED WITH AQUATIC WEED

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ABSTRACT

The Earthworm *Eisenia fetida* was cultured using garden leaf litter (Control), Aquatic weed waterhyacinth (*Eichornia sps*, (E1) and Waterhyacinth+Probiotic enriched *E.fetida* (E2) for 60 days. Tissue protein was calculated by Lowry *et al*., (1951) method. The results of the study confirmed that worms grown on *Eichornia sps*, with probiotic enriched earthworm showed 88.60% increased length, 128.00% increased weight and 84.90% higher tissue protein than the control group of earth worm. Earthworm is viewed as a potential ingredient and a good source of protein in animal feed formulation. Vermiculture of probiotic enriched earthworm using aquatic weeds is a cheap strategy to get good source of protein, bio-fertilizer and a most promising way to clean the aquatic habitat.

Key words:- Aquatic weeds, Earthworm, Probiotics, Growth, Tissue protein.

INTRODUCTION

Aquatic weeds (Mukherjee *et al* 2010) are potential dietary protein sources that have been documented. Vermiculture using aquatic weeds have a good nutrient value and earthworm is viewed as a potential ingredient and a good source of protein in the feed formulation. Water hyacinth is the free floating invasive aquatic macrophytes that are known to cause severe damage to the aquatic habitat. Literature revealed that the noxious weeds like water hyacinth are resisted to the all physical, chemical, biological as well as hybrid methods that have been applied to eradicate it (Abbasi and Ramaswamy, 1999).

The role of earthworms in bioconversion of different types of waste materials has been already reported in literature (Sangwan, *et al* 2008). Even, the technology for bioconversion of water hyacinth into vermicompost has also been already established (Gajalakshmi, *et al* 2002). Ngyuon and Yang, (2007) reported that earthworm powder-based diet contains 6-11% of fat, 5-12% of carbohydrate and 2-3% of minerals and various types of vitamins. Earthworm contains 60-70% protein and high in essential amino acid (lysine and methionine) compared with meat or fish meal (Sigh *et al*., 1978).

Probiotic bacteria are defined as living microorganisms that exert beneficial effects on human health when ingested in sufficient quantity; these effects include improved intestinal microbial balance, immunomodulation, and extended life span (Park, *et al* 2014). The idea of using yeast as an animal probiotic emerged because it has several enzymes that have important functions, protease and zymase. Protease can be used to improve the quality of animal feed, because it has a role to degrade the protein content in animal feed to simple molecule, so it can be absorbed easily by the animal cells. Earthworms contain many compounds with potential medicinal properties and have been administrated to treat inflammatory, hematological, oxidative, and nerve disease (Chen, *et al* 2010). Ground-up earthworm powder has been used

as oral administration to support circulatory health and treat blood diseases (Pan, *et al* 2010).

With this background the present investigation was aimed to find the amount of protein in the earthworms cultured on aquatic weed water hyacinth and Probiotic enriched earthworm cultured with waterhyacinth.

Materials and methods

Earthworms and substrates

Earth worms of *Eisenia fetida* were obtained from a culture bank maintained in Agricultural college and Research centre, Killikulam. Aquatic weeds *Eichornia sps* was collected from Thamirabarani river near Eral. Leaf litter was collected from the college Campus. Cow dung was obtained from a local farm yard.

Experimental design

Earthworm fed with *Lactobacillis sporogens*

Lacto bacillus sporogens was obtained from the commercially purchased Sporlac sachet (150million spores in 1gram powder). 100mg of Sporlac powder was dissolved in 10ml deionized water. The earthworms were rinsed with deionized water to avoid contamination from the mucus on their bodies. For probiotic enrichment of *Eiseneia fetida*, ten earthworms were allowed in 1kg size plastic box with filter paper (wet with deionized water). Close the box with perforated lid. Chopped tissue papers (100mg) of 1mm size were added. The tissue paper pieces were wet with 10ml sporlac solution containing 15million spores. The earthworms started to eat the tissue paper after 24-36 hours.

Vermiculture

Vermiculture of *Eisenia fetida* was conducted using aquatic weed water hyacinth under laboratory conditions in darkness at an average temperature of 25^{o}C ±2^{o}Cand a substrate moisture content of 70-75 percentages. The weed and cow dung ratio was 70:30 on dry weight basis. 50 earthworms, *Eisenia fetida* were released over the mixture. The experiment was conducted for 60 days after the release of earthworms.

The culture was carried out in wooden box of 3feet breadth and 2feet height. The weed biomass was chopped and heaped under the sun for about 10 days. A thin layer of soil was placed at the bottom of the box. Partially decomposed cow dung was placed over the soil layer. Chopped weed was spread over the cow dung layer.

