TO FORMULATE LOW COST FEED BY USING NON-CONVENTIONAL INGREDIENTS TO IMPROVE GROWTH OF *LABEO ROHITA*

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**To formulate low-cost feed by using non-conventional ingredients to improve the growth of *Labeo rohita***" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr. Neeta Raj Sharma, working as Professor and Dean, in the Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “**To formulate low-cost feed by using non-conventional ingredients to improve the growth of *Labeo rohita***” submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)**in the Bioengineering and Biosciences, is a research work carried out by Manju Rani, registration no. 41900778, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

23/1/2024

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ABSTRACT

Background: Aquaculture is becoming the fastest growing food-producing sector in the world, which plays a significant role in mitigating future global demand-supply gap for aquatic food. However, successful aquaculture sector requires, lower production costs, efficient production systems and environmental sustainability measures. Fish feed often accounts for 60% to 70% of the operational costs in intensive and semi-intensive aquaculture systems, making it one of the most expensive inputs in aquaculture production systems. Therefore, it is crucial to offer cost-effective, eco-friendly and nutritionally rich fish feeds. Moreover, there is a possibility to fulfil the demand for fish feed ingredients by replacing conventional protein-rich ingredients through locally available quality ingredients like silkworm pupae, earthworm and housefly maggot meal. Again, these ingredients are rich in all the essential nutrients and fulfil all the nutritional requirements of fish to achieve optimum growth. Moreover, these alternate ingredients are affordable for all fish farmers and can support the sustainable development of fish culture in a cost-effective way.

Aims and Objective: To determine the potential of silkworm pupae, housefly maggots, and earthworm meals as an alternative to soybean meal in the diet of *Labeo rohita* with the following major objectives:

i) To formulate low cost feeds using non-conventional ingredients from animal sources for fresh water fish (*Labeo rohita*).

ii) To assess the growth of *Labeo rohita* using different formulated feeds.

iii) To evaluate the nutritional quality and fatty acid profile of the cultured *Labeo rohita* fed with formulated feeds.

Materials and Methods: Four isonitrogenous (~ 35% crude protein) and isolipidic (~ 6% crude lipid) experimental diets were prepared: control (with 30% soybean meal), T1 (with 30% silkworm pupae meal), T2 (with 30% housefly maggot meal), and T3 (with 30% earthworm meal). One hundred ninety-two *Labeo rohita* fingerlings (initial average body weight: 5.07 ± 0.01 g) were randomly distributed in 12 experimental tanks (16 fish per tank) following a completely randomized experimental design. The fish were fed twice daily (07:00 h and 16:00 h) with the respective experimental diets to reach satiation levels. About 30% of the water from each tank was replaced at every three days' intervals to remove the faces and maintain the physico-chemical parameters of the water. The potential utilization of insect meal-based diets in *Labeo rohita* was assessed through growth indices, whole body proximate and fatty acid composition.

Results: At the end of the feeding trial, final body weight (FBW), specific growth rate (SGR), percent weight gains (WG %), feed conversion ratio (FCR), and protein efficiency ratio (PER) were significantly affected ($P < 0.05$) among the experimental groups. The values of FBW, SGR, WG%, and PER were significantly higher ($P < 0.05$) in the T1 and T2 groups compared to the control and T3 groups. However, there were no significant ($P > 0.05$) differences observed between the control and T3 groups and the T1 and T2 groups. A significant ($P < 0.05$) opposite trend was observed for FCR values. A significantly higher crude protein content was observed in T2 group, while the value of control group was significantly ($P>0.05$) similar with the T1 and T3 groups. The sum of monounsaturated and polyunsaturated fatty acid composition did not show any significant ($P>0.05$) difference. While, a significantly higher sum of saturated fatty acid content was observed in the control and silkworm pupae meal-based diets than the earthworm meal and housefly meal-based diets. Among the muscle fatty acid compositions of different experimental groups, palmitic acid (16:00), linolenic acid (18:3, n-3), Gondoic acid (20:1, n-9) and Docosahexaenoic acid (22:6, n-3) showed significant ($P < 0.05$) differences among the experimental groups.

Conclusion: Hence, it can be concluded that the use of silkworm pupae and housefly maggot meals in the diet of *Labeo rohita* provides better growth performance and feed utilization efficiency than soybean and earthworm meal-based diets, besides use of housefly maggot meals in the diet of *Labeo rohita* can improve the whole body proximate and fatty acid composition.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Description
%	Per cent
@	At the rate of
ADG	Average daily gain
°C	Degree Celsius
Cm	Centimetre
CP	Crude protein
DM	Dry matter
DMRT	Duncan’s multiple range test
DO	Dissolved oxygen
DORB	De-oiled rice bran
DSBM	De-fatted soybean meal
EE	Ether Extract
et al.	And others/Co-workers
FBW	Final body weight
FCE	Food conversion efficiency
FCR	Food conversion ratio
Fig	Figure
G	Gram
GNOC	Groundnut oil cake
HMM	Housefly maggot meal
i.e.	That
K cal	Kilo calories
Lit.	Litre
Mm	Millimetre
MOC	Mustard oil cake
PER	Protein efficiency ratio
pH	Potential hydrogen
S.E	Standard error of mean

SGR	Specific growth rate
SWP	Silk worm Pupae
Viz.	Namely
WG	Weight Gains

1. INTRODUCTION

Despite recent advances in per capita food supply, under-nutrition remains a chronic problem, particularly in developing nations (FAO, 2018). One of the most critical issues that humanity will confront is feeding the world's 9 billion people by the middle of the twenty-first century. Under these conditions, there is a pressing necessity to boost food production. Agriculture and associated sectors, according to FAO (2018), should be technologically prepared to meet this challenge. Fish is a high-quality protein source for humans, accounting for more than 20% of animal protein intake (FAO, 2020). As capture fishery production has been largely unchanged since the late 1980s, the global aquaculture sector has risen faster than other food production sectors to play a vital role in nutritional security and poverty alleviation. Between 1990 and 2020, total global aquaculture grew at a 6.7% annual rate.

Indian fish production has increased dramatically over the last few decades, rising from 0.75 MMT in 1950-51 to 5.66 MMT in 2000-2001, 8.67 MMT in 2011-12, and 14.73 MMT in 2020-21 (MFAHD, 2022). In the most recent year (2020-21), the inland and marine sectors contributed 11.25 and 3.48 MMT, respectively, to total production (MFAHD, 2022). The sector provides 1.1% of GDP and 4.7% of agricultural GDP, accounting for around 4.4% of world fish production. In inland fisheries, a change from capture to aquaculture has been witnessed during the previous two and a half decades. Freshwater aquaculture's share of inland fisheries increased from 34% in mid-1980 to around 76% in recent years. In India, inland aquaculture has emerged as a rapidly expanding industry and a viable alternative to the relatively steady catch fisheries. In 2017-18, India's aquaculture production was 7.1 million tonnes, placing it second in the world (FAO, 2020). Aquaculture's growth rate is steadily increasing, reaching

10.14% in 2017-18. In 2017-18, the inland finfish aquaculture sector contributed 6.3 million tonnes (FAO, 2020), with Indian Carps like rohu, catla, and mrigal accounting for roughly 70-75% of production. Simultaneously, other exotic carps such as silver carp, common carp, grass carp, and catfishes account for 25-30% of total freshwater aquaculture production.

However, successful aquaculture sector requires, lower production costs, efficient production systems and environmental sustainability measures. Fish feed often accounts for 60% to 70% of the operational costs in intensive and semi-intensive aquaculture systems, making it one of the most expensive inputs in aquaculture production systems (Singh et al., 2006). Therefore, it is crucial to offer cost-effective, eco-friendly and nutritionally rich fish feeds. Moreover, aquaculture's inability to address the widening disparity between fish supply and demand in India is attributed to several factors, chief among them being the shortage of high-quality feeds. Typically, compost cribs and occasional use of animal droppings constitute the primary feed sources for pond-raised fish. Regrettably, these resources can only sustain limited growth, hampered further by a deficiency of essential nutrients derived from primary production (Edwards et al., 2000). For any potential expansion to occur, additional feed is imperative to meet the escalating nutritional requirements. Presently, Indian fish farmers resort to feeding their fish with cereal bran, kitchen leftovers, and green foliage, given the unavailability of commercial feeds in the country. Even if these were accessible, their cost would render them unaffordable for most fish farmers. India grapples with a substantial shortfall in fish production relative to demand, a gap that cannot be feasibly bridged through conventional fish farming practices. Consequently, the adoption of advanced techniques in fish farming is indispensable to narrow this demand-supply chasm. As noted by Hecht (2007), the economic hardships faced by farmers in Asia constitute a primary obstacle to the progress of aquaculture. Both protein and energy are vital components in fish diets, serving roles in maintenance, growth, and reproduction. Unfortunately, these resources are often either unaffordable or clash with food security concerns, and they tend to be irregularly and inadequately supplied. Moreover, commercially available feeds are occasionally compromised by adulteration, contamination by pathogens, and harmful chemical additives detrimental to human health. Conversely, if fish farmers could produce their own alternative feeds, they could consistently provide nutritious and hygienic sustenance to their fish stocks. These homemade feeds would be fresh, virtually free of pathogens and harmful chemicals, and come at a more affordable price. Moreover, there is a need for sufficient quantity of cost-effective fish feed to support future fish production.

Wheat gluten meal, soybean meal, canola meal, cottonseed meal, corn gluten meal, peanut meal, mustard oil cake and sunflower seed mealare all typical examples of plant-based protein sources extensively employed in aqua feed. Complementing these proteins are oils of plants, including soybean oil and canola oil. Plant-derived proteins serve as the primary source of protein for lower level fish species and rank as the second most critical source of lipids and protein for marine shrimps, trailing only behind fish oil and meal. The specific quantities of plant protein meals and oils incorporated into aqua feeds exhibit significant variation, contingent on the target species and species groups. Among these, soybean meal stands out as the most prevalent plant protein source utilized in compounded aqua feeds, commonly substituting fishmeal in aquaculture feeds. The selection and utilization of plant-based proteins and oils are now determined by a blend of factors including local market availability, cost considerations, and nutritional profiles, encompassing anti-nutrient content and levels. As fishmeal prices continue to escalate, concentrates of plant protein such as pea protein, canola protein, wheat gluten meals and soybean protein are expected to surpass conventional plant protein meals in aqua feeds designed for high-trophic-level cultured species and crustaceans. However, several factors, including low protein content, amino acid imbalances as noted by Tacon (1987), the presence of anti-nutritional components, and elevated crude fibre content consisting of cellulose, hemicellulose, pectin, and lignin (NRC, 2011), impose limitations on the integration of plant-based ingredients in fish feeds. Due to a lack of fibre digesting enzymes such as cellulose, hemicellulose, and others, fish cannot utilise dietary fibre; also, high dietary fibre interferes with appropriate nutrient utilisation, resulting in poor performance (Glencross et al., 2012). As a result, removing anti-nutritional factors and correcting nutrient imbalances in plant-based ingredients, particularly amino acid imbalances, with proper techniques should improve their assimilation in fish feeds (Gabriel et al., 2007; Barrows et al., 2008). Moreover, there is a possibility to fulfil the demand for fish feed ingredients by replacing conventional protein-rich ingredients through locally available quality ingredients like silkworm pupae, earthworm and housefly maggot meal. Again, these ingredients are rich in all the essential nutrients and fulfil all the nutritional requirements of fish to achieve optimum growth. Moreover, these alternate ingredients are affordable for all fish farmers and can support the sustainable development of fish culture in a cost-effective way.

In India, sericulture is a well-established rural agribusiness that generates roughly 40,000 metric tonnes of silkworm pupae annually. Pupae have been discovered to be a great feed component for freshwater fishes since they have high quantities of protein and fat (Jayaram and Shetty, 1980). Silkworm pupae contain a high concentration of unsaturated fatty acids. This element is extracted as a result of the increased demand for oil from numerous sectors. Defatted silkworm pupae with high protein content are being employed in the poultry sector. Other useful animal feed ingredients, such as clam flesh, squid meat, and meat meal, are only available in modest quantities, despite the fact that they are all important sources of human food. A little amount of these pupae is sun-dried and used in animal feeds, but a large proportion is dumped in open areas. In addition to nutrient loss, the sun-drying and disposal of pupae promotes environmental contamination. In the laboratory, a suitable fermentation ensiling procedure was devised to produce pathogen-free pupae silage (Yashoda et al., 2001). When fed to carp, it outperformed plant protein sources (Swamy et al., 1994). When fed to fish (batrachus), the crude protein in silkworm meal digested similarly to fish meal (Borthakur and Sarma, 1998). Moreover, it has been proven that silkworm meal could be used in carp diets in place of fish meal.

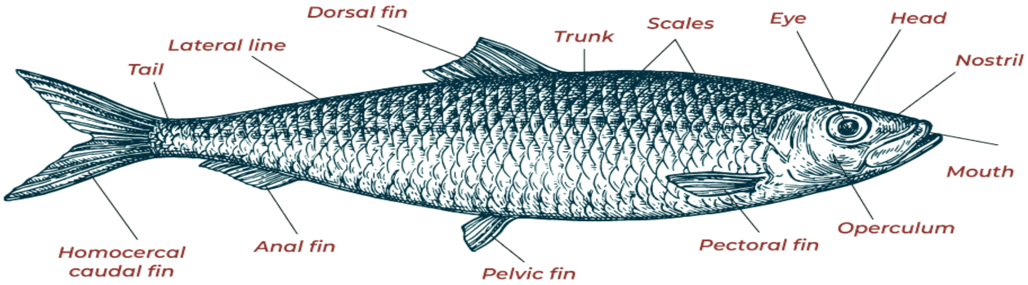
Incorporating insects as a protein source in fish feed nutrition is a relatively novel strategy. Maggots are used to process magmeal and are made from the larval stage of *Musca domestica*. Bondari and Sheppard (2001) emphasised that insects at various stages of life have been employed to feed fish. Interestingly, research on the use of housefly maggot meal as a replacement for fish meal in fish diets has increased recently (Fasakin et al., 2003; Ajani et al., 2004; Adesulu and Mustapha, 2000). According to research, magmeal has a significant biological value. The percentages of crude, fat, protein, and crude fibre range from 39 to 61.4%, 12.5-21%, and 5.8-8.2%, respectively. Magmeal contains phosphorus, trace minerals, and B vitamins (Teotia and Miller, 1973). Ogunji et al. (2009) tested house fly maggot meal digestibility in tilapia and carp using a reference diet (including fishmeal as the principal protein source) and a test diet (containing 70% reference diet + 30% maggot meal). An examination of the amino acid profiles in fish and fly larvae protein showed that no essential amino acid was deficient. Spinelli et al. (2009) utilized magmeal protein in the diets of rainbow trout.At substitution levels ranging from 25-100%, the protein provided growth and feed conversion levels comparable to fish meal. The results of nutrient composition research revealed that magmeal has a better amino acid profile than fish meal, but it has a higher crude fat content (19.8%), which altered the fat level and fatty acid composition of diets and fish. Feeding *Oreochromis niloticus* fingerlings magmeal-containing meals can result in growth comparable to that obtained with fishmeal as the primary protein source. However, enough quantities of n-6 and n-3 fatty acids should be incorporated in magmeal diets to improve the ideal fatty acid profile required for metabolic processes. So far, using magmeal in fish nutrition hasn't shown any economic advantage. However, magmeal is considered a viable alternative to all fish meal in fish diets due to its cost-effectiveness, availability, biological value, and feed conversion ratio.

