EFFECT OF FEED SUPPLEMENT CONTAINING EARTHWORM (*Eisenia foetida*) ON GROWTH PERFORMANCE OF COMMON CARP (*Cyprinus carpio*)

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Batch: 2073

A thesis submitted in partial fulfillment of the requirement for the award of the Degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture

Submitted to:

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2019

TRIBHUVAN UNIVERSITY
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RECOMMENDATIONS

This is to recommend that the thesis entitled **"Effect of feed supplement containing earthworm (*Eisenia foetida*) on Growth performance of Common Carp (*Cyprinus carpio*)"** has been carried out by Miss Nima Sherpa for the partial fulfillment of Master's degree of science in zoology with special paper of fish biology and aquaculture, this is her original work and has been carried out under my supervision. To the vest of my knowledge, this thesis has not been submitted for any degree in any institution.

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LETTER OF APPROVAL

On the recommendation of supervisor Prof. Dr Kumar Sapkota this thesis is submitted by Nima Sherpa entitled "**Effect of feed supplement containing earthworm (*Eisenia foetida*) on Growth performance of Common Carp (*Cyprinus carpio*)**" is approved for the examination has been carried out by Miss Nima Sherpa for the partial fulfillment of Master's degree of science in zoology with special paper of Fish Biology and aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis submitted by Nima Sherpa entitled "**Effect of feed supplement containing earthworm (*Eisenia foetida*) on Growth performance of Common Carp(*Cyprinus carpio*)**" has been accepted as a partial fulfillment of Master's degree of science in zoology with special paper of **Fish Biology and Aquaculture.**

EVALUTION COMMITTEE

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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ACKNOWLEDGMENT

This study would not have been completed without the support of the staff of central department of zoology and central fishery promotion and conservation centre.

During my thesis study, I have been indebted to many people without whom this dissertation could never have been completed. First of all, I would like express my sincere thanks to **Prof. Dr Kumar Sapkota**, my supervisor, for his unreserved support and encouragement in the conduct of this study.

I would like to thank **Mr. Baikuntha Adhikari**, chief fisheries development officer, who gave me permission to work in Central Fishery Promotion and Conservation Centre. I would also like to thank **Mrs. Sumitra Laudari,** senior fisheries development officer, for providing me help throughout my field work. I appreciate the support given by the **farm workers** too**.**

I would also like to thank **Prof. Dr. Ananda Shova Tamrakar,** for providing me with basic knowledge of vermicomposting and also assisting me in making protein powder. Similarly, I would like to thank **Associate Prof .Dr. Chitra Bahadur Baniya,** for his immense help in data analysis. Also, I would like to express my sincere thanks to **Prof. Dr. Surya Ratna Gubhaju**, for his help in feed formulation.

Personally, I am most grateful to my **family** who has always supported me with their love and concern. I could not have finished my study without their encouragement, patience, sacrifice and sympathy.

Finally, I would like to thank my friends, **Dipa Rai , Shruti Shakya and Niraj Khadka**, for their help and substantial assistance during the field trial.

ABSTRACT

Several studies have conducted of utilizing alternative source to substitute fishmeal, as earthworm contains the essential amino acids and crude protein. Its special attributes have been demonstrated by chemical studies, animal experiments and digestibility examination. The main aim of this study was to explore the effect of feed supplement containing earthworm (*Eisenia foetida*) on growth performance of Common Carp (*Cyprinus carpio).* Four types of feeds were formulated, control diet, which was formulated with the commercial granulated feed and the rest three were formulated by result of the experiment, the biggest values of Specific Growth Ratio (SGR) were registered at the group fed with 6% earthworm meal in comparison with other treatments, spiking from the initial average mean weight of 7.13g to final average mean weight of 37.22g. The control diet and 2% and 4% earthworm meal has given almost same result showing no significance difference between them. The food conversion ratio (FCR) recorded by 6% earthworm meal was better (1.77±0.18) from economic point of view. Similarly, Protein Efficiency Ratio (PER) and Economic Conversion Ratio (ECR), have shown significant growth and reduction cost at 6% inclusion of earthworm meal. From this result, it can be concluded that *Eisenia foetida* can be used successfully in common carp culture as protein supplement source by partial replacing the fishmeal.

Keywords: Earthworm powder, *Eisenia foetida*, growth performance, *Cyprinus carpio,* Common carp.

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LIST OF ABBREBIATION

Abbreviated foam	Details of abbreviation
AFCR	Apparent Feed Conversion Ratio
CP	Crude Protein
DFTQC	Department Of Food Technology and Quality Control
DO	Dissolve Oxygen
DOFD	Directorate of Fisheries Development
FAO	Food and Agriculture Organization
FM	Fishmeal
EM	Earthworm Meal
PER	protein efficiency rate
PWG	percentage weight gain
SR	Survival Rate
IMW	Initial Mean weight
FMW	Final Mean Weight

1. INTRODUCTION

1.1 Background

Fishmeal has immense contribution in fish's health, as it provides crude protein and also has essential amino acids, vitamins contents, palatability, growth factor, and attractant properties. Because of presence of all crucial nutrients, required for the better growth of fish, has made the fishmeal the only peerless protein ingredients, most globally used in aqua feed (Goddard et al. 2008, Hardy and Barrows 2002). But the fishmeal has limited reach to fulfill the demand of aquaculture (Ng 2001). And it is becoming more expensive due to declining stocks of fish. Therefore, for the sustainability of the aquaculture, it has become a matter of concern to acquire the alternative to fishmeal (Sogbesan 2008). Eagerly seeking the alternative feed, as global fish consumption rate has been increasing last few decades (FAO 2012). Several studies have been conducted on earthworm with the hope to acquire the sustainable food source for aquaculture. Also in recent years, vermiculture is grabbing attention as they are the supplier of vermicompost and also they are the source of earthworm (Kostecka and Paczka 2006). Earthworms have economic value as they possess the potentiality of ameliorating of some basic issues related with organic waste (Dedeke et al. 2007, Elizabeth et al.1985, Sogbesan & Madu 2008,Pucher et al. 2014, Mohanta 2016). Earthworm can be the source for protein supplement in aquaculture because it has high crude protein content and essential amino acids (Hilton, 1983). According to Medale et al. (2013), in replacement of the fish meal, the proteinic sources must bring the ten essential amino acids (EAA) required for fish. Earthworms are easy to produce at reasonable cost due to the fact that also be reared on a small scale in boxes or container made from different materials (Sogbesan et al. 2006)**.** They breed and multiply in large number within month. (Sogbesan and Madu 2003). Common carp, on the other hand, is the world's third most cultured fish species and has highly adaptive capacity to both environment and food nutritional value (soltani et al. 2010). In view of the high nutrient value of the earthworm, the current study was conducted to see the effect of feed supplement containing *Eisenia foetida* on growth performance of *Cyprinus carpio*.

1.2 *Eisenia foetia*

Eisenia foetida is a species of earthworm, which is globally utilized for vermicomposting. It is the smallest species among the other species of earthworm. These worms are usually

grown by farmers and folks who are into gardening who use their compost as a fertilizer. They are found all around the world except Antarctica. *Eisenia foetida* is also used by fisherman as a bait for fishing trout, pan fish etc. Besides, people who own fish tank use these worms as fish food. These worms, which are the capable of making the soil fertile for improving soil health and for plant health has 60-70% crude protein and all the ten essential amino acids and protein higher than hen egg and higher than that of cow milk and soyabean. eprotein supplement in the diet of fish (Hilton 1983, Sogbesan and Madu 2008). Which are required for the proper growth of the fish (Ebadi et al. 2008, Tuan 2010). Therefore, the earthworm can be the alternative source for protein in aquaculture and, it is easy to produce at low cost due to the fact that also is grown on a small scale in boxes made from different materials (Kotechka and Paczka 2006). So, this study is designed to evaluate the potential of earthworm meal *(E .foetida*) as the replacement of fish meal for culture of Common carp (*Cyprinus carpio*).

Cyprinus carpio

Globally, the most important fish species produced in fish farming is carp. The common carp are artificially breed in the farm. In everyday life, commercial, artisanal and sport fishers use the short scientific name *Cyprinus carpio* in their publications.