Garden leaf residues were taken as control to culture Earthworm. Waterhyacinth was used as feed for the first set of experiment (E1). For the second set of experiment earthworms were fed with *Lactobacillus sporogens* to decompose Waterhyacinth (E2).Three replicates were setup for statistical analysis of the results. The experimental set up was kept under shadow and covered with jute sheet and moistened twice in a day. The physical parameters such as pH, temperature and moisture content were monitored with utmost care. The tissue protein of the earthworm was analyzed after 60 days.

Estimation of protein: (Lowry *et al*., 1951)

10 gram of powdered sample was homogenized with 2ml of 10%TCA. The homogenate was centrifuged at 3000rpm for 30 minutes. The residue was dissolved in 0.1N NaOH and again centrifuged for 10 minutes at 3000rpm. From this 1ml of supernatant was taken and added with 5ml of biurette reagent. Then 0.5ml of freshly diluted folin phenol reagent was added. After 30 minutes the intensity of the color developed was measured at 650nm using colorimeter. Respective volumes of the reagents cited above are added to distilled water instead of the supernatant to serve as control and the BSA served as standard. Amount of protein was expressed in mg/g.

Results

Table 1 : Length, Weight and Protein content of Earthworm E*isenia fetida*

Earthworm feed	Body Length gain			Body weight gain			Tissue protein gain		
	Initial (cm)	Final (cm)	Gained Length (cm)	Initial (mg)	Final (mg)	Gained Weight (mg)	Initial (mg)	Final (mg)	Gained Protein (mg)
Garden Leaf litter	7.2±0.6	10.5±0.4	3.3±0.8	340±25	460±20	120±20	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±45	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±60	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 (% Increase from the initial)	45.83	35.29	29.34
2	Eichornia *Sps* (% Increase from the control)	36.72	73.16	31.90
3	Eichornia &Earthworm with Probiotics (% Increase from the control)	88.60	128	84.90

Results and Discussion

Results of present investigation (Table -1) clearly indicated that worms cultured on aquatic weed water hyacinth and probiotic enriched earthworm had far superior in protein content than worms cultured on garden leaf residues and waterhyacinth without probiotic enriched bacteria. Vermiculture of *Eisenia fetida* using garden leaf residues showed increased length of 45.83%, increased weight of 35.29% and increased tissue protein of 29.34% from the initial values (Table-2).

Earthworms grown on Eichornia *sps,* gained 36.72% increased length, 73.16% increased weight and 31.90% higher tissue protein from the control values after 60 days of experimental period. According to (Lee, 1985) if the organic materials pass through the gut of earthworms then unavailable forms of phosphorus are being converted to such forms that are available to plants. Manyuchi, *et al* (2019) reported that vermicomposting of water hyacinth presents an opportunity for management of lignocellulose waste to

value added bio products like vermicompost. The present study concluded that earthworm fed with aquatic weeds considerably improved the worm biomass and the worms could be used as dietary source to enrich the protein requirement.

Earthworms enriched with probiotic bacteria *Lactobacillus sporogens* grown on Eichornia *sps*, gained 88.60% increased length, 128% increased weight and 84.90% higher tissue protein from the control values after 60 days of experimental period.

Microbial biofilms are three-dimensional structured communities of adherent microorganisms encased in a self-produced extracellular matrix, containing networks of channels for nutrient supply and long-distance cell-to-cell communication (Van Gestal, *et al* 2015). *Bacillus subtilis* is a model beneficial bacterium with the ability to display many distinct cell types under developmental control, including the ability to form robust and sophisticated biofilms (Vidhyalaxmi, *et al* 2014). Interestingly, a recent study showed that planktonic *B. subtilis* modulates the longevity of the bacteriovorus nematode and model organism *Caenorhabditis elegans* independent of its role as a food source. Worms fed on planktonic *B. subtilis* cells live longer (an approximately 15% increase in lifespan) than worms grown in the presence of other food sources, that is, E. coli (Kim, *et al* 2013). These evidences support the probiotic role to increase tissue protein of the *Eisenia fetida* in the E2 group of the experiment.

Earthworms contain high levels of protein and that this protein is rich in the amino acids, considered essential for food of domesticated animals and humans. However, the protein content varies with the earthworm species and experimental food of earthworms, and the protein contents reported range from 48 to 71% (dry weight basis). E. fetida is a species with relatively high protein content of 58–71% dry weight or about 9.7% of its live weight (Serna-Cock et al., 2017). Earthworm protein is easily dissolved by enzymes into free amino acids. Production of earthworms with heavy body mass seem a promising source of protein supplementation not only for animal feed but also for human food.

Conclusion

The challenges associated with sustainability of protein feed ingredient sources in view of cost, nutritive value and resources have necessitated further research on viable animal protein replacers. Non-conventional protein sources, such as earthworm, have gained interests to provide an alternative protein source thanks to its nutritional values that are close to that of fish meal. Earthworm enriched with Probiotic bacteria is an effective strategy to increase the protein biomass of earthworm. Vermiculture of probiotic enriched earthworm with aquatic weed waterhyacinth is a universal remedy for reducing pollution hazard, getting surplus organic manure and sufficient protein.

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