Earthworm is the popular term for the biggest members of the phylum Annelida's class Oligochaeta. They thrive in decaying foliage, compost, and manure. Chakrabarty et al. (2009) used vermin-composting as a direct application organic manure in fish farming ponds with success. Nandeesha et al. (1989) developed a culture trial with common carp utilising dried worm meal derived from *Eudrilus eugeniae* as a fish meal replacement. *Cyprinus carpio* (Linn.) and increased muscular fat were discovered in fish bred on an oil-rich diet. When earthworm meal was fed to fowl, researchers saw conflicting outcomes. Some studies showed it to be a good protein source for growing broilers (Reinecke et al., 1991), while others found it to be a poor substitute for fish meal (Koh et al., 1984). Earthworm meal was shown to be superior to fish meal in grill applications (Cariaga, 2003), while others found it to be comparable (Das et al., 1991). There were no variations in performance in layers when it was used to replace soybean meal and fish meal (Mekada et al., 2009; Das et al., 1991; Ulep, 1982). In young growing chicks, the diet was similar to fish meal and casein (Koh et al., 1984). When earthworm meal was fed to quails in place of fish meal, it increased weight gain and feed conversion (Silvestre, 1984;

Dioson, 1984). The growth performance of the freshwater cat fish, *Catla catla* (Linn.), was assessed for 45 days under optimal and identical laboratory circumstances using three different ingredients: fish meal, earthworm (*Perionyx sansibaricus*), pila (*Pila bengalensis*), and goat liver. The earthworms fed group grew faster, demonstrating their appropriateness. The difference in growth rate of fish fed earthworms demonstrated a significant (p 0.01) difference from the population fed other meals. Rameshguru et al. (2011) discovered that vermin-wash has an important impact in the proliferation of *Oreochromis mossambicus*. Guin et al. (2009) evaluated the use of vermin-compost as a component of integrated nutrient management in aquatic systems. The study found that vermicomposting could be an efficient strategy for increasing resource utilisation in aquaculture by recycling organic waste and reducing large-scale chemical fertiliser use. Dioson (2015) discovered that Japanese quail given 100% earthworm meal as their protein source performed best. In this instance, vermin-wash might be an effective direct application meal for juvenile fish. Chakrabarty et al. (2009) discovered that fish growth and survival rates were significantly higher in vermin-wash treated aquariums, possibly due to the inclusion of various micronutrients, vitamins (Pro vitamin D and B complex), free amino acids and metabolites in vermin-wash (Ping and Boland, 2004; Springett and Syers, 2009; Kale 1998). In comparison to zooplankton or artificial feed of larger dimensions, juvenile fish can consume very minute food particles contained in vermin wash. Vermiwash contains major and micronutrients, as well as cocoons, small worms, and body part detritus, all of which are edible. Common food particles, such as live plankton, freeze dried tubifex, or market available feed pallet, are much larger in size (at least three times larger when compared to particles present in vermi-wash under an ocular microscope) than food particles that juvenile fish can consume (Chakrabarty et al., 2009). Vermiwash has been shown to include growth hormones, antibiotics, and vitamins that are advantageous to fish growth (Atlavinyte and Daclulyte, 1969; Lee, 2012; Ismail, 2007). In a laboratory experiment conducted by Chakrabarty et al. (2009), these chemicals most likely assisted the fish in remaining illness free. The utilization of detritivorous, aquatic andterrestrial in the organic waste decomposition process is on the rise in popularity, as highlighted in studies by Edwards and Densem in 1980 and Burrows in 1984. The resultant worm castings are well-recognized as valuable organic fertilizer for agriculture. Furthermore, the by-products derived from worm biomass have proven to be a valuable source of protein for fish, as demonstrated in research conducted by Tacon and colleagues in 1983 and Stafford and Tacon in 2015. Within the fish farming sector, there is a growing effort to substitute the costly fish meal traditionally included in fish diets with more economical protein sources.

The systematic position of *Labeo rohita*, also known as rohu, is:

- **Phylum**: Chordata
- **Subphylum**: Vertebrata
- **Class**: Teleostei
- **Order**: Cypriniformes
- **Family**: Cyprinidae
- **Genus**: *Labeo*
- **Species**: *rohita*



Labeo rohita

Labeo rohita, with a compressed, fusiform body, a distinct head with a short, blunt snout, thick fringed lips, a single pair of maxillary barbels, and large overlapping cycloid scales covering its body, its coloration is typically silvery on the sides and darker on the back, with a deeply forked caudal fin and a lateral line running along its body; its mouth is subterminal and positioned with a lower labial fold that is complete and fimbriated, often displaying a slightly concave dorsal fin profile.

Labeo rohita is a freshwater fish that are farmed for food in several regions of the world and are frequently farmed in ponds. In the Indian subcontinent, rohu, *Labeo rohita* is the main species raised in carp polyculture systems together with the other two major carps, *Cirrhinus mrigala* and *Catla catla*. In comparison to the other two species, rohu is typically stocked at relatively higher densities due to its greater feeding niche, which stretches from column to bottom. The most significant and prevalent species cultivated in other nations, including Bangladesh, Pakistan, and Myanmar, is the rohu, one of the three main Indian carps (FAO, 2012). However, rohu is domesticated because of its excellence of food, which is sweet in taste and cheap also. It is accepted as highly nutritious as protein substitute due to high quality of protein content and easily assimilation properties of all the amino acids present in it. It is highly resistance to various type of common disease like as gill rot. Hence, it is most acceptable and recommended fish across the country (Uauy and Birch, 2011).

Objectives: -

Keeping in view the above points the research program was conducted with the following major objectives:

i) To formulate low cost feeds using non-conventional ingredients from animal sources for fresh water fish (*Labeo rohita*).

ii) To assess the growth of *Labeo rohita* using different formulated feeds.

iii) To evaluate the nutritional quality and fatty acid profile of the cultured *Labeo rohita* fed with formulated feeds.

2. LITERATURE REVIEW

2.1. Status of the aquaculture and world fisheries

One of humanity's greatest challenges is to feed the projected 9 billion people by the mid-21st century (FAO, 2020). The 2030 Agenda for Sustainable Development, along with its 17 Sustainable Development Goals, offers a new, transformative, and holistic approach to advancing towards a more sustainable and resilient future, ensuring no one is left behind. In line with the FAO Code of Conduct for Responsible Fisheries (FAO, 1995), the 2030 Agenda also sets objectives for the role and practices of fisheries and aquaculture in enhancing food security and nutrition.

In recent decades, the global fisheries and aquaculture sector has significantly supported global food security and nutrition. It was projected that global fish production would reach about 178 million tonnes in 2020 (FAO, 2022). Of this total, global catch fisheries contributed 90 million tonnes (51%), while aquaculture provided 88 million tonnes (49%). Approximately 89% of the total production (157 million tonnes) was allocated for human consumption, with the remaining portion mainly used for producing fishmeal and fish oil (81% of the remaining 20 million tonnes). The overall first-sale value of global production in 2020 was expected to be USD 406 billion, with aquaculture contributing USD 265 billion and world capture fisheries accounting for USD 141 billion.

In addition to the foregoing, past production data from global capture fisheries and aquaculture suggested that the capture fishery has been essentially steady since the late 1980s. Aquaculture, on the other hand, has been responsible for the continued significant development in the supply of fish for human consumption. Farmed finfish production reached

57.5 million tonnes in 2020, with inland aquaculture accounting for 49.1 million tonnes and, marine culture and coastal aquaculture accounting for 8.3 million tonnes. Other farmed aquatic animal species produced included 17.7 million tonnes of molluscs, predominantly bivalves, 11.2 million tonnes of crustaceans, 0.52 million tonnes of aquatic invertebrates, and 0.54 million tonnes of semi-aquatic species such as turtles and frogs (FAO, 2022). Furthermore, global animal aquaculture production increased by 2.7% in 2020 compared to 2019 (FAO, 2022). Between 1990 and 2020, total global aquaculture grew at a 6.7% annual rate. However, the average annual growth rate significantly declined from 9.5% in 1990-2000 to 4.6% in 2010-2020. Furthermore, the annual growth rate of aquaculture has been lowered to 3.3% in recent years (2015-2020). Despite this, it is crucial to highlight that global aquaculture production has increased in absolute terms during the last three decades.

Aquaculture accounted for 56% of aquatic animal food products accessible for human consumption in the same year. Despite advances in per capita food supply over the decades, under nutrition (including inadequate consumption of protein-rich animal food) remains a large and chronic problem, particularly in developing countries rural areas. The worldwide number of chronically undernourished persons was 815 million in 2016, down from almost 900 million in 2000, with Asia and Africa having the highest numbers and proportions (FAO, 2018). As a result, the circumstances must be addressed in order to close the gap between the demand for and supply of animal protein, especially fish. This is only achievable with the sustainable increase and intensification of available resources.

2.2. Status of Indian aquaculture

Globally, India has surpassed China in yearly fisheries and aquaculture production in 2020 (FAO, 2022). Indian fish production has increased dramatically over the last few decades, rising from 0.75 million metric tonnes (MMT) in 1950-51 to 5.66 MMT in 2000-2001, 8.67 MMT in 2011-12, and 14.73 MMT in 2020-21 (MFAHD, 2022). In the most recent year (2020-21), the inland and marine sectors contributed 11.25 and 3.48 MMT, respectively, to total production (MFAHD, 2022). In inland fisheries, a change from capture to aquaculture has been witnessed during the previous two and a half decades. Freshwater aquaculture's share of inland fisheries increased from 34% in mid-1980 to around 76% in recent years. In India, inland aquaculture has emerged as a rapidly expanding industry and a viable alternative to the relatively steady catch fisheries. In 2017-18, India's aquaculture production was 7.1 million tonnes, placing it second in the world (FAO, 2020). Aquaculture's growth rate is steadily increasing, reaching 10.14% in 2017-18. In 2017-18, the inland finfish aquaculture sector contributed 6.3 million tonnes (FAO, 2020), with Indian Carps like rohu, catla, and mrigal accounting for roughly 70-75% of production. Simultaneously, other exotic carps such as silver carp, common carp, grass carp, and catfishes account for 25-30% of total freshwater aquaculture production. Furthermore, recent efforts have been made to diversify traditional carp cultivation to high-value prawns, sea bass, and other prospective species produced in the country's inland regions.

2.3. Rohu (*Labeo rohita*) culture

The Indian large carp is a native of the rivers of northern and central India, as well as those of Bangladesh, Pakistan, and Myanmar. Traditional carp culture has been practised for hundreds of years in the little ponds of the eastern Indian provinces. Until the breakthrough of induced breeding in 1957, riverine seed collecting was the only way to meet the demand for species culture. Following that, consistent seed supply makes carp culture prevalent in Asian subcontinent freshwater tanks and ponds, particularly in India. Catla (C*atla catla*), rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*) account for 70 to 75% of total freshwater fish production in India, with rohu alone accounting for 35-40% (FAO, 2018). Because of its column feeding habits, Rohu (*Labeo rohita*) can assess a wider food niche, making it the most preferred species for carp polyculture systems in India. The increased consumer preference and market demand also recognise rohu as India's most significant culturable freshwater species.

2.4. Role of aqua feeds in aquaculture growth

To meet the world's expanding demand and needs, aquaculture production should be doubled by 2030. To maintain such a rapid rate of growth, a corresponding rise in fish feed production is required, as prepared fish meals is a key aspect in aquaculture for increasing growth and productivity (Abdel-Tawwab et al., 2007). In aquaculture system, feed accounts for more than half of overall production costs. Any reduction in cost of fish feed directly increases profitability (Pandian et al., 2001). The protein source is the costly component of a fish feed, has the greatest influence on feed cost (Singh et al., 2006). To continue fish farming, high costs, variable quality, and unknown availability of fish meal have prompted the replacement of fish meal in aqua-feeds with plant feedstuffs. At the same time, traditional feedstuff costs rose to the point that their incorporation in a required quantity completely wiped out the farmers' predicted profit (Njidda and Isidahomen, 2010).

2.5. Aqua feed ingredients

Based on their availability and acceptance, the principal components utilised in aqua feed production can be divided into two categories: conventional and non-conventional ingredients. Agro-industrial by products are routinely, commonly, and widely used in aquaculture as conventional feed additives. The majority of these are inexpensive and widely available throughout the year (Tacon et al., 2011). Their application is standardised and widely recognised. They might be of either animal or plant origin. Non-traditional feed components have the ability to supplement or replace fishmeal in aqua feeds. These include feed additives that are not standardised but are regionally available. Their incorporation in aqua feed varies depending on availability, nutrient content, processing technology, fish species, and agricultural pattern typical in the area. They, like traditional feed additives, can be of animal or plant origin (Tacon et al., 2011). Soybean meal, ground oil nut cake, mustard oil cake, rapeseed meal, canola meal, fababean meal, cottonseed meal, sunflower seed meal, corn gluten, wheat gluten and other interesting alternative protein components have been explored. Aquaculture's rapid development results in a high demand for feed and a scarcity of feed materials. Scarcity and competition with the human food and animal feed industries drive up the price of regularly used ingredients (Tacon et al., 2011). Another major problem is the quality of the materials, since the nutritional composition, pathogen and contamination of biogenic amines of common animal origin, such as fishmeal, can vary greatly (Dersjant-Li, 2002). As a result, a plant-based ingredient that is abundant may provide a solution to the high cost and quality of ingredients. As a result, there is a need to develop plant protein supplies that are less competitive with humans, nutritionally appropriate, and effective in lowering aqua feed costs (Saha and Gosh, 2013). In this case, non-edible oilseed cake/meals would be the best option if they were free of inherent toxic and anti-nutritional factors (Marrufo-Estrada et al., 2013), because all plant-based ingredients, without exception, have one or more anti-nutritional factors that limit their use in fish feed preparation.