According to different authors (Jhingran and Pullin 1985,Pintér 1989, Kuznetsov, Aminova and Kuliev 2011), scientists have identified four subspecies of common carp. These are as follows: *Cyprinus carpio carpio* – European Transcaucasian common carp (from the Danube River basin in the natural waters up to the Ural Mountain range), *Cyprinus carpio aralensis* – Aral common carp (Central Asia) *Cyprinus carpio haematopterus* – Amur common carp (Amur River basin to southern China) and *Cyprinus carpio viridiviolaceus* – North Vietnamese common carp. The body of native forms and improved strains may vary from elongated to deep oval. There are two basic forms of common carp: *Cyprinus carpio* morpha hungaricus and *C. carpio* morpha acuminatus. The first has an elongated torpedo-like body, while the body of the second is short and stocky, with a high shoulder (Pintér 1989). These basic forms are presented by Balon (1995) as typical wild common carp and a feral form from the Danube delta. There are four basic types of scaliness: scaly carp, mirror carp, linear carp (also called frame carp) and leather carp (Figure 2, from upper left to bottom right). The transitional forms are the

strongly or slightly-scaled irregular mirror or scattered carp (Pintér 1989, Bakos and Gorda 2001). Body shapes of the different forms and strains are described by the profile, head and width indexes. The profile index is the ratio of body length to body height, the head index is the ratio of body length to head length, while the width index is the ratio of body height to body width. The colour of the body varies from gray through silver to bronze with a yellowish or reddish belly. Common carp has one long dorsal fin which possesses 2–3 hard and 17–22 soft rays. The first (largest) hard ray is sharp and is serrated on its posterior margin. Additional morphological characteristics include 2–3 anal spines, 5–6 anal rays and 36–37 vertebrae (Froese and Pauly, 2011). The mouth is large and opens in an accordion-like fashion. There are two pairs of barbels, one pair on the upper lip and the other pair at the corners of the mouth. There are 5–5 molar-like pharyngeal teeth serving to grind the food. Common carp occur within the temperature range of 3–35 ^{o}C (Froese and Pauly, 2011). The optimum water temperature for growth and propagation is 20–25 ^{o}C. In nature, common carp live in the middle and lower sections of rivers and in areas where the water is shallow (only a few meters deep) and the bottom is muddy. Common carp has been introduced into practically all countries where there is a chance for successful reproduction. In many of the natural waters where it has been introduced, the common carp is considered as an invasive species whose populations should be reduced or even eliminated. Still, common carp is one of the most widely cultured freshwater fish species in the world (Welcomme, 1988; Hasan *at al.*, 2007; FIGIS, 2011). Aquaculture production of common carp increases parallel to the increase of global aquaculture production of freshwater fishes. According to FAO (FIGIS, 2013), production of freshwater fishes was 31 839 573 tonnes in 2005, and increased to 45 335 385 tonnes by 2011 (an increase of more than 42 percent). During these years, the contribution of common carp to the global aquaculture production remained at about 8–9%.

1.4 Fish food

Fishes are species specific; they consume various sorts of food on fish species and its feeding behavior. It may feeds on foods of plant, animal or bio waste origins. In modern aquaculture system, fishes are fed with supplementary foods along with natural food for maximum yield. The supplementary foods are made according to the species reared. Some of the byproducts like poultry, animal husbandry, fish farm, waste food materials etc. has been utilized as alternative sources of fish food.

1.5 Common feed ingredients used in aquaculture

1.5.1 Ingredients of plant origin

Fish are species specific, therefore, the type of food they prefer are also specific according to the species. Some are herbivorous, some are carnivorous and some are omnivorous. In modern aquaculture system fishes are fed with supplementary food are more according to the species reared. Some of the byproduct like poultry, animal husbandry, fish farm, waste food materials etc. has been utilized as an alternative sources of fish food.

Various plant origin ingredients has been used in fish feed researches in order to evaluate its efficiency to replace animal stuffs such as fodder plants, oil cake and oil meals, aquatic weeds (El – sayed 1991), Luecaena leaf (Wee and Wang 1987). Legume seed (Keembiyehetty 1993) roof and tubers (Tusan et al. 2013) and cereal and cereal by products (Santis et al. 2016). Some of the mostly used plant origin ingredients in aquaculture are mustard oil cake, soybean, rice barns, maize and wheat flour etc.

1.5.2 Ingredients of animal origin

Animal origin ingredients are comparatively rich in protein, essential amino acids, especially lysine and methionine that are limiting in plant sources. These includes fresh and dry meat of various fish (casteneres 1990), blood, earthworms (Hilton 1983, Rawling et al. 2012) and insects (Fasakin et al. 2003)

1.5.3 Ingredients in bio waste origin

The bio waste origin ingredients includes fish silages, milk by products, kitchen by products and slaughter house by products (Cheng et al. 2015) which are regarded as the waste are being used as supplemental feed in aquaculture to reduce the feed expenses and biological waste management practices.

1.5.4 Miscellaneous feed ingredients

It includes cane molasses, brewer's yeast, leaf protein concentrate and grain distiller by products, algae, yeast (Ran and Zhou 2016) and bacterial protein etc.

OBJECTIVES

General objective

- To investigate the effect of feed supplement containing earthworm on the growth performance of common carp (*Cyprinus carpio*)

Specific objectives

- To explore the growth performance of Common carp fed with different proportions of earthworm meal.
- To find the effect on Feed Conversion Ratio (FCR) of Common carp fed with different proportions of earthworm feed.
- To compute Specific Growth Rate (SRG).

1.7 Rational of the study

Earthworm, *Eisenia foetida*, has protein content comparable to that of fishmeal (Sogbesan et al. 2007, Kostecka and Pączka 2006) and suggests that the combination of palatable and highly nutritious earthworm protein is superior to plant-based proteins, which frequently lack sufficient sulfur-based amino acids and are often unappetizing to cultured aquatic animals. Replacement of fishmeal with earthworm meal may also be beneficial to piscivorous humans. The FAO estimates that aquaculture production worldwide will need to increase to 80 million tons by 2050 to sustain the current demand (Subasinghe 2012). The raw materials for cultured fish feed are becoming increasingly scarce and higher in cost, so alternative protein sources are being investigated. The use of earthworms as a protein source for cultured aquatic species (fish and shrimp) is gathering substantial attention worldwide. Producing earthworms locally, using organic wastes, is increasingly seen as an inexpensive method for supplying high-quality protein for farmed fish.

LITERATURE REVIEW

Nepal has tremendous ability for aquaculture as it has wide varieties of water bodies lying at altitudes ranging from 60m to 8848m which facilitate good commercial culture of varieties of fishes with little supplementary feed. Nepal is self-reliance in food cereal and mill by products such as maize bran, wheat bran and rice bran for fish feeding but in terms of major protein source for fish diets is imported fishmeal because oil seeds are not grown in sufficient quantities in our country, which are used as the protein sources in the fishmeal. The lack of domestic sources of protein feed has been and will continue to be a major constraint in the development of the feed mill industry (FAO 2012).

Earthworm *(E. foetida*) is one of the alternatives that can be used as a substitute, because earthworms have a protein content of 60.4% and amino acids and also essential minerals (Hilton 1983, Shakthika 2014) comparable to fishmeal. (Nandessha 1988) has indicated the possibility of using earthworm meal at low levels in common carp diets. However, about the usage of earthworm meal in different proportions in fish feed has shown both negative and positive influence on growth rate and on protein and energy efficient usage. Which had recorded reduced growth rate and feed utilization in rainbow trout when high levels of worm meal are included in the fish feed (Hilton 1983).

Study on Protein from earthworm meal had shown that it influences reproduction performance and stimulates fry appetite (Nguyen et al. 2010). Another, research by Kostecka and Paczka(2006) depicts that feeding guppy fish (*Poecilia Recticulata*) with earthworm influences both growing and body development of the obtained products. There are some research which had shown some contradiction, inclusion of earthworm meal by 0%, 30%,70% and 100% in the diet of Common carp, *Cyprinus carpio* has got no growth effect rather the control diet gave better result(Krome and Focken 2016). Fish fed diets containing high levels of whole frozen worms suggested an adverse effect of worm incorporation, probably due to dietary energy/protein imbalance (Periera and Gomes 1995).

