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Efficacy and suitability of earth worm M*egascolex* sps as supplementary feed for cat fish *Pangasius hypophthalmus* in response to different animal protein sources

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Abstract
Experiments were conducted to determine the relative efficacy of three different animal protein sources (the earthworm muscle (Megascolex sps), foot muscle of snail (Pila sps) and pig liver) was used as supplementary feeds in fish nurseries ponds. The survival and growth performance of catfish *Pangasius hypophthalmus* fingerlings was studied over a period of 60 days in the aquarium tanks. Among the experimental diets, advanced fry of the catfish showed better growth when fed earthworm muscle as supplementary feed with comparison of the foot muscle of snail and pig-liver. Survival percentage, weight gain, feed conversion ratio and growth performance showed significant difference among the experimental diets. Present study suggests that in nursers stages of fingerlings fed with earthworm muscle is better supplementary feed as its showing better growth performance in comparison with other animal protein sources.

Keywords: megascolex sps. P. hypophthalmus, supplementary feed

Introduction
Aquaculture operations especially finfish culture are now-a-days gaining momentum in order to meet the global demand of proteinacious food. And it is easily digestible protein comprising of essential amino acids with high biological value for human consumption. Globally fish provides about 16% of the animal protein consumed by human beings. Due to an ever rising population of India, there is an increased interest in the development of aquaculture production for food security and for larger economic benefits. Furthermore the intake of fish in our country population diet is relatively less compared to international average consumption. The global fish supply is increasing the demand for feed resources, especially for high quality protein and high quality lipid feed resources such as fishmeal and fish oil [1, 2]. The price of fishmeal has increased greatly within the past decade [3], due to the high demand which inhibits small scale aquaculture enterprises in rural areas to increasing the fish production by using higher quality feed inputs. This, in turn, leads to the search for alternative highly nutritious feed ingredients in aqua feeds [3].
Vermiculture, a century old practice is now being revived worldwide for waste management, sustainable organic agriculture, and aquaculture [4]. Detritivorous terrestrial and aquatic oligocheate worms are well known for their capacity to break down and utilize human and animal wastes. Among the various terrestrial worms, earthworm (Megascolex sps) has been proven the most promising and successful species for culture. The earthworms has been found to be a good source of protein with favorable amino acids [5]. Earthworm meal has been investigated as a one of the best feed for freshwater culture species. Under laboratory conditions and without access to natural food resources, partiall replacement of fish meal by earthworm protein feed for common carp (*Cyprinus carpio*), Rohu (*Labeo rohita*) and Buenos Aires tetra (*Hemigrammus caudovittatus*) had a positive effect on growth performance [6]. But it is unknown whether full replacement of fishmeal by on-farm produced earthworm meal may be beneficial for growth under pond conditions where fish also have natural food resources which are known to be of high nutritional value and contain high levels of protein and essential amino acids [7]. Insufficient availability and high cost of nutritionally balanced

supplementary feeds are the major impediments in increasing fish production. Protein is one of the most important nutrients used for preparation of fish feed. It is necessary to ascertain the quantitative requirements of dietary protein in order to reduce cost has been to develop low cost diets. The need of the hour is raise fish on relatively cheap, readily available and nutritionally rich ingredients from which fish diet can be formulated earth worm has crude protein up to 72% which can be cheaply procured and effective protein source [8].

P. hypophthalmus is a commercially important fresh water cat fish and widely distributed throughout India. It's belongs to family pangasiidae, order cypriniformis and cat fishes are most important food fishes in India. Successful and sustainable culture of fin and shellfish culture depends upon the provision of nutritionally balanced and economically viable supplementary feed. Feed is the major operational input and feed costs normally range from 30-60% of the operational expenditure in finfish and shell fish culture systems[9].Present day culture systems faces one of the major problem that the appropriate low cost input and easily, cheaply available complete supplementary feeds. A number of cheap raw materials have been tested to evolve a suitable supplementary feeds for different fishes with variable success in cat fishes [10, 11, 12, 13]. These alternative sources of feeds can be utilized to provide the essential nutrients and energy needed to fuel the growth of culture organisms. The supplementary feeds are formulated using locally available animal source ingredients considering their nutrients profiles and cost. Several low cost feed formulations are in use and their selection, depending on farming system and type of culture species. Rearing of fry in the nursery ponds over a period of 60-80 days before stocking in the production ponds to prevent lose of organisms due to mortality is a common practice. The need for an alternative species in freshwater aquaculture has generated lot of interest on catfish *P. hypophthalmus.* But, so far the available literature on use of earthworm in formulating the finfish diet is very limited. Considering the high nutrient value of earthworm and its huge production potential in the coming years as by-product of vermiculture and waste management processes, a study was undertaken to evaluate the possibility for use of different forms of earthworm in formulating the diets of fish larvae.