2.6. Prospects of insect meal

In recent decades, fish meal has been replaced by plant meal, but due to unbalanced protein and amino acids, it creates health problems that damage our immune system and enhance the chances of infectious diseases (Oliva-Teles, 2012). Plant meals are also a reason to create health issues in farmed fish due to starch components and anti-nutritional compounds (Francis et al. 2001). Generally, plant meal (soybean meal, sunflower meal) sources are not used as a part of natural fish diets (Moutinho et al., 2017). The plant-originated protein derivatives, namely soybean, have low levels of methionine with high prices and a smaller amount according to the requirement (Gamboa et al., 2013). Likewise, sunflower meal also consists of low levels of methionine, phenylalanine, lysine, and phosphorus, is poorly appetising, and has low energy (Ogello et al., 2017).

The protein derivatives obtained from the plant are generally used to make fish feed that does not have the required amount of amino acids. Anti-nutritional factors are also present in it, which decrease its ability to be used in fish (Hossain et al., 2003). Along with this, plant-based protein derivatives have the lowest level of appetising (Refstie et al., 2000) and high fibre and ash contents (Olvera et al., 2002). When the plant-based protein material is used in making fish feed at a high level, decreasing the protein conversion ratio, digestibility, and pellet quality of the fish meal (Drew et al., 2007; Ogello and Munguti, 2016). The whole factors are decreasing the nutrients digestibility in the fish, decreasing feed utilization, and increasing the feed conversion ratio of the fish, resulting in reduced profitability in fish farming (Hossain et al., 2003; Shao et al., 2002).

All these barriers are related to the feasibility of protein meal resources related to price, nutritional importance, and sources, which increase the necessity for further research on locally available animal protein sources to replace fish meals (FAO, 2016). In comparison with plant meal sources used in fish nutrition, insect farming under the required conditions can be another profitable substitute source of protein in fish meals. Other than insects, non-conventional protein sources such as earthworms are also alternative protein sources, as their nutritional importance is similar to fish feed (Vielma-Rondón et al., 2003; Fadaee, 2012). Global beef production is being taken over for the first time by aquaculture production on a large scale (Larsen and Roney, 2013). The high demand for fish meal has caused the price of fishmeal to increase in past decades, inhibiting small-level fish culture in particular areas and increase in fish production by accessing the good quality of meal inputs (Tacon and Metian, 2009; Naylor et al., 2009). To overcome these issues, alternative feed ingredients are required in aquaculture systems (Hardy, 2010).

The small rural level of fish culture in developing countries is necessary for providing food assurance to the needy (Tacon et al., 2010). Rural small-scale aquaculture often controls and manages integrated fish farming as per the traditional management system using cheap, quality feed (Dongmeza et al., 2009; Pucher and Focken, 2013). The omnivorous common carp represent a higher fish production level and more advantages than traditional fish culture in semi-intensive pond aquaculture systems. To increase fish production in rural areas, semi-intensive pond management must be done according to locally available feed sources

(Pucher and Focken, 2013). In developing countries, the feed formation segment is accordingly an underdeveloped area in the aquaculture process (Gabriel et al., 2007).

In the aquaculture system, ingredients play an important role in fulfilling their nutritional value. Along with financial development in the fish meal formation process, fish feed operational costs are generally high in aquaculture process (Biswas et al., 2006). So, fish farmers need to minimise fish feed costs by using cheap feed ingredients like silkworm pupae. There are many feed ingredients that have been investigated as alternate parts for fish feed (Ogunji et al., 2008), including animal protein sources like earthworms, silkworm pupae, maggots, etc. (Ogunji et al., 2006). These feed ingredients are cheaper and more locally available than fish meal, which is available at a higher cost (Bureau et al., 2000; Bharadwaj et al., 2002). The new approach uses insects as an animal protein source in the fish feed (FAO, 2013). It can be considered that silkworm pupae and other animal sources are good protein alternatives for fish meals. It is easily available from sericulture units as waste. Earthworms, house fly larvae (maggots), and silkworm pupae meal now have the potential to substitute fish meal ingredients, which are available at a higher cost for fish feed. So, these are considered alternate protein sources that are locally available for Indians.

2.7. Silkworm pupae

To ensure fish culture is profitable, sustainable, and green in the final analysis, there is a requirement to find alternate protein sources carrying the same level of nutritional ingredients. Silkworm pupae play a major role in fulfilling the requirement for protein in fish. Sericulture emerged in the twenty-seventh century BC in China and now grows in other countries around the world. In 2011, silkworm cocoon production across the world was approximately 485,000 metric tonnes (FAO, 2012). Only India produces silkworm pupae per year of around 40000 metric tonnes on the basis of dry weight, as it is a well-established rural agribusiness. At present, if we find the largest consumer of silk in the world, it is India, and on the other side, the second-biggest producer of silk is also India. In India, mainly four types of silk are produced: Eri, Tassar, Mulberry, and Muga. The silkworm is common feed, providing one-third or more production in India (Heuze et al., 2017). Silkworm (*Bombyx mori*) culture on the leaves of mulberry is used for the silk yarn production through the process of heat killing (heat-killed pupae). After the process of reeling silk fibre, the pupae are treated as waste material and can serve as feed for other animals (Nisha et al., 2014). A certain quantity of silkworm pupae can be used as animal feed after the process of sun drying, while a balanced amount is disposed of in the open area. Disposal of the pupae results in environmental pollution and the loss of nutrients (Wei ZJ et al., 2009). Because of the deficiency of fish feed, the waste of silkworm pupae can be a substitute for protein sources that give nutritional ingredients (essential amino acids, phospholipids, and fatty acids) to fish feed, which has been extensively explored.

Due to their high levels of protein and fat, silkworm pupae have been found to be an excellent dietary ingredient for freshwater fish (Jayaram and Shetty, 1980). Dried silkworm pupae's and fishmeal's nutritional values are comparable, but in terms of cost comparisons, the fishmeal is costlier than the silkworm pupae. Its raw protein ingredient ranges between 52% on the lower side and 72% on the higher side, and deoiled meal approximately ranges between 65% on the lower side and 80% on the higher side. The essential amino acids like methionine, phenylalanine, and valine are available in the silkworm pupae protein (Table 1). In 2007, the FAO/WHO mentioned the nutritional requirements for fish and the ingredients of essential amino acids such as methionine, phenylalanine, and valine in silkworm pupae protein. The details of essential amino acids such as lysenin, isoleucine, methionine, valine, and cystine levels are mentioned in tables. Silkworm pupae are rich in unsaturated fatty acids. Defatted silkworm pupae are presently used in the poultry industry with a high percentage of protein.

A suitable process of fermentation has evolved in the laboratory to make some disease-free silkworm pupae (Yashoda et al., 2001). According to Kling and Wohlbier in 1977, in China and Japan, the pupae of silkworms have been a major nutritional ingredient in fish diets. The exoskeleton includes chitin as a component, which consists of a 25% lower and 32% higher range of raw protein content. The higher fat component in the silkworm pupae meal reveals its use limit, but if the fat amount decreases in silkworm meals, then more feed can be fed. The quantity of silkworm meals in the diet depends on the fish species. In many ways, more silkworm meals can be provided to carp (Gohl, 1981). It was observed that if fish meal alters in different proportions or in all quantities, then the development of fish in length and weight is similar to the development of fishmeal (Nandeesha et al., 2000). The silkworm meal was superior when fed to carp as compared to plant protein sources (Swamy et al., 1994). The acceptability of the raw protein in fish meal is the same as in silkworm meal when it is provided to fish (Borthakur et al., 1998).

The silkworm pupae (*B. mori*) in the Indo-Pacific (the bio-geographic of seas, including tropical water of sea of India, central and western part of sea attached with two part of Indonesia) region has been used as major fish meal constituents with highly dietary protein source (Hasan, 1991). The incorporation of increased silkworm pupae in future produces offodor and unpleasant taste (Sawhney, 2014). Therefore, these dead silkworm pupae and moths can be provided as a protein component for fish diets and make the environment pollution free.

Table 1: Amino acid concentration of non-deoiled silkworm pupae

Amino acid	**Non-deoiled SWP (g/16 g Nitrogen)**	**Deoiled SWP (g/16 g Nitrogen)**
Ala	5.60	4.40
Arg	5.80	5.10
Asp	10.40	7.80
Cys	1.00	0.80
Met	3.50	3.00
Lys	7.00	6.10
Ile	5.10	3.90
Leu	7.50	5.80
Phe	5.10	4.40
Thr	5.20	4.80
Trp	0.90	1.40
Glu	13.90	8.30
His	2.60	2.60
Pro	5.20	5.20
Ser	5.00	4.50
Gly	4.80	3.70
Tyr	5.90	5.50
Val	5.50	4.90
Reference	**Miles et al. (2006)**	**Mahata et al. (1994)**

As a conventional fish feed specially in India and China part of Asian region, pupae of silkworm (animal by product) have been provided as a diet either exclusively or otherwise. In the town of Turkey like as Central Anatolia and Cifteler disclosed that diets of silkworm pupae was the main component which provide good result in feed conversion rate and growth (Umalatha et al., 2018). It was noticed that raw silkworm pupae and dried silkworm pupae have their own taste, in case of dried the taste is better than raw silkworm pupae. Due to presence of good compounds in silkworm pupae (deoiled) like as good quality of proteins, amino acids, fat, carbohydrates, vitamins and appetite stimulants could have been accountable for fish growth (Nandeesha et al., 1990). Non-deoiled dried silkworm pupae is another source of protein available at low cost to counter the costly fishmeal (Rana et al., 2009). For outstanding growth of fish, required to include other components as well like as rice bran and mustard oil with control animals fed when mostly Indian carps fingerlings fed silkworm pupae integrated diets (Bose and Majumder, 1990).

2.7.1. Effect of silkworm pupae on different fishes as feed

2.7.1.1. *Catla catla*

For outstanding growth of Indian carp's fingerlings, recommended to inclusion of other components like as rice bran and mustard oil cake with control animals fed when fed silkworm pupae integrated diets (Bose and Majumder, 1990). *Catla* fish fed with 30% non-deoiled silkworm pupae diet revealed high level of growth in contrary *Labeo* fed with same diets revealed lower growth. Because of low fat requirement as fat quantity is higher than required in silkworm pupae diet. Examination on keeping quality of the diet, digestibility of nutrients, impact of feed on body structure (Jayaram et al., 1980). An examination shows that when pelleted feed incorporated with cheaper proteins as silkworm pupae through supplementary diets in the carp culture, mix accordingly with shrimp or clam waste revealed remarkable growth in mrigal, but no remarkable growth revealed in *Labeo rohita* (Borthakur, 1983). Further examines outcome of animal waste like as silkworm pupae and eliminated substance in the diet of the *Labeorohita* and *Catlacatla* proved the major influence of silkworm pupae on the positive progress and revealed that silkworm pupae are the probable component in carp feed (Nandeesha et al., 1990).

In the further studied it was found that when the hybrid catla- rohu consumed same food as provided to rohu as routine diet founded that when pupae of silkworm and fishmeal used with at a level of 15% and 10% respectively, emerged higher level of growth of fish. Market available fish meal is costlier than animal waste like as silkworm pupae, by the above statement it can be said that the silkworm pupae can be utilized as major ingredient to formulate cheap and nutritionally rich fish feed (Nandeesha et al., 1989). In a research it was found that if 100% pupae of silkworm (crude protein source) incorporated to formulate the nutritionally balanced diet for catla (*Catla catla*) fingerling, the remarkable development revealed. Therefore, silkworm pupae could be substitute diet instead of fishmeal. (Hasan,1991). As above discussed, 100% incorporation of pupae provide remarkable growth in case of catla (*Catla catla*) fingerling, but in case of rohu (*Labeo rohita*) fingerlings, approx. 50% incorporation of pupae with fishmeal formulate a diet for rohu, resulted remarkable weight size increased as growth (Begum et al., 1994). Incorporation of pupae at a 43.8% in feed of rohu (*Labeo rohita*) fingerlings resultant better size increased and other factor improved as well like as survival or existence rate of rohu contrast with different experimental feed with different oil cakes. All these experiments revealed the potential of pupae can be a better alternate for rohu protein requirement

(Olaniyi et al., 2013). Therefore, it can be said that most of Indian carps can be a better option with silkworm pupae, altering fish meal up to 50%, with the same growth.

2.7.1.2. Mahseer

Incorporation at 60% silkworm Pupae in the feed of mahseer revealed improves growth. Same as 50% inclusion of de-oiled silkworm pupae increased the growth performance of mahseer in the diet (Shyama et al., 1993). It was revealed that feed incorporated with inclusion of pupae for mahseer fingerlings resultant an outstanding growth (Sawhney et al., 2014). All above facts, it was suggested that silkworm pupae were cost effective and nutritionally balanced. Commercially, it could increase the returns for fish farmers if partial replacement of fishmeal with silkworm pupae in case of masheer fingerlings. Adding raw silkworm pupae to feed at a rate of 60% increases the growth rate of Deccan mahseer. Likewise, adding 50% of deoiled silkworm pupae in the diet significantly improved the growth performance of mahseer (Shyama et al., 1993). It has been observed that mahseer offspring exhibited outstanding growth when fed with silkworm pupae based feed (Sawhney et al., 2014). It has been suggested that silkworm pupae are less expensive and could improve economic returns for fish farmers by partially substituting fishmeal for feeding mahseer juveniles.

2.7.1.3. Ornamental fish

When 38% of silkworm pupae changed to fishmeal as the better feed for silver barb fingerlings, the said fingerlings got better results with the diet (Majoankar and Biamber, 1987). Incorporation of 60% silkworm pupae as a proportion in fish meal may be a better option for cultured red zebra fingerlings with positive growth. Silkworm pupae in fish diets not only reduced the cost of feed formulation but also increased the growth of red zebra fingerlings

(Rahman et al., 1996). The growth performance of other fish, such as the Rainbow shark, when fed a diet containing 30% silkworm pupae was reported. This replacement provides ideal growth compared to the control fed with only fish meal (Keshavappa, 1988). Hence, it might be decided that cultured ornamental fish through pupae as a substitute for fish meal from 30% lower side to 40% higher side can be successful for fish farmers to get better performance.