Study had shown that the addition of earthworm meal in the diet of *Clarius gariepinus* fry; give no adverse effects on growth and nutrient utilization (Dedeke et al. 2013). Even, Combination of earthworm and maggot were formulated in the ratio 2:5; 1:4; 1:12 and 0:1 on growth performance of *Clarius gariepinus* fingerlings in which it was noticed that the

ratio of2:5 between the two worms is used to entirely replace fishmeal (Arnauld et al. 2016)

(Georgescu et al., 2016) had registered better growth in 10% inclusion of earthworm meal than the commercial feed and 20% earthworm meal.

The fish feed on earthworm meal prepared from water hyacinth showed 51.87% increase in Body weight gain, 22.22% increase in Specific growth rate, 51.43% decrease in Food conversion ratio and 55.29% increase in Protein efficiency ratio than the control group of fish (Sakthika et al. 2014)

Feeding *P.excavatus* meal to mirror carp decreases some aspects of the innate immune response, but at the same time gives rise to significant enhancement of growth and feed utilization efficiency (Rawling 2012). The diet with 5% sardine oil induced the best growth of fish, in comparison with the diet containing the earthworm (Nandeesha 1988).

Culture and utilization of earthworm as animal protein supplement in the diet of *Heterbranchus longifilis* fingerlings, resulted that agro-allied waste substrate could be better culture substrate for *H. euryanlos* than soil substrate and 7.5 to 25 % earthworm meal inclusion is recommended in the diet of *H. longifilis* fingerlings for profitable and sustainable aquaculture practices (sogbesan et al. 2007)

Study on earthworm meal as fishmeal replacement in plant based feeds for Common carp in Semi-intensive aquaculture in Rural Northern Vietnam, in which fishmeal protein was replaced by 0 %, 50 % or 100 % of protein from sun dried earthworms (*Perionyx excavatus*). At both stocking densities, control groups of fish fed only on natural food resources. The growth rate of fish increased with rising replacement of fishmeal by earthworm meal at both feeding rates. Large zooplanktons were the predominant natural food resource. With increasing availability of large zooplankton, sun dried earthworm meal in plant-based supplemental feeds seemed better able to meet the nutritional requirements of common carp (Pucher et al. 2014)

Formulation of fish feed with optimum protein-bound lysine for African catfish (*Clarias gariepinus)* fingerlings, Earthworm powder and fish meal have contributed the largest portion of lysine in fish feed due to high lysine content which were 4.48 % w/w and 3.60 % w/w, respectively. The optimized fish feed shows high lysine content of 23.39 % w/w

which doubles the lysine content in commercial fish feed (11.21 % w/w). The composition of fish feed obtained from this study can be used as guidelines for formulation of high lysine fish feeds for African catfish fingerlings (Hamid et al. 2016)

Also number of Several researchers have reported the use of earthworm as dietary protein source either alone or in combination with other feed ingredients in formulating the fish diets (Edwards and Niederer 1988, Guerrero 1981, Hartenstein et al. 1979, Sogbesan and Madu 2008, Mohanta 2016, Chaves et al. 2014, Kim et al 2016, Fahah et al. 2017, Chiue et al. 2016 and Beg et al. 2016). As we know, the fish meal cannot support the aquaculture for long; it has become crucial to look for alternatives to substitute it. Earthworm has many essential amino acids and high crude protein contains which has attracted attention. Numerous studies have been conducted on earthworm (*Eisenia foetida*). Hence, vermicomposting can be a good idea for overcoming the problems related with waste management and at the same time can be the best supplement for the better growth of fish, if the production rate of earthworm extends locally.

3. MATERIAL AND METHODS

3.1 Experimental design

The study was carried out in Central Fishery Laboratory and Conservation Department, Balaju, Kathmandu, Nepal. The experiment was conducted in happa fixed in one of the commercial fish production earthen pond. Twelve nylon happa of same size 2m × 1.5m × 1.5m were used for experimental purpose. All these happa were suspended in four different clusters in a single pond. Pond was pre-limed and pre- fertilized. Happa in this pond were suspended by tying to bamboo poles with brick pieces as sinkers at the bottom. Freshwater from a bored tube well was filled in pond at regular interval to maintain the water depth of about 1.5 m in pond and 1m in happa.The experimental design for present research was completely randomized design (CRD) with four feeds as control diet T0 (Feed I), treatment T1 (Feed II), T2 (Feed II) and T3 (Feed VI). Each treatment was replicated thrice different cluster. Altogether 20 fish were stocked in each happa. Feeding of fingerlings started from next day of stocking in happa. The experimental fish were fed at 3% of their body weight per day for two months continuously. Feed for next month was adjusted according to monthly weight sample. Feeding was done twice, in the morning at 10.00 am and in the evening at 4pm.

3.2 Collection of fingerlings

The Fingerlings of Common carp were collected from the production pond of "Central Fisheries Promotion and Conservation centre" Balaju, Kathmandu, using a sein. Fingerlings were sorted and only uniform sized were selected for stocking. Individual weights of 20 fish were taken and batch weights of fish to be stocked in a happa were also recorded. The mean stocking weight of fish was 7g.

3.3 Vermiculture

Matured *Eisenida foetida* were collected from local vermicomposting farm. Total of 10 plastic containers of sized (3feet× 3feet ×3feet) were used. At first, small pebbles layers were made at the bottom. And soil bed layer was made on it, then, cow dung was spread over the soil and organic wastes of 1 to 2 inch layer were made at the top. Big quantity of organic waste at once was avoided to halt the heat secretion inside the box or container

and small holes were also made on it. *Eisenia foetida* were released on the prepared layers. For moisture, water was sprinkled twice a day and the earthworms were fed with all types of organic waste except citrus waste. The compost bin was placed in corridor.

3.4 Feed preparation

3.4.1 Cleaning, Drying and Grinding

Earthworms were washed and kept in warm water for 1 hour. After that, it was shade dried for 4 days under observation. And grinded using mixture and sieved to obtain earthworm meal power in homogenized form.

3.4.3 Feed Formulation

Four experimental diets were formulated using mustard oilcake, Soy bean, rice bran, Fishmeal, wheat flour and Earthworm as diet ingredients which were procured from local market, earthworm by local vermicomposting. Diets were prepare with about 30% analyzed crude protein .Vitamin and Mineral premix (Agrimin) was also mixed in each type of feed at the rate of 1%. Crude protein content of different feed ingredients and formulated feed were analyzed by ANTARI II DR (FT-NIR Analyzer), India. Feed formulation was done using Pearson Square Method. Feed ingredients were mixed according to requirement and feed was prepared.

Table 1. Formulation and composition of experimental diets

S. No.	Ingredients	Inclusion percentage			
		Control Diet(T0)	Diet I(T1)	Diet II(T2)	Diet III(T3)
1	Mustard Oilcake	23	23	23	23
2	Fishmeal	23	21	19	17
3	Rice Bran	15.5	15.5	15.5	15.5
4	Soybean	23	23	23	23
5	Wheat flour	15.5	15.5	15.5	15.5
6	Earthworm meal	0	2	4	6

Table 2. Proximate composition of different feed ingredients.

Ingredients	Crude protein	Crude fat	Ash	Fiber	Moisture
Mustard oil cake	34.41	13.36	11.83	1.11	8.29
Fishmeal	44.61	3.81	15.50	1.79	12.30
Rice bran	12.71	16.31	6.52	2.23	9.96
Soybean	38.13	7.17	13.12	1.06	10.67
Wheat flour	11	7.24	6.51	1.65	12.36
Earthworm meal	54.54	7.77	18.09	0.44	10.86

3.5 Feeding

The experimental fish were fed at 3% of their body weight per day for two months continuously. Feed for next month was adjusted according to monthly weight sample. Feeding was done twice, in the morning at 10.00 am and in the evening at 4pm.

3.5 Growth check

All the fish were scooped out using scoop net for growth check. Growth check was done in interval of every 15 days during which weight and length of individual fish was recorded by digital balance (model) and measuring scale (stainless scale). The growth checking was done in the morning period around 6.00 to 7.00 am to avoid stress to fish. No mortality was recorded during these periods.