Materials and Methods

The experiments were conducted at the Aquarium tanks in Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur District, Andhra Pradesh, during the months of August and September, 2016. The advanced fry of cat fish average weight 5.4 $\pm$ 0.5 were collected from Krishna River (at Prakasam barrage) and are transported to the department and were acclimated laboratory conditions for 15 days. Fishes were divided into 3 groups and each group consisted of five sets (10 individuals in each set). Each set of individuals were introduced into rectangular glass aquaria (75 x 35cm) containing 40 liters of water. Before starting the feeding experiment, they were allowed to starve for 12 hours to empty their gut. From these fishes almost uniform sized individuals were selected, weighed and stocked @ 10 individuals (5.4 $\pm$ 0.5 average individual weight) filled with water. First group of *P. hypophthalmus* were fed on a muscle of earthworm, Megascolex sps, second group was fed on the foot muscle of snail and 3rd group fed on the pig liver were given 2 times per day. Before feeding the gut of earthworms were evacuated by keeping 24 hours in water [16] and then cutting into small pieces and then washed carefully in tap water to remove the unwanted sand particles. Snail foot and pig liver also washed carefully and cutting into small size pieces. There were three diets with three replications for each treatment. The fishes were fed on the respective diets for 60 days. The feed was offered in the morning (8.00 am) and evening (5.00 pm). After 4 hours of given feed, the unfed feed particles siphoned out every morning and evening. Water quality in the experimental tanks was monitored [17] every day to maintain water quality.

Results and Discussion

The results of water quality parameters were recorded during experimental period of 60 days, with no significant difference in water quality parameters. The water temperature ranged from 24.0 to 28.50C with average of 25.90C. The PH values did not show any marked variation in between 7.6 to 7.9. The total alkalinity in between 88 to 112 with an average of 99.6 mg/l and total hardness in between 85-110 with average of 90.4 mg/l. The level of dissolved oxygen 4 to 5.7 with average of 5.04 mg/l. The final weight gain, survival and growth performance of this experiment was given in Table No. 1. It can be observed from Fig. 1 that growth parameters differed significantly ($P < 0.05$) in all feeding levels in treatment groups. Highest average growth was recorded in case of worm muscle were 28.9 gms, snail foot muscle was 26.4 grams and pig liver was 25.8 grams. Highest survivals were recorded 96.5% in case of worm muscle, snail foot were 95.4% and pig liver meal were 94.2%. Proper feed and feeding are critical factors which decade the early stages of growth and survival of organisms during early part of their rearing. Protein is most essential component of diet and is used as growth enhance and source of energy in fish. Fish required diets relatively higher in protein than those of commercially reared terrestrial homeotherms [18]. Earth worm is the major source of protein and it is the most important nutrient promoting growth in animals, because it has direct influence on growth. The earthworms are generally described as resources with high protein content and protein quality but these parameters have been shown to differ according to earthworm species and, to a lesser extent, the feed substrate [19, 20, 21, 22, 23].

Recently, nutritional value of earthworms has considerably taken attention of fish nutritionists as they search for alterative animal protein source to fishmeal, which is characterized by seasonal availability, and inconsistent supply from world has dwindled fisheries stock. Protein quality of earthworm has been reported at par with that of fishmeal [22, 24, 25]. Survival and growth of the larvae of many forms are known to be influenced by the availability of right type of food in right concentration. High nutrients and energy rich diets accelerate proper utilization of feed making energy available for new tissue building of fishes. The nutritional value of the food is another important aspect of to be evaluated to analyze influence of food on growth. The requirement of nutrients for different fish species generally reflects different dietary and metabolic adaption's to a given habitat. The rate of food consumption is depend up on the capacity of stomach rate of digestion and gastric evacuation have been reported to influence rate of food consumption [26, 27], which is turn is dependent upon the nature of food material [10]. Sriwastwa [28] have shown the improved growth rate of fish *Mystus vittatus* when fed on eighter dry or fresh worms. Reimmers [29] has reported a comparatively short