2.7.1.4. Exotic carps

The pelleted feed formulated with animal waste such as silkworm pupae and prawn waste for common carps revealed better growth without any adverse effect on the quality of the fish (Ji et al., 2010). The diet, which includes 50% raw protein and is formulated with sun-dried pupae as a source of protein, might be utilised as an entirely different fishmeal without affecting the protein efficiency ratio, size, or food conversion ratio (Nandeesha et al., 2000). It was further revealed that the exchange of protein from fishmeal with 50% of pupae meal as a diet for mirror carp fingerlings found positive effects on the nature of entire body protein (Ji et al., 2015). Likewise, the exchange of protein from fishmeal with 50% of pupae as a source of protein in the diet of Jian carp (*Cyprinus carpio*) did not show any adverse effect on the health and growth of the fish, and the exchange of more than 60% of fishmeal protein revealed comparatively low growth in Jian carp fingerlings (Jintasataporn et al., 2011). In finge-lipped carp (*L. fimbriatus*), when silkworm pupae are used as a protein source, the protein absorb ability becomes greater at 20% inclusion and becomes lesser at 40% inclusion, though nitrogen-free extract and fat absorb ability are higher from 20% minimum and maximum up to 40% inclusion, respectively. The common carp revealed better protein absorbability as well as fat absorption from 10% minimum and maximum up to 30% incorporation of pupae in fish feed (Gangadhar et al., 2017). Hence, it might be decided that carp can be cultured with silkworm pupae as animal waste and exchange fish meal from 30% minimum and maximum up to 50% without adversely affecting the fish's growth.

2.7.1.5. Catfish

An experiment was conducted that revealed that the metabolism activity and development or growth of catfish (*Clarias batrachus*) fingerlings size depend on diets with different protein alternatives, in which dried silkworm pupae are a better alternative to raw pupae in the fish feed (Watanabe et al., 1996). A diet containing 100% incorporation of silkworm pupae as meal yielded effective growth, a ratio of feed conversion, and protein usage in said fingerlings and the same fingerlings in Africa (Oso et al., 2014). A feed formulated with 80% incorporation of non-deoiled pupae gave better growth performance in catfish fingerlings from Asia (Habib et al., 2001). It was revealed that silkworm pupae could be provided as a replacement protein ingredient for up to 50% of catfish fingerlings in African countries (Kurbanov et al., 2015). So, silkworm pupae as fish feed can be utilised in Asian and African countries for successfully culturing catfish fingerlings at a cheaper price with effective growth by exchanging fish meal for more than 75% and up to 100%.

2.7.1.6. Marine fishes

The energy digestibility of 73% and CP digestibility of 85% in Japanese seabass (*Lateolabrax japonicus*) for non-deoiled pupae as a protein ingredient was cheaper than that of poultry by-product meal, blood meal, feather meal, and soyabean meal (Ji et al., 2012). It was found that the replacement of meat and bone meal, or combined both as meal and silkworm pupae meal with fishmeal, affected the effective growth of juvenile olive flounder fingerlings. It was revealed that the replacement of fishmeal with 20% promate meal and 10% pupae meal had no adverse impact on better performance (Lee et al., 2012). On the basis of the above, it could be concluded that, without affecting the growth performance, marine fish can be effectively cultured with silkworm pupae after exchanging fish meal up to 10 percent.

2.7.1.7. Shellfish

As studied by Cho, protein sources in animals and plants play a major role in the replacement of market-available fishmeal for growth and other positive impacts on juvenile abalone fingerlings and revealed that the combination of silkworm pupae meal and soya meal on a DM basis (16.9% and 29%, respectively) could entirely replace market-available fishmeal (Cho, 2010). Hence, it could be concluded that without affecting the growth performance, the shellfish fingerlings can be effectively cultured with silkworm pupae by exchanging fish meal up to 30% to 40%.

2.8. Housefly maggots (*Musca domestica*)

Musca domestica, belonging to Diptera. Maggots can grow on a broad range of spoiled organic wastes (Hogsette et al., 2000). Housefly maggots contain a heterogenous organic substance, including chitins, antimicrobial peptides, and lectins (Hou et al., 2007; Fu et al., 2009). Recently, chitosan obtained from maggots has been used in medicines and beauty products (Jing et al., 2007; Ai et al., 2008). In recent times, maggot meal usage has increased as an alternate source for fish feed (Ajani et al., 2004; Adesulu et al., 2000). Magmeal has a high protein and lipid content instead of soybean and fish meal (Ogunji et al., 2006; Adesulu et al., 2000; Ajani et al., 2004).

As the maggots easily grow on the organic waste, they have a low cost, a high crude protein concentration, and are locally available, making them an efficient source of protein for fish. The maggots have raw protein of 40% to 55%, lipids of 13% to 21%, and crude fibre of 6% to 8%, approximately. In addition, maggot meal is rich in vitamin B complex, phosphorus, and trace elements (Totia et al., 1973). There are many variables that determine the favourable outcome of rearing fish. Among all of the factors, feed is the major factor that affects the capacity of the reared fish to grow. Quality of feed, feed consumption, water temperature, daily ratio size, and actions of fish are the important factors by which fish culture is also influenced (Bascnar et al., 2007). Fish fed on maggot diets observed improved growth responses, which may be explained by the required quantity of crude protein in maggots (Olaniyi et al., 2013). Ogunji et al. found in 2009 that a good result was obtained when maggot meal was replaced with fish meal in fish.

However, the present findings concluded that when a large amount of fishmeal is exchanged with animals, it mostly results in a reduction in fish growth (Cabral et al., 2011; Ogunji et al., 2007). As an absence of required amounts of amino acids in any prescribed diet can be a reason for opaqueness in fish (Cowey, 1994), Magmeal is simply broken down and absorbed by fish due to its high crude fibre content (Jhingram, 1983). (Fagbenro and Arowosoge, 1991), Maggots, due to their nutrients, play an important role in feed assimilation. If we compare maggot meal with fish meal, then due to its biological value, it is equivalent to fish meal (Ajani et al., 2004). Mass production of larvae of maggots can be possible in less time (in a week) by using agricultural leftovers and substituting fishmeal, which reduces the production cost of fish feed. The above states are the key reasons for the existence of sustainable and productive fish cultures in developing countries.

2.8.1. Maggot's Importance in Fish Feeding

The high cost of fish feed adversely affects the development of fish production. The favourable outcome of commercial fish rearing and rapid expansion is mostly based on the better quality and low cost of fish feed. The use of animal ingredients as protein sources for aquaculture feeds will decrease the cost of fish rearing. The cost comparison shows that the cost of producing 1 kg of magmeal is 20% less than the producing cost of 1 kg of fishmeal (Ogunji et al., 2006). There are many aspects, like cost, availability of feed, biological value comparison, and conversion ratio of feed, from which it can be assumed that maggots as a meal are a low-cost protein source. It is a feasible substitute source of protein for fish farming, which gives fish farmers a better opportunity to process low-cost diets for fish than the import of fish meal, which is costly and not available at the right time, especially in developing countries (Ogunji et al., 2006). Its production cost is very low compared to other animal protein sources. Therefore, the rearing or processing of maggots fulfils both purposes: firstly, it saves from environmental issues, and secondly, it provides the best nutritional feed for fish (Teguia et al., 2005).

2.8.2. Chemical Composition of Maggots Meal

The chemical composition of maggot’s meal is approximated at 48% crude protein, 25% fat, 7.5% crude fibre, 93% dry matter, 6% ash, and the same amino acid profile as fishmeal (Table 2) (Aniebo et al., 2009). It was also described in another study that the maggot meal contained approximately 64% crude protein, 5% moisture, 24% ether extract, 1.25% carbohydrate, and 5% crude ash (Hwangbo et al., 2009). This chemical composition is differ depending on age at the time of harvest (Aniebo et al., 2009), larval feed (Newton et al., 2004), and different drying formulas (Fasakin et al., 2003) mentioned by the researchers (Aniebo et al., 2009; Newton et al., 2004; Fasakin et al., 2003).

Table2. Proportion of amino acids and minerals in fishmeal and maggot's meal

Nutrients	**Fish meal**	**Maggot meal (%)**
Aminoacids	**(%)**	**(%)**
Valine	5.7	5.6
Tyrosine	3.5	8.1
Tryptophan	1.3	1.5
Threonine	4.6	4.5
Serine	4.6	4.5
Proline	4.4	3.7
Phenylalanine	4.4	6.9
Methionine	2.8	2.2
Lysine	7.9	7.4
Leucine	7.4	6.9
Isoleucine	4.4	4. 1
Histidine	2.2	3.5
Glycine	5.9	5.4
Glutamine	13.3	12.2
Cysteine	0.7	0.8
Asparagine	9.3	10.8
Arginine	5.8	5.4
Alanine	6.3	6.2
Minerals		
Calcium (%)	0.40	0.36
Copper (ppm)	--	21.47
Iron(ppm)	162	1129
Lead (ppm)	--	1.08
Magnesium (%)	0.02	0.21
Manganese (ppm)	86	15.41

Potassium (%)	0.08	0.45
Sodium (%)	0.55	0.31
Zinc(ppm)	173	49.63

2.8.3. Effect of maggot meal on Heteroclarias hybrid fingerlings

It was suggested that as an alternate to fishmeal, maggots be used in different proportions, like a half-fish meal replacement or a complete replacement of fish meal for Heteroclarias hybrid fingerlings. It was noted that in the case of half replacement of fish meal, there was a high rise in length, mean weight, feed conversion efficiency, and protein intake. So, to achieve good growth and utilisation of nutrients, maggots can be used as a good substitute for fishmeal (Omoruwou et al., 2011). It was also shown that adding a supplement containing 390 grams of protein from maggot's meal per kilogram to the feed increase the antioxidant capacity in gibel carp (Dong et al., 2013). Further, it was reported that the maggot (a good protein source) components at 10%, 15%, 20%, and 30% per kilogram were given as diet to change the fish meal. The outcome showed that up to 30% per kilogram of *Musca domestica* (maggot meal) can be used to alter the fish meal (protein) without any harm to the development of Lates calcarifera juvenile barramundi (Lin et al., 2017).

2.8.4. Role of a maggot's meal on Nile tilapia (*Oreochromis niloticus*)

For richly required nutrients and the development of fish, maggot's meal is one of the best and cheapest sources (Sogbesan et al., 2006). No oxidative stress or any other negative impact on fish metabolism was revealed after the inclusion of maggot's meal in Nile tilapia diets. The feed efficiency and good growth performance of fish fed on maggot diets have high nutritional value (Sogbesan et al., 2006). In Nile tilapia diets, incorporation of maggot meal in fish feed does not reveal any oxidative stress on the metabolic activity of fish. So, the maggot's meal can be effectively used as a replacement for fish meal as a protein source in the rearing of fingerling tilapia (Ogunji et al., 2006). It was recorded that Nile tilapia has good growth performance on diets that consist of half and three-fourths of maggot components, respectively, instead of market-available fishmeal in both cases, although the fingerlings have the lowest growth performance on diets that consist of complete maggot meals (Mustapha, 2001).

Further research revealed that in Nile tilapia (*Oreochromis niloticus*), complete fishmeal can be changed by the maggot's meal in fish diets. The biological importance of the fish meal was equivalent to the importance of a maggot's meal. As harmful and anti-nutritional components were not present in maggot's meal that are found in the sources of protein in plants (Ajani et al., 2004). Moreover, it was reported that, for the fingerlings of *Nile tilapia*, seven different test diets prepared by maggot's feed were fed as a substitute for fishmeal. The outcome of the experiment showed that growth parameters, haematological parameters, protein utilization, and stress indicators found no major impact between all these groups of feeding in fish. The analysis indicates that if the fish meal is entirely exchanged with maggot feed in the diets of Nile tilapia fingerlings, then it can fulfil all the requirements of nutrition in fish (Ogunji et al., 2006).

Moreover, it was revealed that one fifth of the fish meal diet can be easily exchanged for 25% of housefly maggot feed without adversely impacting the efficiency of the feed or the development of Nile tilapia (Alofa and Abou, 2019). Additionally, Nile tilapia fed feed prepared by the maggots decreased fish development and enhanced feed conversion levels if it matched the feed on the control diet (Slawski et al., 2008). It was also revealed that the fish meal can be semi- or fully exchanged with the maggot's components in the same proportion as feed for fingerlings (Nile tilapia) for more development and feed effectiveness. The feed conversion ratio decreases in fish as if the maggot component amount expands from a quarter to a full part of the diet, reducing the protein efficiency ratio (PER) and also reducing the feed conversion ratio (FCR) (Mustapha and Kolawole, 2019).

2.8.5. Effect of a maggot's meal on African catfish (*Clarias gariepinus*)

It was revealed that the catfish fingerlings, which also belong to African fish farming, had a high survival rate or growth rate when maggot meal was used as fish feed (Faturoti and Ifili, 2007). Further, it was revealed that the maggot's meal was exchanged with fishmeal as a half and, in another case, as a complete exchange. Then there will be no negative impact on fish development and better-quality feed. It was revealed that the maggots are a better protein replacement if compared with fishmeal in the case of catfish fingerling feed. So, maggot compound usage can decrease the price of fish feed for fish farmers (Aniebo et al., 2009). Furthermore, it was revealed that *Clarias gariepinus* fingerlings fed on a maggot-based diet. The result revealed that the use of a maggot-based diet from 30% as minimum inclusion and up to 100% as maximum exchange with conventional feed showed that a fish diet of 55% as conventional with an inclusion of 45% as maggot components got good growth in fish along with high survival. It was also revealed that when the maggot's component is used as a supplement for catfish at the relevant ratio, it will enhance growth (Okore et al., 2016).