3.7 Water quality parameters

The water quality parameters like temperature, dissolve oxygen, pH, chlorophyll, ammonium, ammonia, chloride, orthophosphate and conductivity reading were studied weekly using Multiparameter Water Quality Sonde (model 6600V2). Manufactured by YSI (USA). The readings were taken at morning hour sharp at 11am.

3.8 Data analysis

Data analysis were done by using following formulae

$$\text{Specific growth rate(SGR)} = \frac{\text{In (FMW)} - \text{In (IMW)}}{\text{Culture period (days)}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Total harvested number}}{\text{Total stocked num ber}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Quantity of feed intake (g)}}{\text{Net weight gain(g)}}$$

$$\text{Protein efficiency rate (PER)} = \frac{\text{Wet body mass gain}}{\text{Protein intake (g)}}$$

$$\text{Protein Weight Gain (PWG) (\%)} = \frac{\text{final mean weight}}{\text{Intial mean weight}} \times 100$$

$$\text{Economic Conversion Ratio (ECR)} = \text{FCR} \times \text{feed cost}$$

Statistical analysis

Statistical differences between diets were evaluated by one way analysis of variance using R Software of version R-5.3.2. Experimental data were evaluated as mean ± SD.

4. RESULTS

Growth parameters

Positive result had been acquired by the present experiment as the fish showed good growth performance by quintupling their body mass during the period of 8 weeks. The initial average mean weight of 7.13g spiked to final average mean weight of 37.22g. The control diet, 2% and 4% earthworm meal has given almost same result showing no significance difference between them. No mortality was observed during the experimental period and diets were all consumed by the fish.

Specific growth rate (SGR)

The SGR of fish in different treatments during the interval of 60 days of data sampled is shown in figure 1. Overall, the highest inclusion of earthworm meal has given the better result. The 6% supplement of earthworm has given the highest value of SGR 2.75±0.18 and control has given the least value of 2.19±0.15. From initial day to day 15 the SGR pattern is in ascending order of different treatments T0, T1, T2 and T3. After day 30 there was a bit difference in the SGR, as the value of T1 and T2 is almost same. By the day 45 surprising result can be seen as the control diet has given the higher value than the T1and T2 but T3 is still the highest. By the end of day 60 the value of T0,T1 and T2 has shown almost close values and the T3 still continued to be the highest.

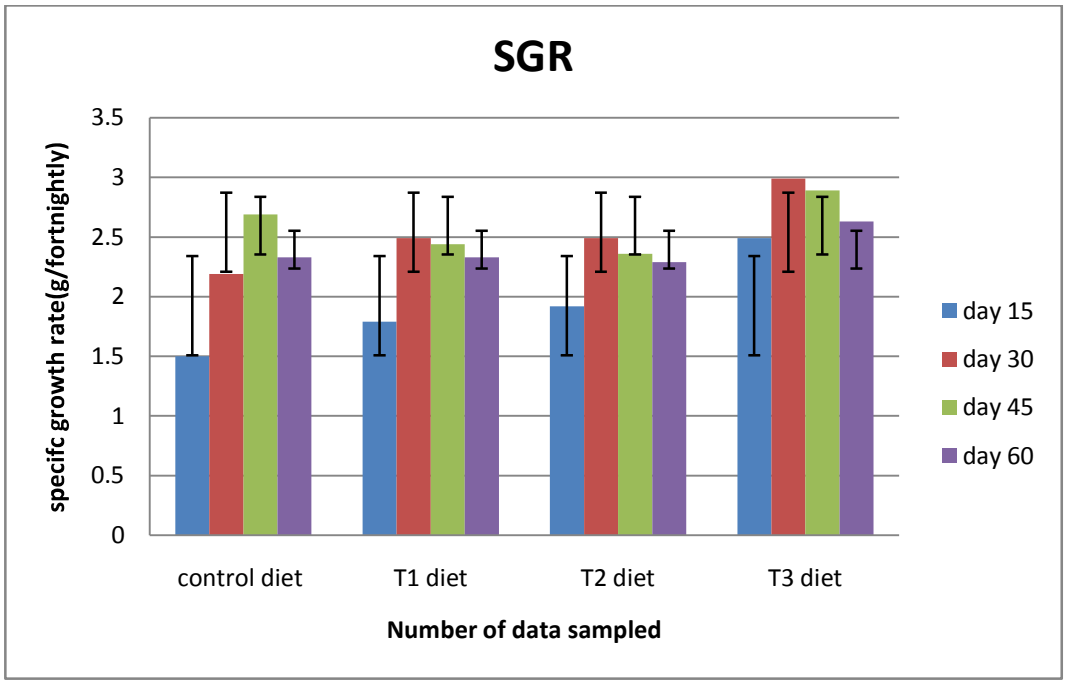

Figure1. Specific Growth Rate of fish in different treatments during sampling.

Feed conversion ratio (FCR)

The Feed Conversion Ratio of fish in different treatments during the 15 day interval of data sampled is shown in figure 2. As a whole, higher earthworm meal has the best FCR value and low earthworm meal has the worst FCR. The initial 15 day FCR was in descending order of T0, T1, T2, and T3 with value of 2.37 ± 0.62^{a}, 2.31 ± 0.44^{a}, 2.25 ± 0.31^{a} and 1.77 ± 0.18^{b}. After day 30 the value was decreased by in each treatments and 6% has the best FCR among the 4 treatments. Day 45 has shown fluctuation in T1 and T2 and by day 60 again T1 and T2 has given the reverse value than the previous FCR, 6% earthworm inclusion has given the better FCR but not as the day 30, which was the best FCR calculated during the whole experimental period.

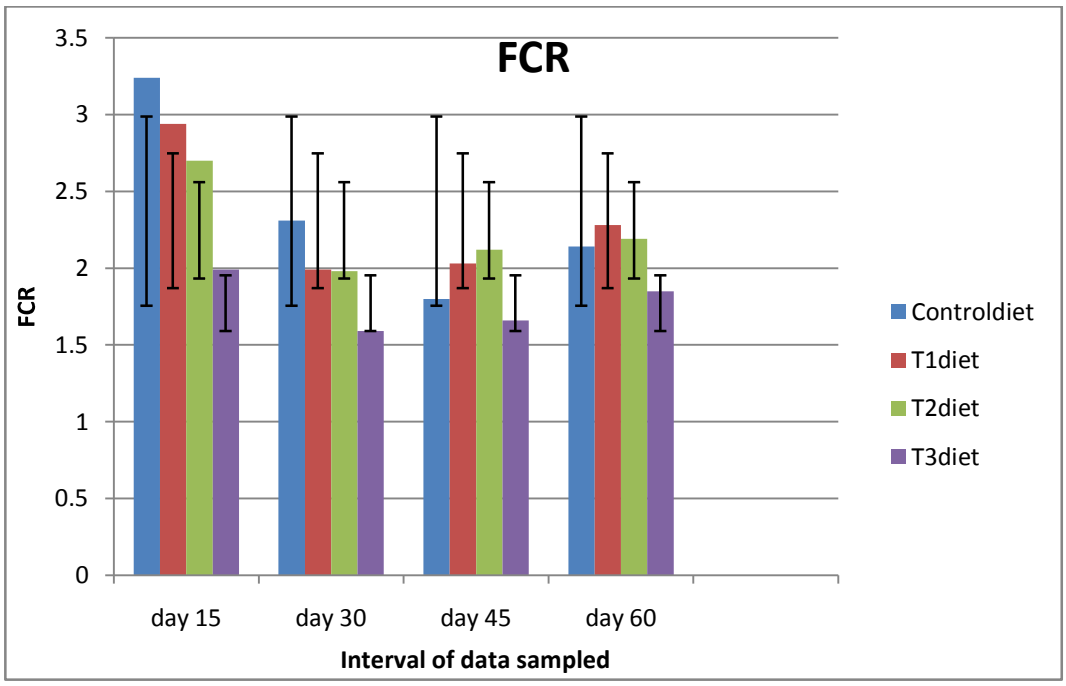

Figure 2. FCR of fish in different treatments

Protein efficiency ratio (PER)

Protein Efficiency Ratio in different treatments is shown in table 2. PER has shown better in addition of 6% earthworm meal, which is related with the weight gain with intake of a particular food protein during the test period. The value recorded were1.43 ± 0.02^{a}, 1.43 ± 0.01^{a}, 1.41 ± 0.03^{a} and 1.77 ± 0.06^{a}.