period for gastric evaculation for meal of oligochetes. Bisht and kaushal [30] reported that earthworm protein are found sixteen amino acids including eight essential acids. Sastri and Monika [8] reported that the effect of earthworm meal on the growth of catla, catla fry. Sinha *et al*, [13] observed that the superiority as well as suitability of verm meal over the other meals molluscan meal and goat liver for growth and development of cat fish *Clarius batrachus*. In the present study higher consumption of earthworm muscle by the experimental fish *P. hypophthalmus* showed better growth, when compare with other meals of snail foot muscle and pig liver. Jhingran [31], Krishna [32] reported that addition of supplementary feed enhanced the growth and survival of fry in the necessary ponds. The earthworm (*E. foetida*) meal has high protein content and the amino acid compositions are close to fishmeal and hen egg [5].

Moreover, the protein and lipid contents of the earthworm are species specific. In addition, the culture environment (nutrient/medium) plays a great role in determining the nutrient composition of earthworm. As we do not know the culture environment of the earthworm as reported by the earlier researchers, it is difficult to compare the nutrient composition of the earthworm found in the present study and the earlier reports. Protein is the most important nutrients promoting growth in fishes, because it has direct influence on growth. According to DeLong *et.al*, [33], the protein need for growth and maintenance varies in different species and culture environment [34, 35]. Li et.al, [11] reported that immune response and disease resistance of certain fish species are affected by dietary protein levels. Fish growth is influenced by feed availability and intake genetics, fish age and size environment and nutrition, of these factors, feed intake is perhaps the main principal factor affecting growth rate of fish [36]. Laboratory studies with channel cat fish have shown that feeding frequency affects growth performance. Li *et al*., [11] suggested that feeding two times daily to satiation was sufficient for maximal growth. In our experiments also feed given twice in a day, morning and evening. The feeding behavior and mechanism of feeding in fishes however have been reported to be a very complicated. Several types of stimuli are usually linked with fish feeding [37]. Akiyama et.al., [38] reported improvement in growth and feed efficiency in Chum salmon fry fed on a diet with 5% earth worm power substitution and suggested that earth worms might contain unknown growth promoting factors rather than attractions or appetite stimulants. Supplementation of small amount of earthworm powder can efficiently promote growth in Chum salmon fry and improved tolerance to starvation. Considering the protein content of species like *Lumbricus terrestris* 66.25%. *Apporectodea longa* 62.12%; *Octolasion cyaneum* 60% and *Eisenia fetida* 60-61% reported by [39].

Nutritional composition may influence the growth i.e., the composition of food from proteins, lipids, carbohydrates and other energy. Protein quality of any food and feed in mainly depend upon the number of amino acids. Murray *et al*., [40] reported that an optimum level of protein, fat and carbohydrates components to promote growth of a catfish *Ictalurus punctatus*. Possibilities of using earthworm mean as partial replacement of fishmeal of carp diet was reported by [41, 42] also worked on the earthworm meal. Guerro [43] has observed protein value of 54.77% and fat 13.5% in Perioynx excavatus. Das and Das [44] have reported protein concentrated in earthworm muscle and in terms of nutritious quality and earthworm protein utilization. Rahman *et al.* [45] named large zooplankton as the most important natural food resources for common carp as soon as the supply of the preferred zoo benthos in the pond becomes limited. An increase in production of natural food should be aimed by changing the water management and fertilization management [46, 47] to increase the financial benefit and growth of common carp fed on supplementary on plant-based feeds with earthworm as the animal protein resource. In the aquatic environment, the increase of natural food availability may be promoted by improving methods of fertilization as well as water flow management [48, 46, 47].