In another study, it was reported that when the fingerlings of African catfish were fed on the feed, which consisted of a housefly maggot's meal in the ratio of fully part, three-fourths part, half part, and quarter part, the ratio changed to fish meal. It was found that fish feed consisting of three-fourths wet maggots had good development, feed conversion ratio, and feed efficiency in fish (Ipinmoroti et al., 2019). It was also reported that the fingerlings of African catfish were fed on these diets: half artificial diet, half wet maggots, complete artificial diet, and complete wet maggots. The observation revealed that the specific growth rate and final weight were significantly higher in catfish feeding on half wet maggots with half artificial diet, subsequently complete artificial diet, and complete fresh maggots. This was revealed from the experiment, African catfish fry had better growth performance by using half artificial diet and half wet maggot feed. It also decreases the cost of feed (Saleh et al., 2020).

2.9. Earthworm

An earthworm is a non-chordate that lives on earth and relates to the Annelida phylum. The bodies of earthworms are segmented into external and internal sides. All the segments have setae on their bodies. The existence of earthworms depends on temperature, water, and soil across the world (Coleman et al., 2004)."Earthworm" is the general name for the biggest representators of the class Oligochaeta (Omodeo et al., 2000). Earthworm is a non-conventional source of protein in several studies. It was found that earthworm meal has been used as a protein source by replacing fishmeal in fish diets. (Tuan, 2010). Under the control conditions of the lab, the semi-substitution of fish meal protein by protein present in earthworms satisfied the diet requirements for *Labeo rohita* and *Cyprinus carpio* with a favourable or better growth effect (Deborah et al., 2011).

2.9.1. Culture and meal production of earthworms

Vermiculture is the process of making rich compost with the involvement of different species of earthworms. It is the simplest process to reuse agricultural waste and to form large numbers of earthworms. This process is performed by different methods; pit and bed methods are generally used. We used the bed process, in which composting is done on the pucca floor by preparing the beds. The earthworms were cultured in a combination of buffalo or cow dung, and the animal wastes were collected from farms. During the culture, wetness was controlled by scattering drops of water on the dung on a regular basis. The bed should be enveloped with easily available materials such as grass or others to stop the entry of rats, ants, lizards, and snakes and save the compost from direct sunlight and rain. To avoid the death of the earthworm, pre-composting is very essential (Gunadi and Edwards, 2003). The collected dung was used for this process. After the third or fourth week, a large number of earthworms were produced, and all the raw material was processed into vermicompost. Earthworms were measured, cleaned with purified water, and placed for drying. After the drying process the dry earthworm was ground by a mill, and the collected material was put in a cool, dry place.

2.9.2. Small-scale earthworm production

Earthworms can be cultured with easily obtainable substances such as market and kitchen leftovers, cow dung, wheat crop waste, and other types of usable waste that consist of a high amount of organic material on a small scale (Li et al., 2016; Abbasi et al., 2015). Earthworms are sensitive to sunlight; they eat the vermicompost after it is collected, washed, and provided to fish as feed (Singh & Singh, 2014). The production systems of earthworms on a small scale have been utilised to increase fish yields in systems of spatially intensive farming. It was reported that small-scale vermicomposting can increase fish production in partially intensive farming in North Vietnam (Muller et al., 2012).

In India, Ghosh established an environmentally friendly and economically feasible procedure for managing both pisciculture and vermiculture. The process of vermicomposting produced organic fertiliser along with earthworm biomass in *Clarias batrachus* of catfish in a partially intensive pond with a mean weight gain of approx. 1.64 grams per day as individuals and enhanced the capacity of water holding (Ghosh, 2004). As a result, the production of earthworms by vermicomposting combined with practises of aquaculture can increase fish production, enhance feed usage, and provide financial benefits to small-scale farmers in developing countries with a population living in poverty (Pucher and Focken, 2013). Other economic advantages can be obtained by agro ecosystems, which promote sustainability (Pucher et al., 2014).

2.9.3. Utilization of earthworms as a feed component

As per many reviews, the earthworms are often used as a source of higher-quality protein. According to earthworm species, the protein concentration and quality differ among the species, and feed substrate has been given to them (Dong et al., 2010). As a comparison, *Eisena foetida* was dried by the freeze and found tasteless to trout because hemolytic ingredients exist in the coelomic fluid. But the crude protein of this earthworm represents suitability as a feed ingredient (Kostecka and Paczka, 2006; Medina et al., 2003). The earthworm's coelomic fluid plays an important role, as reported, and also affects the immune responses in chordates more than in non-chordate animals (Kauschke et al., 2007). It was reported that lysenin, as a part of the fluid present in coelom, shows adverse impacts on fish as it binds with sphingomyelin (Kobayashi et al., 2004), but thermally treated fluid of coelom reduced the toxic effects of lysenin and other hemolytic factors present in it. Although the adverse effect of the fluid of coelom was not found in all the species of earthworms (Kauschkeet al., 2007). The high specific growth rate obtained with 25% and 50% diets is similar to that reported by Kpogue (2013) in *P. obscura* fingerlings. The optimum usage level is 25% in *Eudrilus eugeniae* species as earthworm meal in the diet of *Heteroclarias* fingerlings (Monebi et al., 2012). The same output was also revealed in a study by Dedeke (Dedeke et al., 2013). The most favourable usage of the earthworm species as *Hyperiodrilus euryaulos* meal in the diet of fingerlings (*Heterobranchus longifilis*) has ranged from 7.50% to 25% (Sogbessan et al., 2007).

The high feed efficiency was analysed with 50% incorporation, and the low feed productivity was analysed with 100% diet. This outcome can be clarified by hemolytic components in the fluid of coelom in the species (*Eisenia fetida*) of earthworm (Kostecka et al., 2006). Other studies showed that the market-available fish meal can be substituted for carnivorous fish species as a diet with earthworms (Lim et al., 2008; Pham et al., 2007; Hernandez et al., 2006). Particularly, market-available fish meal can be replaced by animal and vegetable proteins (Hlophe et al., 2014; Pucher et al., 2014). The level of protein quality of earthworm meal is similar to that of fish meal, with the best levels of required amino acids for fish (Dong et al., 2010; Tuan 2010, NRC 2011).

This productivity of earthworm meal was also revealed in a report where it was given as a feed to the post-larval stage of tilapia (Chaves et al., 2015). Although this productivity of earthworm meal can depend on the feed substrate and species of earthworm (Sogbesan et al. 2007; Dong et al. 2010). In a study, it was found that *Perionyx excavates* produce adverse effects on fish development based on the methods of preservation and anti-nutritional components present in the different species of earthworm (Nguyen et al.,2010). In another study, the earthworm feed prepared with *Perionyx excavates* did not show any adverse impact on the growth of common carps, which is very likely when the earthworms undergo heat treatment.

2.9.4. Biochemical composition of earthworms for fish feed formulation

Protein with required amino acids, phospholipids, and required fatty acids are known as the major components when selecting a protein source for fish diets (Stankovic et al., 2011). These are responsible for development, tissue growth, and the energy component of fish. Protein is the most-costly source to complete a nutritionally balanced diet for fish, as they are unable to synthesize the vital amino acid (Tacon and Metian, 2008). The raw protein ranges of earthworms are based on the culture substrate, method of processing, environment, and production mechanism. So, for the fish farmers who have limited options to prepare fish diets, they can clean the earthworms and feed them alive to the fish. In formulating fish diets, the essential amino acid profile is necessary to promote development, improve reproduction systems, increase behavioural activities, and develop disease-free fish (Andersen et al., 2016). It was reported that *Eisenia fetida* contains a prescribed quantity of essential lipids and protein nutrients that are important for fish's better performance (De Chaves et al., 2015). The earthworm has an overall energy of about 13.60 MJ/kg (Bahadori et al., 2017), 3.90 Kcal/g (Mukti et al., 2012), and is more metabolizable. The earthworm produces coelom fluid and lumbricin, along with strong antibacterial properties that protect fish (Bansal et al., 2018).

2.9.5. Processing techniques for earthworms

Different processes like reaping, managing, processing, preservation, and protection are essential steps to follow to culture earthworms. Because processing steps affect the nutritional quality, palatability, toxicity, and microbial risks of the earthworm feed, for commercial production, the processing and handling of earthworms has also been a major factor because the earthworms are moist, slimy, and sticky (Dynes, 2003). Some conventional methods, like mechanical handling and hand harvesting, irritate the earthworms, and consequently, the earthworms immediately discharge the coelomic fluid. The protein lysenin is present in the fluid of the coelom and is harmful to the chordates when combined with sphingomyelin and phospholipids of the cell membrane. The coelomic fluid causes damage to red blood cells and smooth muscle contraction, resulting in the death of the chordates sperm (Kobayashi et al., 2004; Ohta et al., 2000).

So, during the processing of earthworm for fish feed preparation, it is advise to separate the coelom from the earthworm and clean it, as the coelom decreases the efficiency of earthworm feed in fish because of its bad smell (Kobayashi et al., 2001; Vodounnou et al., 2016). Lysenin protein present in earthworms is destroyed by heat; washing the earthworm in hot water or baking it decreases the toxicity of coelomic fluid present in earthworm feed for fish (Pucher et al., 2014; Kobayashi et al., 2001).

2.9.6. Utilization of earthworms in fish feed

The high reproductive rate, low cost, effective feed, easy process to produce, and capacity to maintain in various types of climates, because of technological developments and research, we have seen an earthworm being used as a protein component in feed production, fertilizer as another output, pharmaceuticals, bioindicators, cosmetics, sanitary products, and many other processes (Zakaria et al., 2013).

2.9.7. Production systems of earthworms

The fish feed production by earthworms depends on the intensity of the aquaculture. In an intensive aquaculture system, earthworms should be produced in masses for the aquatic animals, which are kept in large quantities and require a good quality of feed to fulfil the demand for fish feed. A large number of vermicomposting or earthworm-producing plants that produce earthworm to fulfil the requirement of fish feed to complete aquaculture systems (Ghosh, 2004). However, artificial feeds are required to fulfil the demand for fish in semi-intensive aquaculture. In Indian carp (*C. carpio*), fish meal can be replaced by up to 50% of earthworm meal in semi-intensive farming (Beg et al., 2016).

Therefore, the vermicomposting process is used on either a large or small scale, depending on the requirement for fish feed (Kostecka and Garg, 2015). The nutritional component of earthworms is based on methods of culture, earthworm age, environment management, harvesting, and collecting mechanisms (Mukti et al., 2012; Zakaria et al., 2013). So, each unit of earthworm production should have the best parameters for better growth performance. The earthworms are distressed by light, so it must be produced in a dark, fully ventilated, and moist atmosphere (Gunadi et al., 2003).

3. MATERIALS AND METHODS

3.1. Experimental site

The experiment was carried out at the M/S. Shah Ji Fish Farm (28.170506 ^{o}N, 77.318258 ^{o}E), Palwal, Haryana.

3.2. Experimental design

Four experimental diets were designed to evaluate the potential of silkworm pupae (*Bombyx mori*) meal, housefly (*Musca domestica*) maggot's meal and earthworm (*Eisenia fetida*) meal in *Labeo rohita* (F. Hamilton, 1822)fingerlings diets in place of defatted soybean meal at 30% inclusion level. A completely randomized design has been followed to carry out the experiment. Three replication of each experimental group was arranged in the study.

3.3. Experimental diets preparation

Different components like silkworm pupae, housefly maggots, earthworm, de-fatted, mustard oil cake, de-oiled rice bran (DORB), soybean meal, wheat flour, groundnut oil cake, vitamin-mineral, and sunflower oil were used for the feed preparation.(Table 3). Out of which, silkworm pupae, housefly maggots and earthworms collected, sun dried there after all ingredients were weighed properly as per the formulation and grinded. All the components except oil and vitamin premix were thoroughly combined to form dough by adding an adequate amount of water. The dough was cooked for half an hour in a pressure cooker. After that dough (pre-cooked) allowed cooling in room temperature. Afterwards, the calculated proportions of the vitamin premix and oil were included in it. The thoroughly mixed dough was compressed through a pelletizer to produce pellets of 1.5 mm. diameter, which were then spread out on a paper sheet and left to air-dry. After air drying process, the pellets were placed in plastic bags, sealed tightly, labelled with treatment tags, and stored in a freezer at -20°C until they were given to the fish.

Table 3: Formulation of the experimental diets with silkworm pupae meal, housefly larve meal & earthworm meal for feeding of *L. rohita*

Ingredients (g 100 g^{-1})	Experimental diets			
	Control	Silkworm pupae meal	Housefly maggots meal	Earthworm meal
Insect meal	0	30	30	0
Earthworm Meal	0	0	0	30
Defatted soybean meal	30	0	0	0
Groundnut oil cake	30	18	30	27
Mustard oil cake	22	8	7	26
De-oiled rice bran	10	23	20	10
Wheat flour	4.5	19.5	12	6
Sunflower oil	2.5	0.5	0	0
Vitamin-mineral	1	1	1	1
Total	100	100	100	100

Composition of vitamin mineral mix (PREEMIX PLUS) (quantity/kg diet) Vitamin A, 11,000 IU. Vitamin D3, 22,000 IU. Vitamin B2, 40 mg. Vitamin E, 15 mg. Vitamin K, 20 mg. Vitamin B6, 20 mg. Vitamin B12, 0.12 mcg. Calcium Pantothenate, 50 mg. Nicotinamide, 0.2 g, Choline Chloride, 3 g. Mn, 540 mg. I, 20 mg; Fe, 150 mg; Zn, 100 mg. Cu, 40 mg. Co, 9 mg. Selenium 2.5 mg. Vitamin C, 50 mg.

3.4. Set up of experiments and animal

The experimental was conducted in 12 uniform sized experimental circular tanks (180 cm X 50 cm) 300 L water volume. A juvenile of *L. rohita* with initial average body weight of 5.07 ± 0.01 g was used in the current experiment with a stoking density of 16 no's /tank. Initially, the tubs were rinsed and filled with $KMnO_4$ solution (4 mg L^{-1}). The tubs were flushed the following day and properly cleansed with clean water. With continuous aeration, the total volume of water in each tub was kept constant throughout the trial.

3.5. Rearing

Before the experiment began, the fish in the tubs were given a control diet. There was no attempt to stimulate or control the environmental state. The experimental conditions remained constant throughout the experiment. To assess growth, the body weight was taken every 15 days. The fish were fasted overnight before weighed.

3.6. Cleaning and draining

The experimental tubs were manually cleaned, and syphoning was performed at every three days to eliminate faecal waste. Throughout the experiment, an equal volume of clean bore well water replaced the syphoned water.