Protein Weight Gain (PWG)

Percentage Weight Gain in different treatments is shown in table 2. Protein contents of the fishes increased significantly for all dietary treatments. However, the highest protein gain was noticed for fishes fed diet T3 and the least protein gain was noticed in T0.

Economic conversion ratio (ECR)

Economic Conversion Ratio in different treatments is shown in table 2. The cost of each experimental feed was determined on the basis of the cost of the ingredients used for its manufacturing. The feed cost decreased but not in high amount because the inclusion percentage of earthworm replacing fishmeal was quite low in quantity. The ECR values recorded were 3.98± 0.54, 3.90± 0.21, 3.69 ±0.28 and 3.18 ± 0.15 of T0, T1, T2, and T3.

Table 2. Growth parameters and feed utilization parameters in different treatments.

Parameters	T0	T1	T2	T3
Initial mean weight (g)	7.13±0.11[a]	7.13±0.11[a]	7.13±0.11[a]	7.13±0.11[a]
Final mean weight (g)	26.69±0.05	27.25±0.20	27.83±0.19	37.22±0.15
Protein efficiency ratio (PER)	1.43±0.02[a]	1.41±0.03[a]	1.43±0.01[a]	1.77±0.06[b]
Percentage weight gain(PWG)	373.5±6.30	381.4±8.87	389.4±4.68	590.9±11.26
Survival rate (SR)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Economic conversion ratio(ECR)	398±54	390±21	368±28	318±15

Note: Mean values with the same superscripts in each row are not significantly different ($p < 0.05$). Values are the means of three replicates ± standard

Water quality parameters

Temperature

The temperature recorded throughout the experimental period is shown in figure 5. The average temperature during the study period was. It shows decreasing trend of temperature throughout the experimental period. The highest temperature registered was 27.02 degree Celsius on 3rd October, 2018 at 11:20 am. And the lowest was registered 18 degree Celsius on 22nd November, 2018 at 11:20 am.

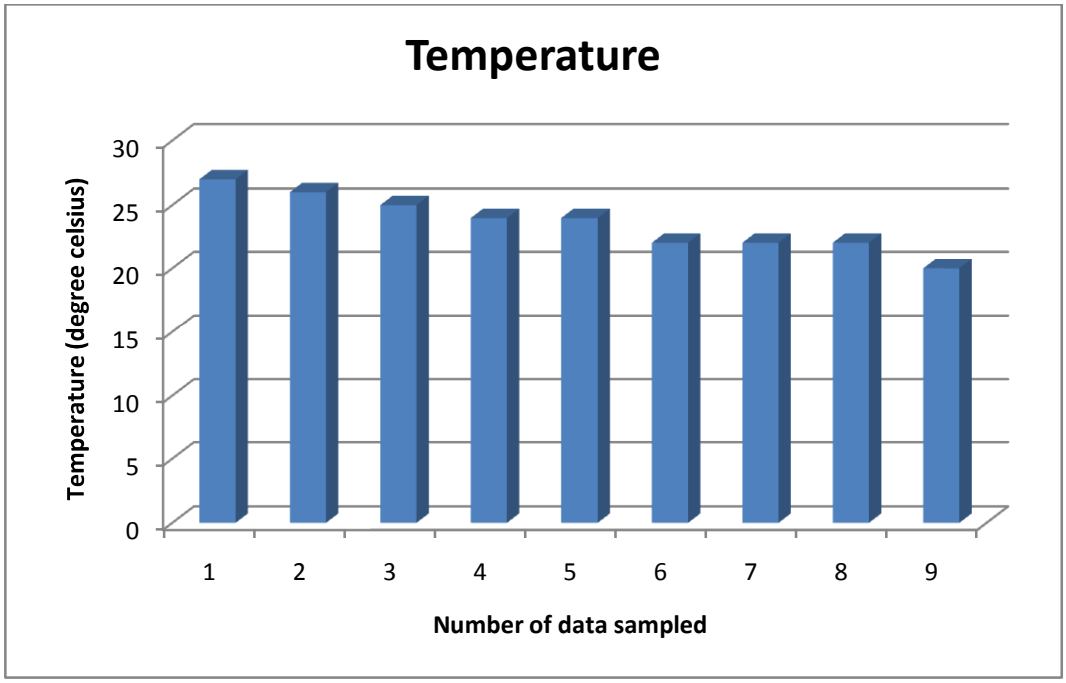

Figure 5. Temperature of the pond water taken weekly throughout the experimental period.

Dissolved Oxygen (DO)

The dissolved oxygen of pond water throughout the experimental period is shown in table 6. The highest DO recorded during the study was 8.5 mg/l on 20th November at 11:20 am and the lowest was recorded was 6.48mg/l on 17th October at 11:20 am.

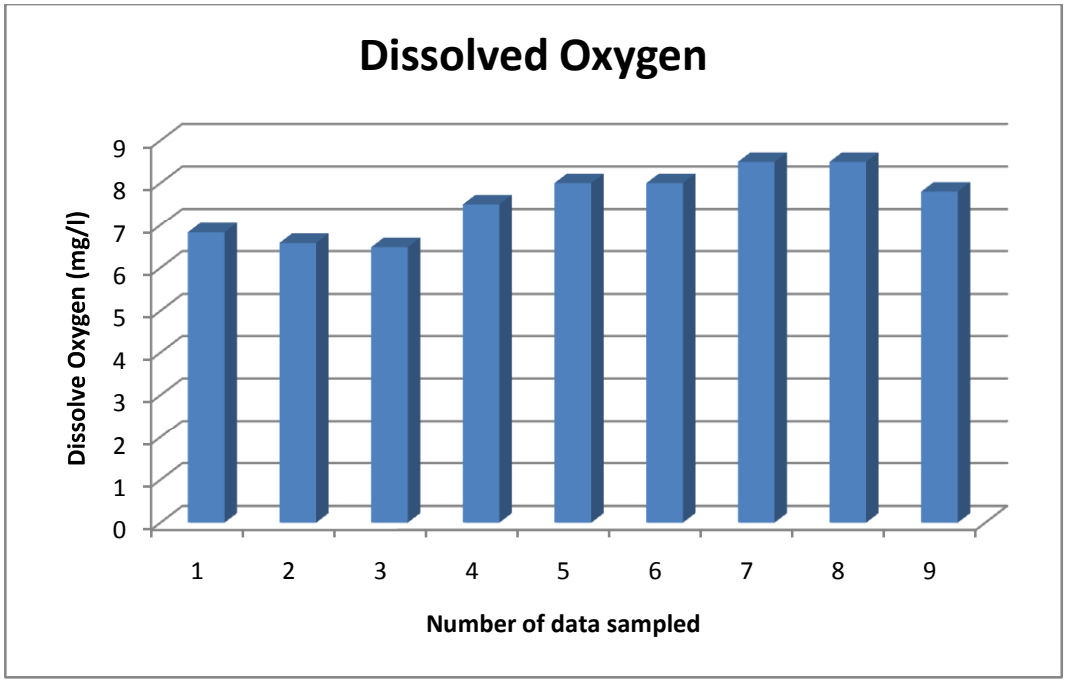

Figure 6. DO of the pond water taken weekly throughout the experimental period.

pH

The pH of pond water taken throughout the experimental period is shown in figure 7. The highest pH value registered was 7.5 on 3rd October, 2018 at 11:20 am. And the lowest was 6.8 recorded on 24th October, 2018 at 11:20 am.