Numerous studies have been conducted on the effect of nutrition on fish health; including of various types of cheap raw materials have been tested to evolve a suitable supplementary feed for different varieties of fishes. Liver as a feedstuff is a by-product of slaughtering animals. Liver meal is a protein feed stuff having a mean protein contest of about 67% and it is a good source of essential amino acids [49]. Subramanian *et al.* [50] reported that effect of beef liver incorporated feed on the growth performance of gold fish, *Carassius auratus*. Suresh [15] worked on the Giant African snail meat as dietary animal protein source for common carp. He concluded that meat of African snail meat could be a cheap and effective protein sources in carp supplementary feeds. Biochemical analysis of food required to find out whether the earthworm muscle contains all required quantity levels of protein, lipid carbohydrates and energy for effecting maximum growth [10]. The overall study indicates that the earth worm muscle yielded better growth than foot muscle of snail and pig liver meal because the earth worm muscle contains all required quantity of nutrients and energy for effecting maximum growth.

The results of present study indicates that there was negligible mortality due to different dietary treatments during course of experiments irrespective levels of protein. It may conclude that the rearing of *P. hypophthalmus* on earthworm muscle is a profitable proposition, economically better supplementary and suitable food. It has high crude protein, which is quite cheaper and easily available. The earthworms might be used by small-scale farmers in rural areas to produce supplemental feeds for omnivorous fish like catfishes. This necessitates the introduction of earthworm production technologies (vermin culture) into rural areas of developing countries as an additional farming activity in integrated farming systems. Vermiculture accelerates nutrient cycling within the farms and generates additional financial benefit due to utilization of underutilized wastes and by-products of low quality [51, 22] by producing a high qualitative feed resources for fish (or other animals) and highly fertile soil (called vermin compost)for gardening or pond fertilization [52, 4, 53]. In small-scale farms in northern Vietnam, Müller *et al*, [54] calculated that vermin culture based on underutilized on-farm wastes (ruminant manure) produces 6-36 kg of earthworm DM per year and farm. Using this amount of earthworm meal in plant based supplemental feeds could produce 18-112 kg of additional common carp per farm, raising the financial yield by 12-75% per year compared to the traditional aquaculture applied by small scale farmers in the research area [55, 48].

Earthworm has high protein content, considerable mineral matter and less of fat and fiber and more palatable to fish. A better dietary source of earthworm feed can meet the demand of protein and other nutrients to fed the fish. This is confirmed in the present study by the increasing growth rate of the fish fed with earthworm than fish fed with snail foot and pig liver.

Table 1: Survival and growth performance of *P. hypophthalmus* different supplementary diets

	Earthworm muscle	Foot muscle of snail	Pig liver
Initial Weight	5.4 ±0.5	5.4 ± 0.5	5.4 ± 0.5
After 15 days	8.3 ± 0.55	7.2 ± 0.54	7.5 ± 0.48
After 30 days	12.5 ± 0.78	11.5 ± 0.79	10.6 ± 0.74
After 45 days	18.3 ± 0.95	17.2 ± 0.91	16.4 ± 0.95
After 60 days	28.9 ± 2.42	26.4 ± 2.51	25.8 ± 2.46
Final Wt. again %	435.1± 7.8	388.8 ± 6.9	386.7 ± 6.6
Survival	96.5 ± 1.00	95.4 ± 2.5	94.2 ± 2.2
Feed conversion	2.32 ± 0.75	2.89 ± 0.85	3.09 ± 0.96

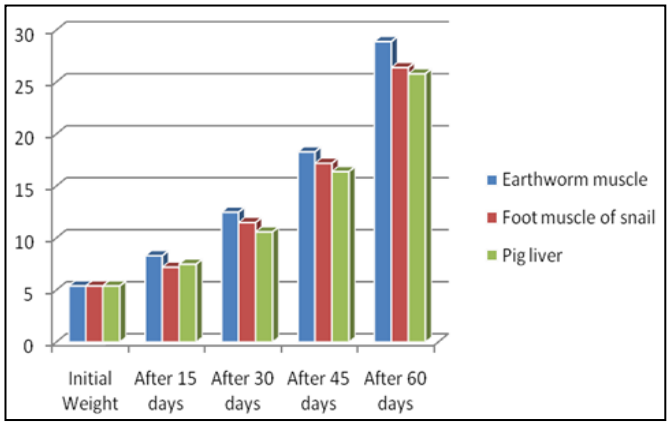

Fig 1: Growth performance of *P. hypophthalmus* different supplementary diets

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