3.7. Water parameters

Throughout the experiment, water quality indices such as pH, dissolved oxygen, temperature, free carbon dioxide total hardness, total alkalinity, total ammonia-N, nitrate-N, and nitrite-N were measured on alternate days by standard methods (APHA, 2005)

3.7.1. Temperature

The water temperature in all of the experimental units was measured with a water thermometer (MERCK, Germany) and expressed in degrees Celsius.

3.7.2. pH

A water digital pH probe (HANNA instruments, Singapore) meter was used to test the water pH of all of the experimental units.

3.7.3. Dissolved oxygen

All experimental units' dissolved oxygen levels were determined using the classic Winkler's method (APHA, 2005). DO concentration was given in milligrams per litre.

3.7.4. Free CO_2

The free CO_2 level in all of the experimental tanks was analysed by measured using the titration method with NaOH solution, using phenolphthalein as indicator and the values were expressed as milligrams per litre, the level was calculated using the formula below.

Carbon dioxide (mg/L) $=(A \times N \times 44 \times 1000)/V$

Where, A = Volume of titrant (NaOH)

N = Normality of titrant (N/ 44)

V = Volume of sample (ml)

3.7.5. Total hardness

The total hardness of the water in all of the experimental tanks was measured using a titrimetric method using Ethylenediaminetetraacetic acid and Eriochrome Black-T (APHA, 2005) and expressed in mg/L.

3.7.6. Alkalinity

The alkalinity was calculated using the titrimetric method (APHA, 2005) and expressed in mg/L using standard H_2SO_4 and phenolphthalein and methyl orange as indicators.

3.7.7. Total ammonia-N

The total ammonia nitrogen concentration in the experimental water was calculated using the Ammonia-Nitrite Test Kit and expressed in milligrams per litre.

3.7.8. Nitrite-N

The nitrite-N concentration in each experimental tank was obtained using an Ammonia-Nitrite Test Kit and expressed in milligrams per litre.

3.7.9. Nitrate-N

The concentration of nitrate-N in all of the experimental waters was obtained using an Ammonia-Nitrite Test Kit and expressed in milligrams per litre.

3.8. Proximate analysis of ingredients, diets and fish

Standard methods as reported in AOAC (2005) were followed for determination of the proximate analysis of feed ingredients for the formulation of diets.

3.8.1. Moisture

The moisture of the ingredients and feeds was assessed by weighing a sample placed in a petri dish and drying it in a hot air oven at 100-105°C until it achieved a constant weight. The moisture content was calculated using the formula based on the change in the sample's weight.

$$\text{Moisture}(\%) = \frac{\text{Initial wt. of sample} - \text{Dried wt. of sample}}{\text{Initial wt. of sample}} \times 100$$

3.8.2. Crude protein (CP)

The nitrogen content of the components, experimental meals, and whole body tissue dried samples was quantitatively measured using the Kjeltec semi-automated method (Kjeltec Auto Distillation, Sweden) and titration. The CP percentage was calculated by multiplying the nitrogen percentage by 6.25.

Crude protein (%) = N (%) × 6.25

3.8.3. Ether extracts (EE)

Soxhlet apparatus was used to estimate ether extract of experimental diets and ingredients using diethyl ether as the solvent. The following computation was performed.

$$\text{EE}(\%) = \frac{\text{Initial wt.} - \text{Final wt.}}{\text{Initial wt.}} \times 100$$

3.8.4. Total ash

A known weight of material was placed in a silica crucible and placed in a muffle furnace at 600°C for 6 hours to measure the ash concentration. The calculation was carried out as follows.

$$\text{Ash}\ (\%) = \frac{\text{Weight of ash}}{\text{Weight of sample (g)}} \times 100$$

3.8.5. Nitrogen free extract

Nitrogen free extract (NFE) of feed and ingredients was calculated by following equation.

NFE (%) =100 – (CP % + CF % + EE % + Total ash %)

3.8.6. Crude fibre

The crude fibre content of various test diets (fat-free samples) was evaluated using Fibro tron (Tulin equipments, India) after acid (1.25% HCL) and alkali digestion (1.25% NaOH), followed by drying (100°C) and incineration (in muffle furnace at 550°C for 4 hrs). The following computation was performed.

$$\text{CF (\%)} = \frac{\text{Weight of dried sample (g)} - \text{Weight of ash (g)}}{\text{Initial weight of sample (g)}} \times 100$$

3.8.7. Digestible energy (DE)

The digestible energy value was estimated using the following formula based on established physiological values (Halver, 1976).

Digestible energy (kcal/100g) = [CP (%) x 4 + EE (%) x 9 + NFE (%) x 4]

3.9. Growth parameters

The fish were sampled at every 15 days to determine their body weight. The fish were starved overnight before being weighed. The weight was measured using an electronic weighing scale. The following formulas are used to calculate the growth and nutrient utilization indices.

$$\text{Weight gain(\%)} = \frac{\text{Final weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e\text{ (Final weight)} - \text{Log}_e\text{(Initial weight)}}{\text{Experimental duration in days (d)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Protein fed (g)}} \times 100$$

3.10. Fatty acid analysis of fish muscle

The fatty acid content of fish muscle (two persons per tank) was evaluated using an Agilent 7820a Series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and the Folch et al. (1957) method. The fatty acid methyl ester synthesis and gas chromatograph analysis were carried out using the procedures published previously (Tian et al. 2014). To extract total lipids, 0.3-0.5 g samples were homogenised in chloroform/methanol (2:1, v/v). The mixtures were filtered using quantitative filter paper after standing for 2 hours. The methanol was then extracted with 4 mL of distilled water. The layered solution was centrifuged for 5 minutes (825 g). The supernatant was then removed, and the remaining mixture was air dried in a 40^{o}C water bath. Following that, 1 mL hexane was added to dissolve the lipids, followed by 1 mL potassium hydroxide methanol (0.4 M) for 1 hour of methyl esterification. Finally, 1 mL of distilled water was added to the mixture to stratify it before collecting and analyzing the upper layer using a gas chromatograph equipped with a capillary column and a flame-ionization detector. The carrier gas was helium; the split rate was 1/50; and the injector and detector temperatures were 250 and 280^{o}C, respectively. The thermal gradient programme began at a temperature of 175^{o}C for 10 minutes, then climbed to 220^{o}C in 20 minutes at a rate of 3^{o}C/min. The temperature was then raised to 240^{o}C in 10 minutes at a rate of 4^{o}C/min. For detection, a total of 1 L of material was injected. By comparing individual methyl esters to established standards, individual methyl esters were identified. The data were represented as a proportion of the total fatty acids identified for each fatty acid.

3.11. Statistical analysis

The statistical significance of various study parameters was assessed using one-way evaluation of variance (ANOVA) in SPSS 22.0 for Windows. Duncan's multiple range test was used for post hoc comparisons of means ($P<0.05$) between different experimental groups. A significance level of 5% ($P<0.05$) was established for all statistical tests.

3.12. Experimental design

Fish	Fingerlings of *Labeo rohita*
Types of feeds	4(Control, SWP, Housefly maggots, Earthworm)
Container	12 tanks (300 L Water volume)
Treatments	4
Replications	3
Stocking density	16 fingerlings per tank
Acclimatization	Experimental setup in which fed with commercial diet for one week and later with experimental diet.
Feeding	3% of body weight
Feeding frequency	Twice a day 9.00 a.m. and 5.00 p.m. hours
Sampling frequency	Once in 15 days
Duration of experiment	60 days excluding from acclimatization period

4. RESULTS

4.1. Nutritional composition of ingredients

The nutritional compositions of the ingredients have been showed in Table 4. The CP contents of the silkworm pupae, housefly larvae, and earthworm meal were 44.24, 54.13, and 52.48%, respectively. While the crude fat content of these ingredients were 25.32, 22.45, and 3.55%, respectively. Digestible energy was recorded as 4.87, 4.93, and 4.82 kcal/100 g, respectively, for the silkworm pupae, housefly larvae, and earthworm meal.

Table 4: Proximate composition of different feed ingredients (% dry matter) used for the preparation of the experimental diets

Composition	Silkworm pupae	Housefly maggot	Earthworm	DSBM	GNOC	MOC	DORB	Wheat flour
Drymatter	93.12 ±0.63^{de}	94.45 ±0.47^{e}	94.63 ±0.58^{e}	90.99 ±0.73^{bc}	92.4 ±0.57^{cd}	91.61 ±0.48^{cd}	89.54 ±0.42^{ab}	88.76 ±0.79^{a}
Crudeprotein	44.24 ±0.32^{d}	54.13 ±0.48^{g}	52.48 ±0.25^{f}	48.59 ±0.65^{e}	49.21 ±0.74^{e}	39.86 ±0.71^{c}	17.23 ±0.24^{b}	11.7 ±0.16^{a}
Crudefat	25.32 ±0.37^{e}	22.45 ±0.45^{d}	3.55 ±0.12^{b}	1.23 ±0.10^{a}	1.19 ±0.15^{a}	7.47 ±0.27^{c}	1.82 ±0.09^{a}	1.16 ±0.05^{a}
Nitrogenfree extract	11.78 ±1.37^{a}	10.26 ±1.36^{a}	18.77 ±0.88^{b}	40.75 ±1.11d	34.14 ±1.43^{c}	34.03 ±1.71^{c}	60.65 ±1.19^{e}	85.33 ±0.36^{f}
Crudefibre	4.56 ±0.17^{c}	6.63 ±0.19^{d}	13.84 ±0.09^{h}	3.67 ±0.16^{b}	9.62 ±0.23^{f}	10.98 ±0.21^{g}	8.43 ±0.25^{e}	1.25 ±0.11^{a}
Totalash	14.1 ±0.51^{e}	6.53 ±0.32^{bc}	11.36 ±0.42^{d}	5.76 ±0.21^{b}	5.84 ±0.31^{b}	7.66 ±0.53^{c}	11.87 ±0.61^{d}	0.56 ±0.04^{a}
Digestible energy	4.87 ±0.27^{c}	4.93 ±0.39^{c}	4.82 ±0.26^{c}	4.81 ±0.22^{c}	4.48 ±0.29^{bc}	4.16 ±0.41^{abc}	3.65 ±0.32^{ab}	3.44 ±0.26^{a}

All values are presented as mean of three. Abbreviation; DSBM; Defatted soybean meal; DORB; Deoiled ricebran, MOC; Mustard oilcake, GNOC; Ground nut oilcake. P=0.01- 0.001. In a row means followed by different letters are significantly different by DMRT

4.2. Nutritional composition of the experimental diets

The nutritional compositions of the ingredients have been showed in the Table 5. The experimental diets were found as isonitrogenous and isolipidic in nature with around 35% CP and 6% crude lipid.

Table 5: Nutrient composition of different experimental diets

Proximate compositions	Experimental diets			
	Control	Silkworm pupae meal	Housefly Meal	Earthworm Meal
Moisture	9.76 ± 0.27	8.84 ± 0.45	9.25 ± 0.19	9.63 ± 0.23
Crude protein	35.17 ± 0.35	35.21 ± 0.29	35.48 ± 0.33	35.24 ± 0.36
Crude fat	6.04 ± 0.19	6.37 ± 0.13	6.22 ± 0.2	6.30 ± 0.18
Crude fibre	6.63 ± 0.28	7.15 ± 0.37	7.56 ± 0.42	7.34 ± 0.46
Nitrogen free extract	44.7 ± 1.04	43.46 ± 1.02	43.71 ± 0.96	43.53 ± 0.83
Total ash	7.46 ± 0.14	7.81 ± 0.19	7.03 ± 0.09	7.59 ± 0.17

All the values are showed as Mean ± SE (n=6). In a row means followed by different letters are significantly different by DMRT

4.3. Water quality metrics

The metrics of water quality in the experimental units have been showed in the Table 6. There was no statistically significant difference (P > 0.05) was found among the metrics of water quality during the entire period. The temperature, dissolved oxygen and pH was nearly about 27°C, 6 mg/L and 7.5, respectively in all the experimental units. The free CO_2 wasn't detected in the experimental groups. The water alkalinity, ammonia, nitrite, hardness, and nitrate values were around 128 mg/L, 0.05 mg/L, 0.027 mg/L, 175 mg/L, and 0.023 mg/L, respectively.

Table 6: Water quality parameters or variables of experimental tanks

Parameters	**Control**	**Silkworm pupae meal**	**Housefly meal**	**Earthworm meal**	***P value***
Temperature (°C)	27.58±0.29	27.86±0.34	27.48±0.34	27.44±0.35	*0.831*
pH	7.5±0.05	7.52±0.07	7.43±0.07	7.42±0.07	*0.670*
Dissolved oxygen (mg/L)	6.83±0.07	6.82±0.06	6.76±0.08	6.39±0.42	*0.483*
Free CO_2	Not detected	Not detected	Not detected	Not detected	-
Hardness (mg/L)	175.89±1.23	176.03±0.68	175.38±0.96	175.19±1.29	*0.943*
Alkalinity (mg/L)	129.54±1.5	128.53±1.64	127.64±1.45	128.25±1.15	*0.810*
Nitrite (mg/L)	0.028±0.001	0.027±0.002	0.027±0.002	0.028±0.002	*0.937*
Ammonia (mg/L)	0.054±0.002	0.044±0.003	0.047±0.003	0.05±0.003	*0.105*
Nitrate (mg/L)	0.024±0.002	0.023±0.001	0.024±0.001	0.025±0.001	*0.787*

All the values are showed as Mean ± SE (n=6). In a row means followed by different letters are significantly different by DMRT

4.4. Nutrient utilization and growth performance

Nutrient utilisation and growth attributes of *Labeo rohita* fed with various formulated diets have been presented in Table 7. At the end of the feeding experiment, SGR, FBW, FCR, WG%, and PER were significantly affected ($P < 0.05$) between the experimental diets. The evaluations of FBW (Fig. 1), SGR (Fig. 2), WG% (Fig. 3), PER (Fig. 4) and FCE (Fig. 5) were statistically significant ($P < 0.05$) high in silkworm pupae meal and housefly meal fed groups contrast to earthworm meal and control fed diets. However, there were no statistically significant differences ($P > 0.05$) found among the control and earthworm meal-fed diets and the silkworm pupae meal and housefly meal-fed diets. A statistically significant ($P < 0.05$) opposite trend was found for FCR utility (Fig. 6). The values of FCR were significantly higher in the control and earthworm meal groups and significantly lower in the silkworm pupae meal and housefly meal groups.