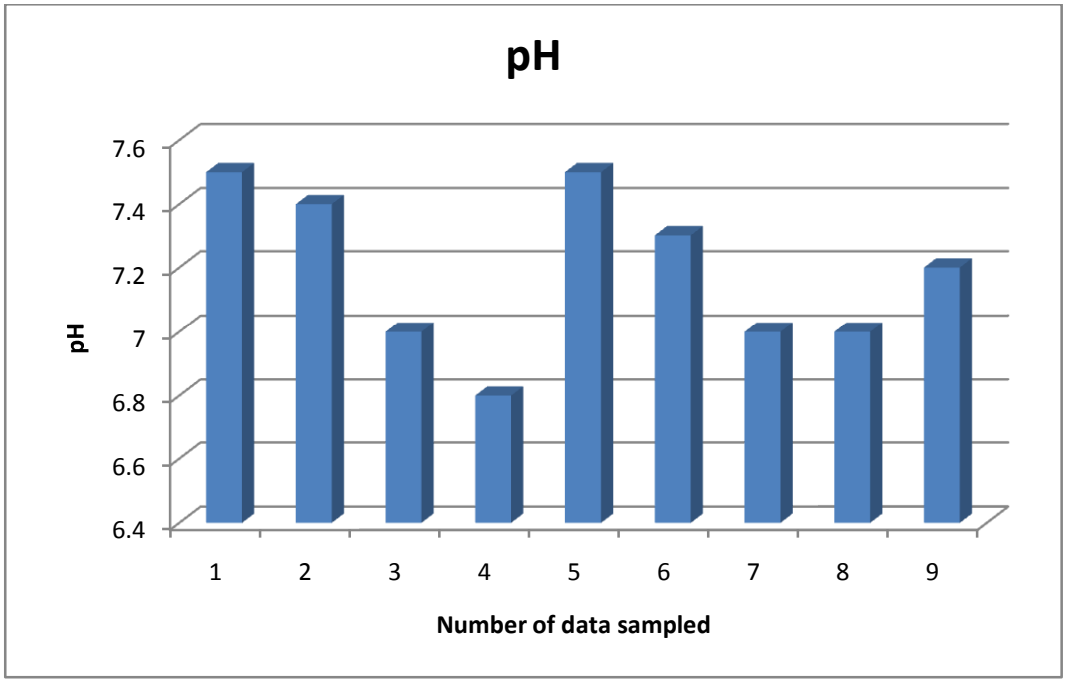

Figure 7. pH of the pond water taken weekly throughout the experimental period.

Ammonia

The ammonia recorded during the experimental period is shown in figure 8. The highest value was registered on 9th November, 2018 at 11:20 am. And the lowest was recorded on twice on 17 the October, 2018 and on 20th November, 2018 at 11:20 am.

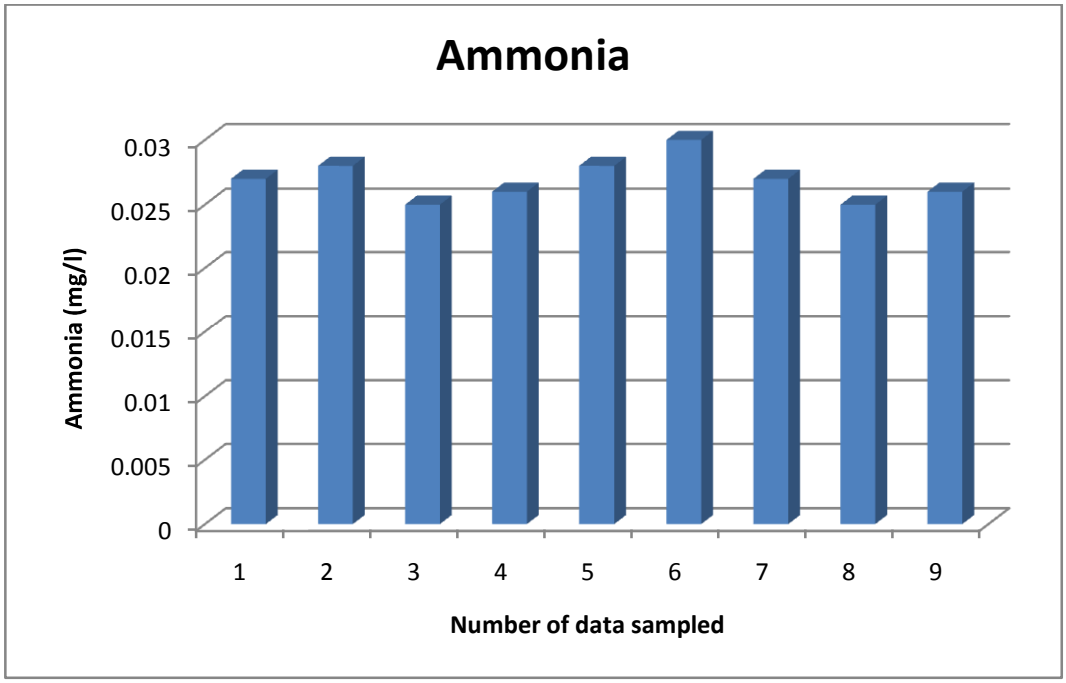

Figure 8. Ammonia of the pond water taken weekly throughout the experimental period.

Chloride

The chloride recorded during the experimental period is shown in figure 9. During the experiment the highest value of chloride recorded was 639 mg/l on 3rd October, 2018. And the lowest value recorded was 602mg/l on 20th November, 2018.

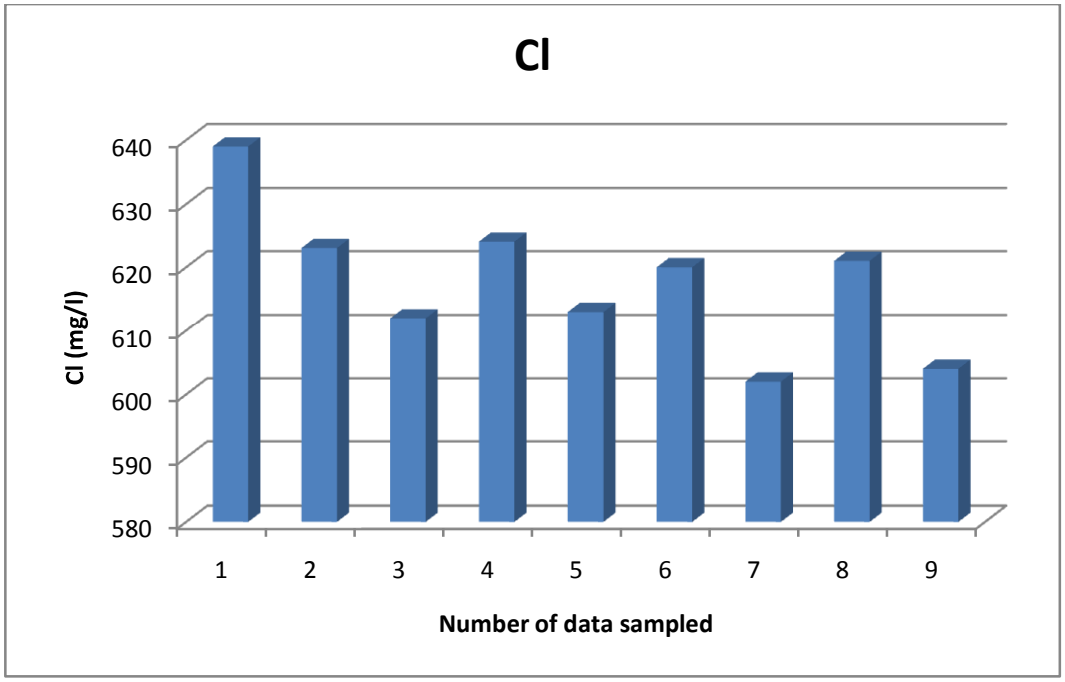

Figure 9. Cl of the pond water taken weekly throughout the experimental period.

Turbidity

The turbidity recorded which was recorded during the experimental period is shown in figure 10. The highest value recorded was 24mg/l on 22nd November, 2018 and the lowest value recorded was 16mg/l on 2nd November, 2018 at 11:20 am.