Table 7: Growth performance and nutrient utilization of *Labeo rohita* provided with different experimental diets

Parameters	Control	Silkworm pupae meal	Housefly meal	Earthworm meal	*P value*
Initial weight (g)	5.06±0.02	5.06±0.02	5.08±0.03	5.1±0.01	*0.492*
Final weight (g) (FBW)	14.58±0.1^{a}	17.21±0.66^{b}	17.8±0.54^{b}	14.4±0.13^{a}	*0.001*
Feed conversion ratio (FCR)	2.11±0.03^{b}	1.85±0.07^{a}	1.85±0.06^{a}	2.14±0.03^{b}	*0.005*
Protein efficiency ratio (PER)	1.35±0.02^{a}	1.54±0.06^{b}	1.54±0.05^{b}	1.33±0.02^{a}	*0.008*
Feed conversion efficiency (FCE)	0.47±0.01^{a}	0.54±0.02^{b}	0.54±0.02^{b}	0.47±0.01^{a}	*0.009*
Specific growth rate (SGR)	1.76±0.02^{a}	2.04±0.07^{b}	2.09±0.04^{b}	1.73±0.02^{a}	*0.000*
Weight gain percentage (WG %)	188.38±3.18^{a}	239.93±13.55^{b}	250.45±9.24^{b}	182.19±2.29^{a}	*0.001*

All the values are showed as Mean ± SE (n=6). In a row means followed by different letters are significantly different by DMRT

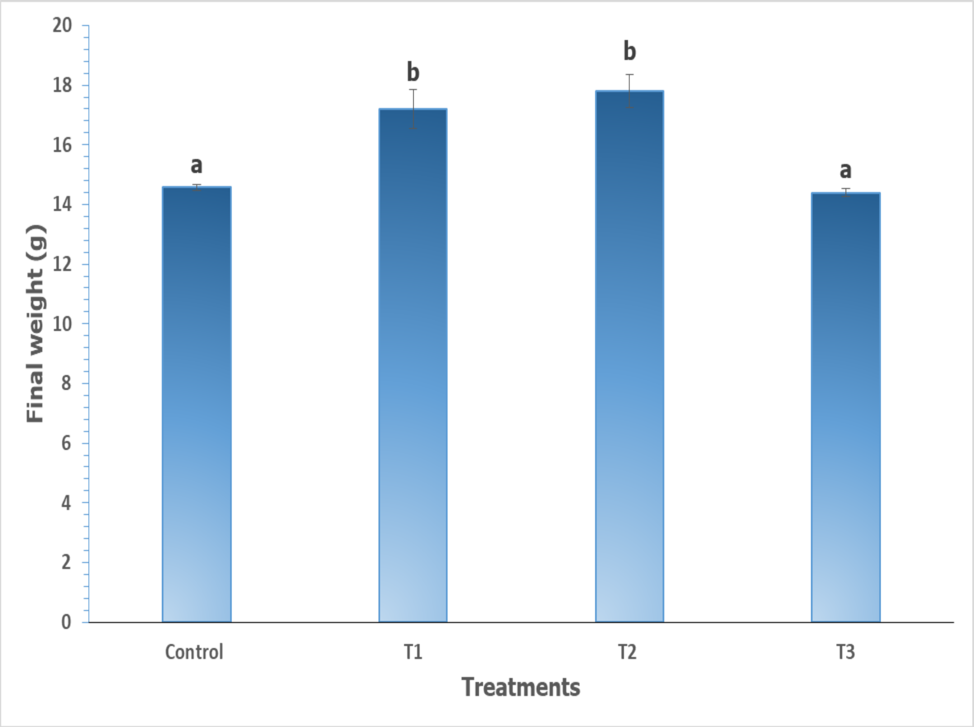


Figure 1: Final weight (grams) of juvenile *Labeo rohita* fed various experimental groups

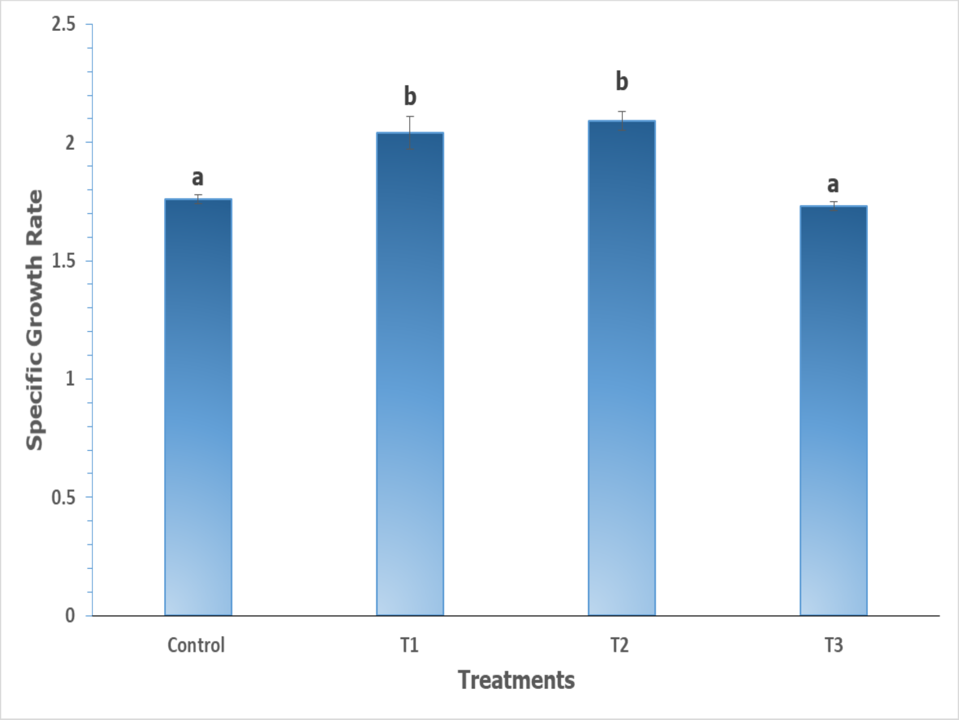


Figure 2: Specific growth rate of juvenile *Labeo rohita* fed various experimental groups

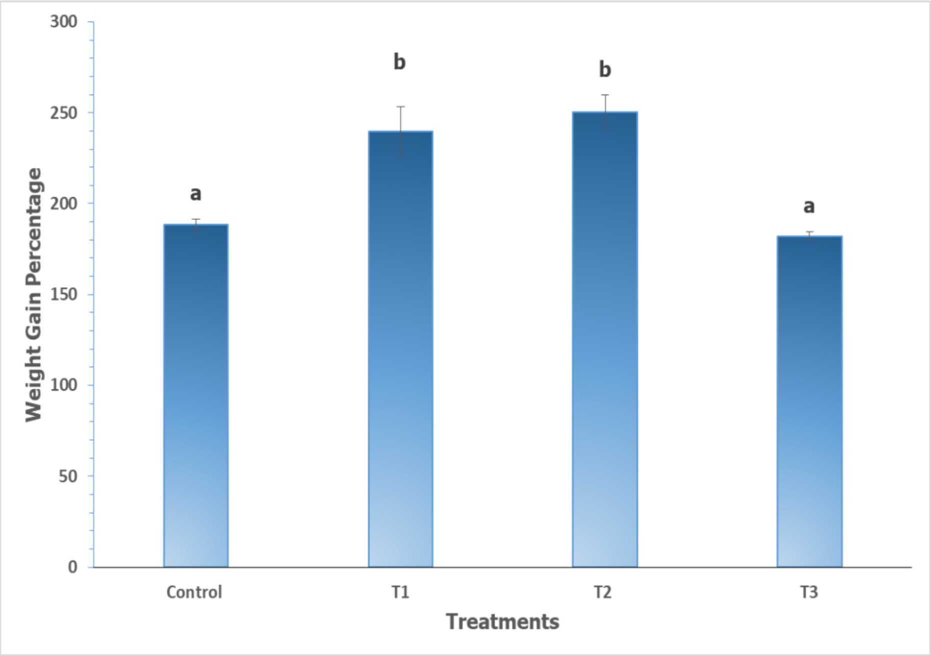


Figure 3: Weight gain percentage of juvenile *Labeo rohita* fed various experimental groups

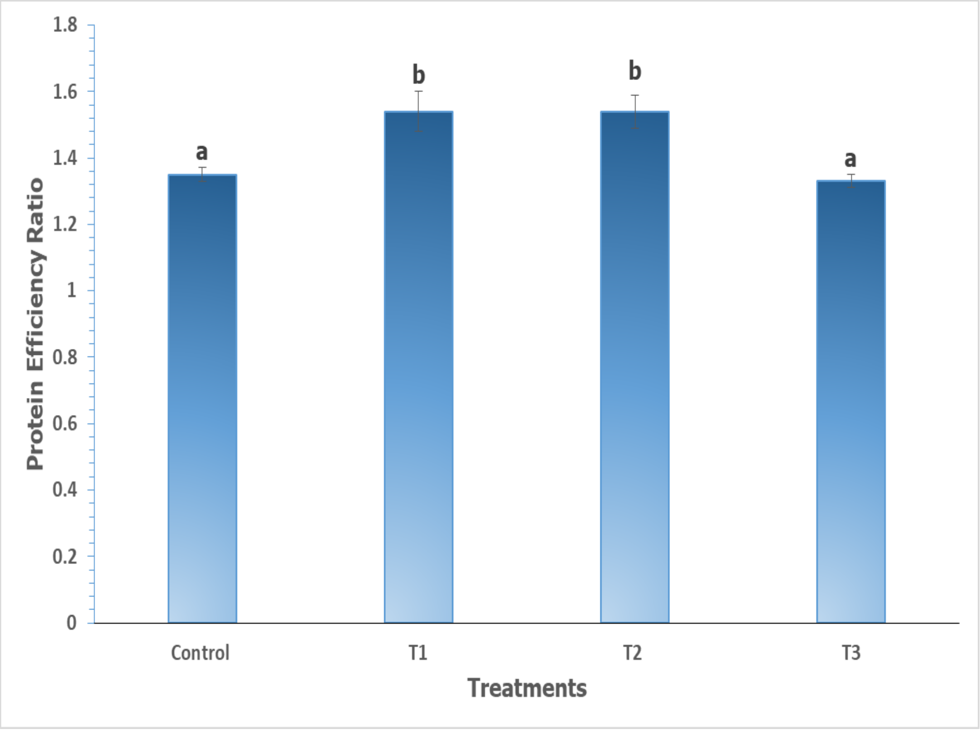


Figure 4: Protein efficiency ratio of juvenile *Labeo rohita* fed various experimental groups

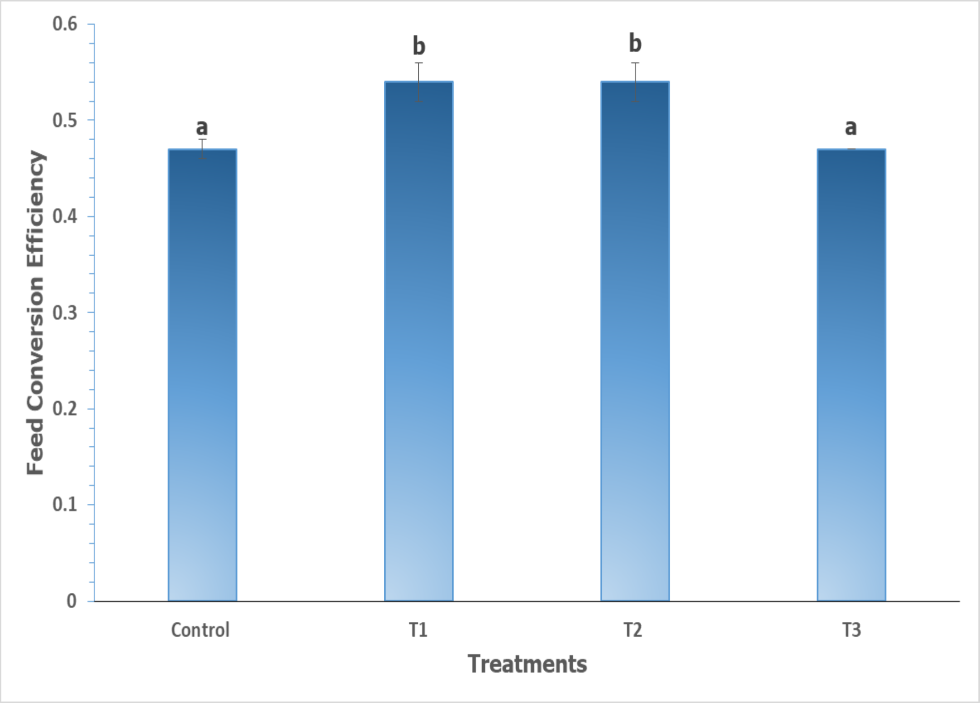


Figure 5: Feed conversion efficiency of juvenile *Labeo rohita* fed various experimental groups

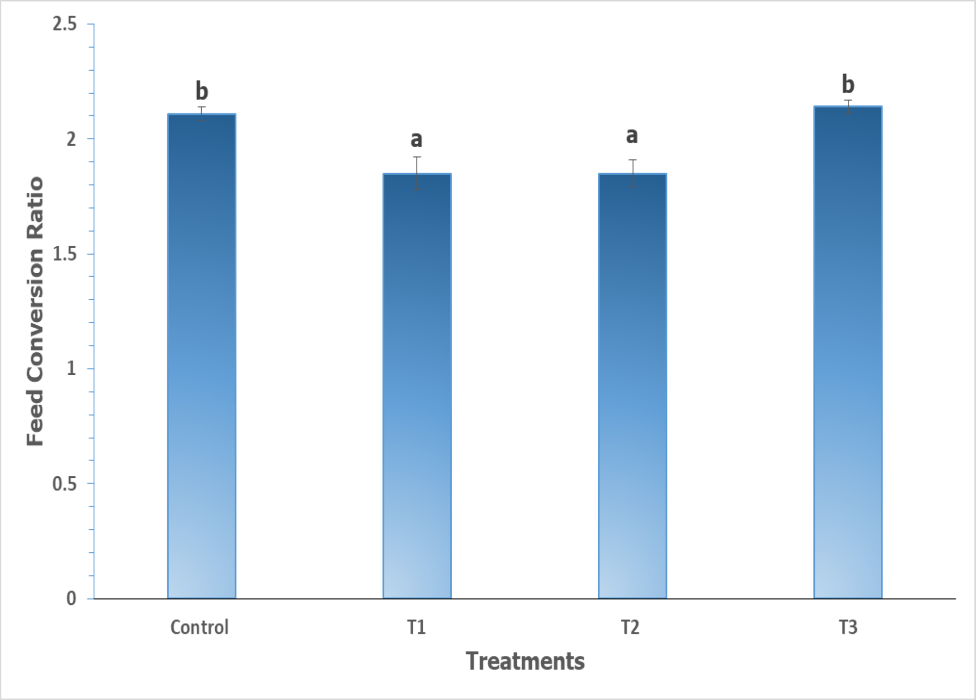


Figure 6: Feed Conversion Ratio of juvenile *Labeo rohita* fed various experimental groups

4.5. Nutritional composition of the entire body of the fish

The proximate composition of the entire body of the experimented fish has been depicted in Table 8. The moisture, crude lipid, and total ash content of the entire body of *L. rohita* showed no statistically significant difference ($P > 0.05$) between the experimental groups. Though feeding insect meal-based diets to *L. rohita* showed statistically significant difference ($P < 0.05$) in entire body CP (Fig. 7) and total carbohydrate (Fig. 8) content, Among the insect meal-based diets, the highest crude protein deposition was observed in a housefly maggots meal-based diet, followed by a silkworm pupae meal-based diet. The total carbohydrate content was significantly ($P < 0.05$) higher in the control and earthworm meal-based diets than in the silkworm pupae meal and housefly meal-based diets.