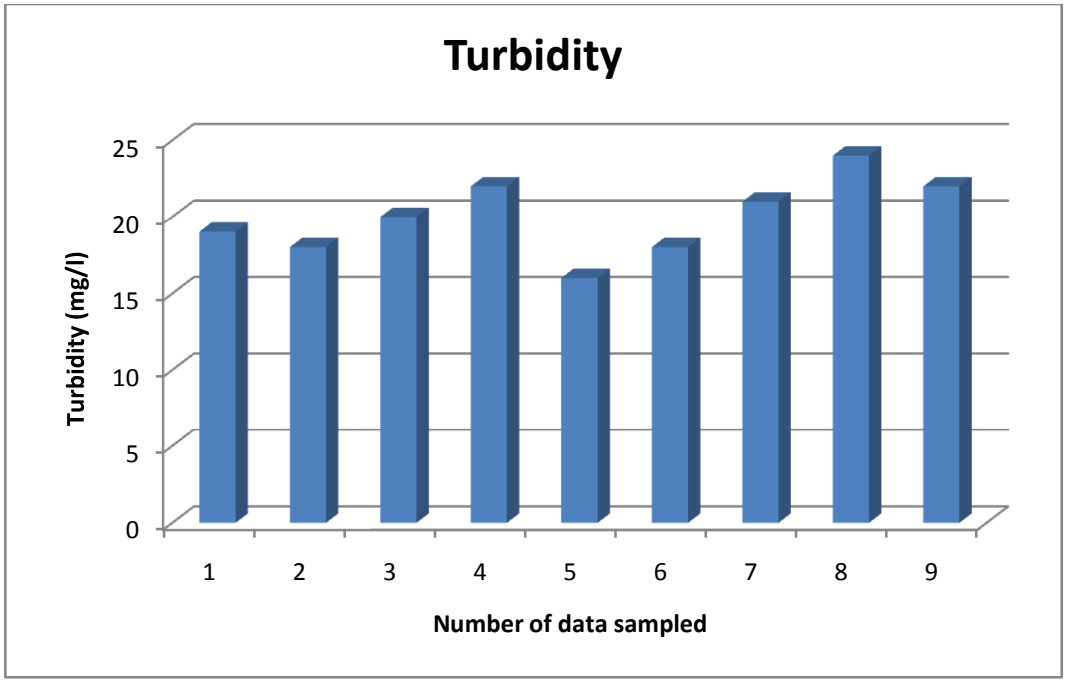

Figure 10. Turbidity of the pond water taken weekly throughout the experimental period.

Chlorophyll

The chlorophyll which was recorded throughout the experimental period is shown in figure11. The highest value of chlorophyll recorded was maximum time from 0.1mg/l which was recorded maximum time from 10th October to 20th November, 2018. The lowest value was recorded on 22nd November

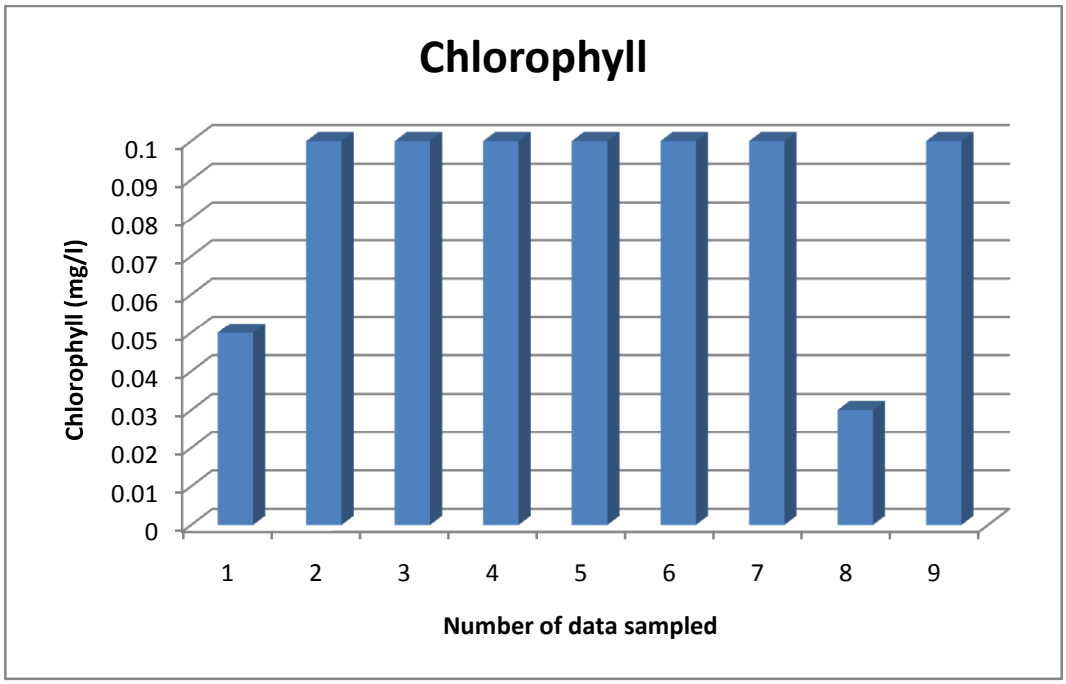

Figure 11. Chlorophyll of the pond water taken weekly throughout the experimental period.

Ammonium

The ammonium recorded during the experimental period is shown in figure 12. The highest recorded value was 0.04mg/l on 24th October, 2018 at 11:20 am. And the lowest recorded value was 0.021mg/l on 22nd November, 2018 at 11:20 am.

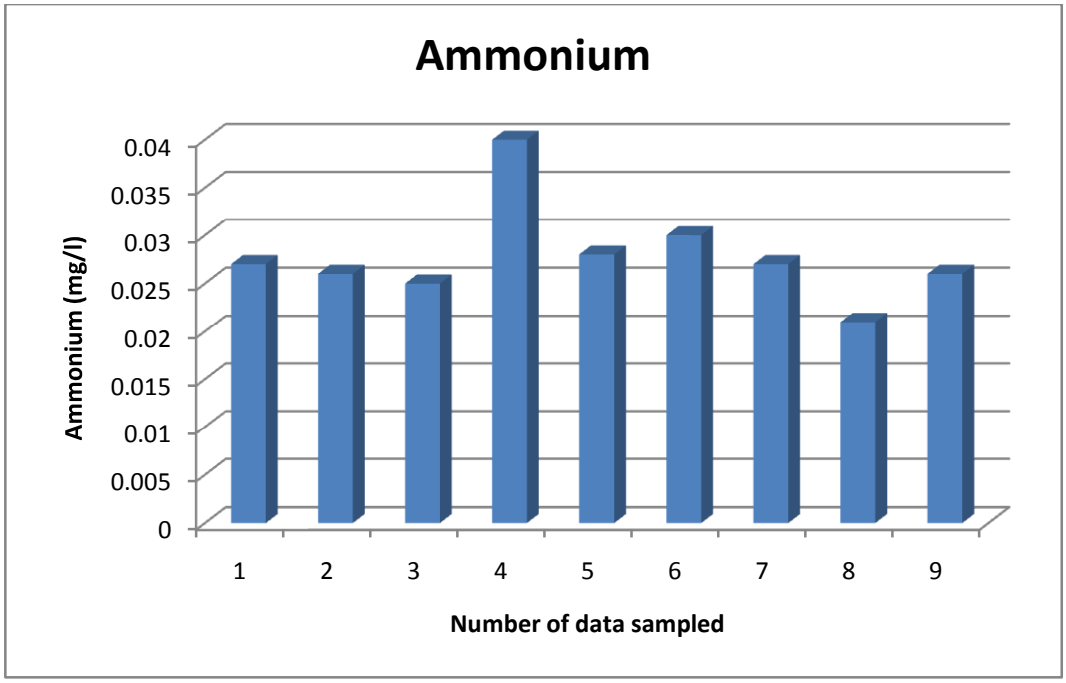

Figure 12. Ammonium of the pond water taken weekly throughout the experimental period.

DISCUSSION

There are several aspects by which the growth of common carp is affected. The main two precursors are environmental conditions and cultural conditions. The present study was carried on good environmental conditions as the experiment was conducted in the natural pond itself and the water quality parameters were to the optimum level. The water temperature for growth and propagation is 20-25degree Celsius (FAO 2019). The food availability was also favored; the fish were fed twice a day, morning and in the evening. The addition of fertilizer in the pond had also assisted for the multiplication of zooplanktons, which further played crucial role in fulfilling the nutritional needs for the fish.

Fishmeal, the only protein source for the growth of fish, has created concern for the search of alternative as it is finite and cannot maintain the sustainability in the aquaculture world. Several studies have been conducted for renewable alternative non-conventional protein source, on earthworm (Georgescu 2016,Focken et al. 2016, Krome and Focken, 2016, Dedeke et al. 2013, Rawling 2012, Sakthika et al. 2014, Hamid et al. 2016, Nguyen et al. 2010,Arnauld et al.2016, Sogbesan and Madu 2008, Mohanta 2016, Chaves et al. 2014, Kim et al. 2016, Fahah et al. 2017, Chiue et al. 2016, Beg et al. 2016, Siti et al. 2016). There are numerous reports that illustrate suitability of different earthworm species as components of aqua feeds while others has advised that the use of only earthworm species can have negative effects on the growth and health of fish (Pucher et al. 2014). However, Inclusion of low level of earthworm meal has given better results (Georgescu 2016, Focken et al. 2016), while high levels of whole frozen worms suggested an adverse effect of worm incorporation, probably due to dietary energy/protein imbalance (Periera et al., 1995). In common carp without free access to abundant natural food resources, the replacement of fishmeal by dried earthworm meal (*Eudrilus eugeniae*, Kinberg 1867) resulted in reduced growth (Nandeesha et al. 1988). And, this was the main reason behind, to design the experiment, to carry out in a pond which was also pre limed and pre fertilized in order to meet the requirements of the live feeds.

The earthworm *Eisenia foetida* is highly nutrious (Kostecka 2006), this positive result can also be reflected in the present study as the SRG of this study is relatively satisfactory despite the low inclusion of the *E.foetida* as the body mass has increased by quintuple.

The lowest FCR value was recorded by the 6% addition of earthworm meal, which showed the significant effect, as it is economical from economic point of view. The highest FCR was observed in the control group (2.37 ± 0.62^{a}), followed byEW2% (2.31±0.44), EW4% (2.25±0.31), while that of EW6% was the lowest (1.77±0.81). This result is relatively satisfactory than previous findings in which the earthworm dose is particularly higher (Ngoc et al. 2015; Kim et al. 2016), but it does not mean increasing the dose can improve the overall growth of the fish species because the study conducted by author (Periera et al.1995) has shown that high inclusion leads to adverse effect in the growth of the fish. Low inclusion of earthworm meal has proven to be conducive in this study. The addition of fertilizer in the pond also helped for the multiplication of zooplanktons, which also played vital role in healthy growth of the fish due to fulfilled nutritional needs throughout the study period. Almost all the fish were healthy and no mortality rates were recorded; this might have resulted because of all the fulfillment of the required nutrients and favorable condition of the pond environment. Similarly, the ECR value has also indicated that inclusion of earthworm supplementing can decrease the feed cost (Arnauld et al. 2016), similar result was indicated in this study too. However, PWG is comparatively lower in present study compared to previous study where significant increment in PWG was observed with increasing ratio between earthworm and maggot meals (Arnauld et al. 2016). Likewise, PER has shown positive results. The PER (1.77) obtained in 6% addition of earthworm in this study is comparable to the PER reported by the author using earthworm protein supplement in the diet of fish (Mohanta 2016).

Vermicomposting, which is environmental friendly process of producing earthworm, has gain a lot of appreciation for its two ways of advantages. Managing waste within own area, and making something useful by it, has made the vermicompost one of the renowned technique of rearing earthworm (Shakthika 2014). Earthworm *(E. foetida*), has a protein content of 60.4% and amino acids and also essential minerals comparable to fishmeal (Hilton 1983).

CONCLUSION

The present experiment on common carp replacing the fishmeal with earthworm protein in order to evaluate earthworm as an supplement dietary protein for *Cyprinus carpio* showed no significance difference among growth parameters like average weight gain, Apparent feed conversion ratio, specific growth rate, protein efficiency ratio and protein percentage gain. The fish fed with 6% earthworm meal has shown better growth than the commercial and 2% and 4% earthworm meal. the physical chemical parameters like temperature, dissolve oxygen, ph, transparency, ammonia, chlorophyll, ammonium, etc were also favorable for the growth of common carp and no mortality were seen when fed with earthworm meal as protein supplement.

Hence, earthworm can be used successfully in common carp culture as protein supplement source by making earthworm (*Eisenia foetida*) locally available though vermiculture in large scale.

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ANNEX

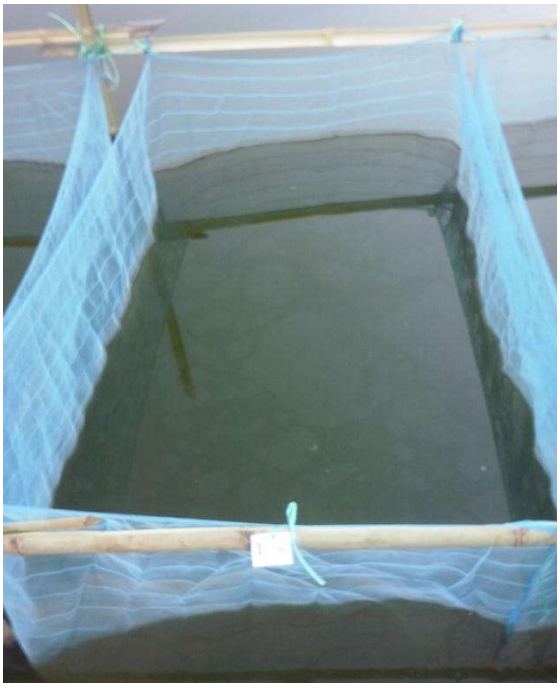
Mixing the different ingredients for fish feed

Measuring the Fish feed

Sorting the fingerlings

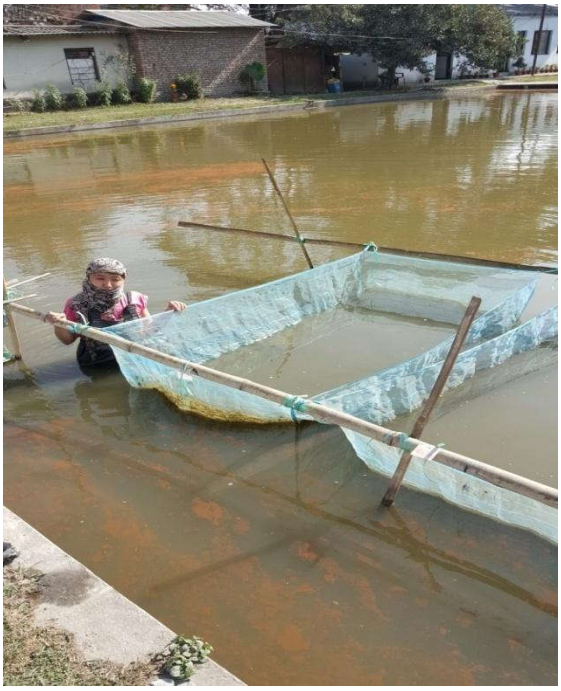
Taking out fingerlings

Completely Randomize Design (CRD)



Hapa with tag

Hapa set in Completely Randomize Design



On the day of data collection

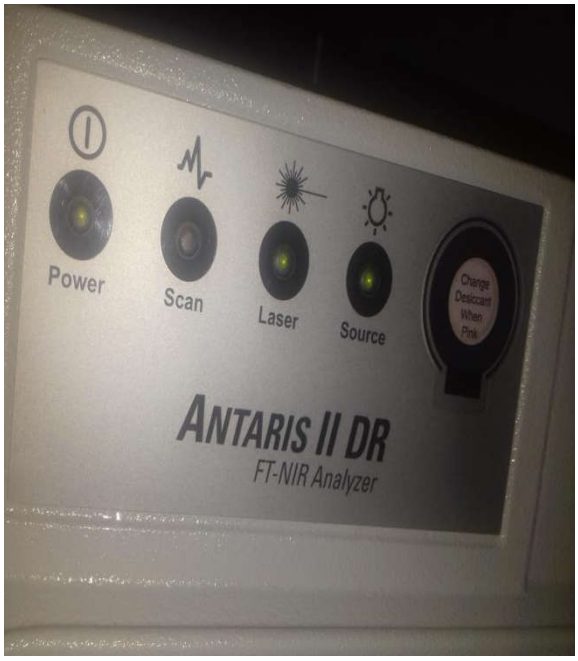
Measuring the fingerlings

On the day of data collection

Measuring the weight of fingerling

On the day of data collection

Multiparameter Water Quality Sonde (model 6600V2)



Feed Analyzer, ANTARI II DR (FT-NIR Analyzer), India

Healthy fingerling

Measuring the length of the fingerling