Table 8: Proximate composition of *Labeo rohita* fed with different experimental diets

Variables	Control	Silkworm pupae meal	Housefly meal	Earthworm meal	*P value*
Moisture	74.18±0.2	74.2±0.15	74.15±0.5	74.03±0.16	*0.975*
Crude protein	15.89 ± 0.06^{ab}	16.22 ± 0.13^{b}	16.78 ± 0.15^{c}	15.52 ± 0.21^{a}	*0.000*
Crude lipid	3.66±0.24	3.72±0.03	3.67±0.27	4.06±0.12	*0.435*
Total carbohydrate	3.54 ± 0.11^{b}	2.86 ± 0.1^{a}	2.56 ± 0.29^{a}	3.73 ± 0.14^{b}	*0.005*
Total ash	2.74±0.09	3.01±0.07	2.84±0.12	2.67±0.18	*0.299*

All values are presented as Mean ± SE (n=3). Within a row, means with different letters indicate significant differences according to DMRT.

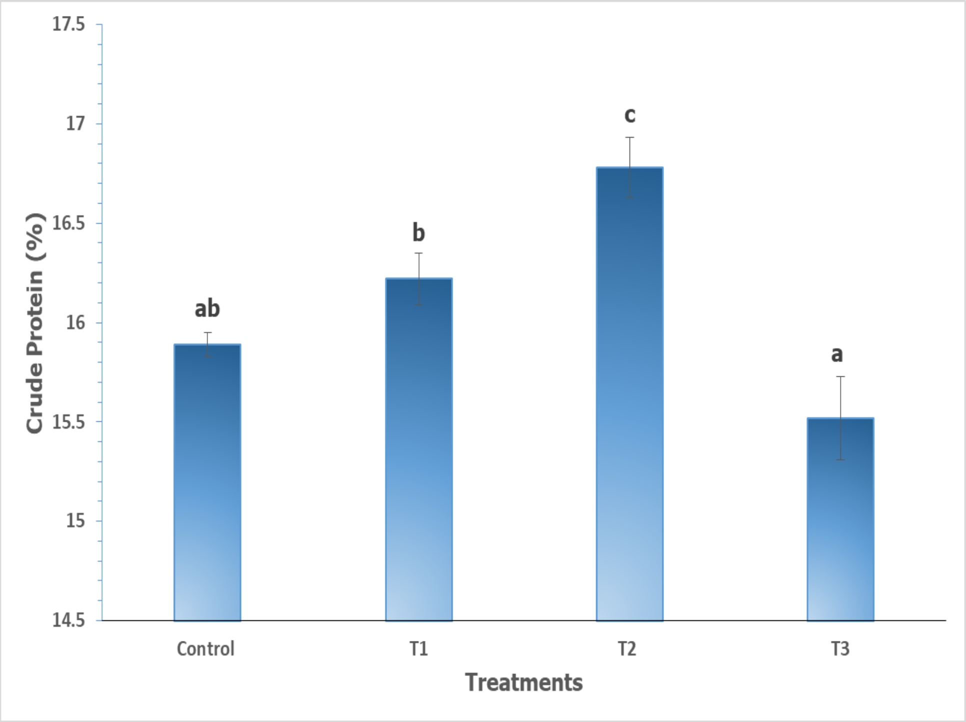


Figure 7: Total crude protein content in the whole body of juvenile *Labeo rohita* fed various experimental groups

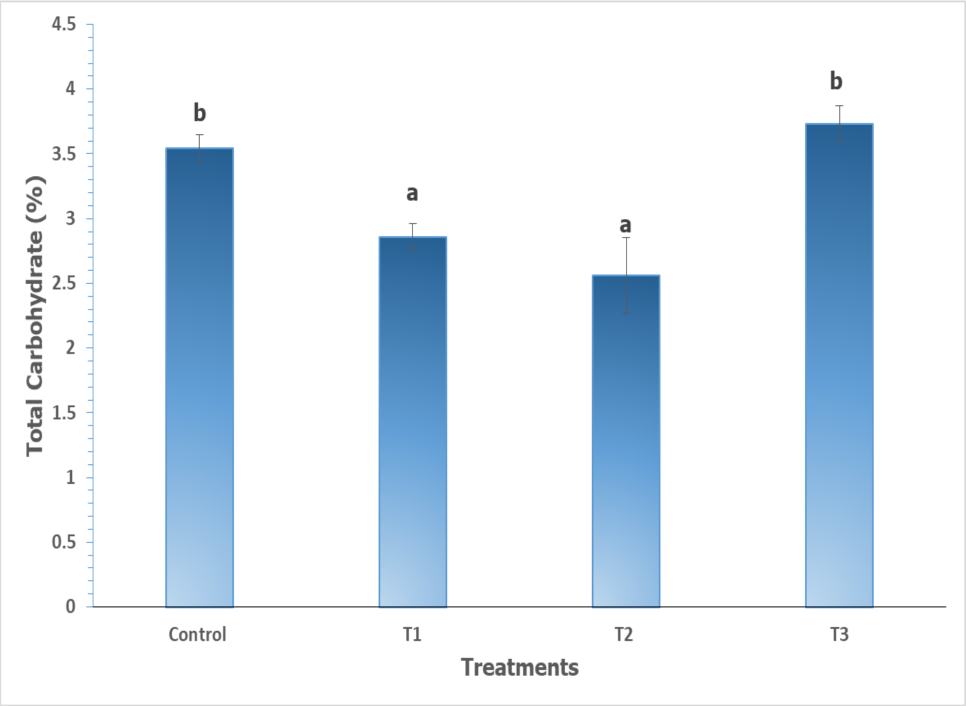


Figure 8: Total carbohydrate content in the whole body of juvenile *Labeo rohita* fed various experimental groups

4.6. Fatty acid composition of the fish muscle

The fatty acid composition of the muscle *L. rohita* has been presented in Table 9. Among the muscle fatty acid compositions of different experimental groups, 16:00 (Fig. 9), 18:3 (n-3) (Fig. 10), 20:1 (n-9) (Fig. 11), and 22:6 (n-3) (Fig. 12) showedstatistically significant ($P < 0.05$) variations between the experimental groups. Besides, the sum of total saturated fatty acids showed astatistically significant ($P < 0.05$) difference among the experimental groups. This sum of total saturated fatty acids (Fig. 13) was significantly ($P < 0.05$) high in the control and silkworm pupae based feeds than the earthworm meal and housefly meal-based diets. Furthermore, there wasn't any observablestatistically significant ($P > 0.05$) change in the sum of mono-unsaturated and poly-unsaturated fatty acids or the ratios of n-3 and n-6 fatty acids.

Table 9: Fatty acid composition of the muscle of *Labeo rohita* fed with different experimental diets

Fatty acids	**Control**	**Silkworm pupae meal**	**Housefly meal**	**Earthworm meal**	***P value***
Lauric acid (12:0)	5.35±0.15	5.57±0.21	4.95±0.14	5.19±0.04	*0.085*
Myristic acid (14:0)	6.27±0.12	5.58±0.44	5.92±0.12	6.19±0.09	*0.238*
Pentadecylic acid (15:0)	2.28±0.12	2.4±0.07	2.51±0.18	2.16±0.07	*0.249*
Palmitic acid (16:0)	11.34±0.15^{c}	11.05±0.18^{bc}	10.4±0.15^{a}	10.49±0.23^{ab}	*0.017*
Palmitoleic Acid (16:1)	5.02±0.05	4.9±0.12	5±0.03	5.3±0.24	*0.261*
Margaric acid (17:0)	1.18±0.05	1.47±0.2	1.2±0.08	1.2±0.11	*0.379*
Stearic acid (18:0)	9.97±0.19	10.18±0.09	10.44±0.17	9.66±0.3	*0.120*
Oleic acid (18:1, n-9)	6.09±0.13	6.23±0.14	6.63±0.22	6.19±0.04	*0.119*
Linoleic acid (18:2, n-6)	4.2±0.1	4.18±0.18	4.37±0.2	3.74±0.25	*0.195*
Linolenic Acid (18:3, n-3)	3.95±0.08^{b}	3.87±0.06^{b}	3.32±0.26^{a}	3.07±0.05^{a}	*0.005*
Arachidic acid (20:0)	8.77±0.25	8.67±0.11	8.16±0.06	8.43±0.16	*0.104*
Gondoic acid (20:1, n-9)	6.1±0.03^{a}	6.66±0.13^{b}	6.19±0.05^{a}	6.21±0.18^{a}	*0.035*
Eicosadienoic acid (20:2, n-6)	2.05±0.12	2.26±0.08	2.16±0.02	2.1±0.07	*0.347*
Dihomo-γ-linolenic acid (20:3, n-6)	3.29±0.18	2.93±0.24	3.11±0.26	3.01±0.15	*0.677*
Mead acid (20:3, n-3)	3.11±0.02	3.33±0.16	3.38±0.12	3.42±0.23	*0.529*
Arachidonic Acid (20:4, n-6)	1.27±0.06	1.79±0.13	1.34±0.05	1.68±0.29	*0.137*
Eicosapentaenoic acid (20:5, n-3)	3.05±0.09	2.83±0.2	2.38±0.27	2.22±0.1	*0.039*

Docosahexaenoic acid (22:6, n-3)	0.81±0.03^{a}	0.85±0.05^{a}	1.13±0.03^{b}	1.02±0.08^{b}	*0.007*
Nervonic acid (24:1, n-9)	4.14±0.06	4.22±0.15	4.36±0.18	4.47±0.24	*0.545*
∑FA	95.44±0.17	96.2±0.41	93.78±1.12	92.32±1.5	*0.081*
Other	7.19±0.03	7.25±0.35	7.3±0.1	7.07±0.08	*0.828*
∑Saturated FA	45.16±0.3^{b}	44.92±0.57^{b}	43.59±0.24^{a}	43.31±0.43^{a}	*0.027*
∑Monounsaturated FA	28.55±0.15	29.24±0.2	29±0.54	28.75±0.63	*0.712*
∑Polyunsaturated	21.73±0.3	22.04±0.48	21.19±0.48	20.26±0.48	*0.089*
∑n-3	7.87±0.09	8.04±0.17	7.82±0.26	7.51±0.22	*0.334*
∑n-6	13.86±0.25	14±0.34	13.36±0.34	12.75±0.28	*0.072*
∑n-3/n-6	0.57±0.01	0.57±0.01	0.59±0.02	0.59±0.01	*0.544*

All values are presented as Mean ± SE (n=3). Within a row, means with different letters indicate significant differences according to DMRT.

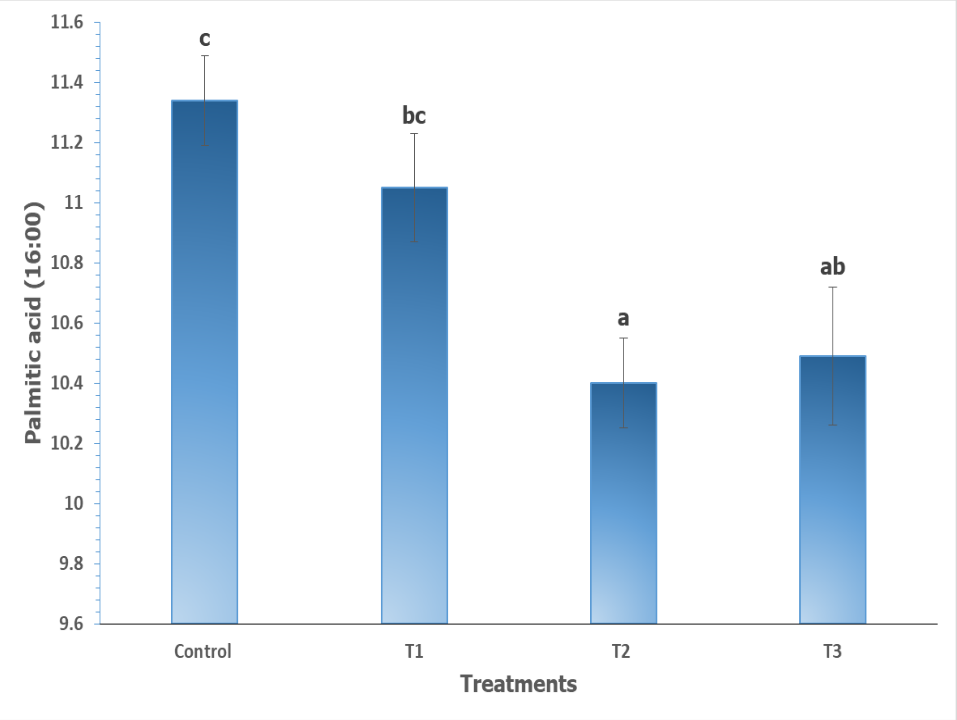


Figure 9: Palmitic acid (16:0) composition in the muscles of juvenile *Labeo rohita* fed various experimental feeds.

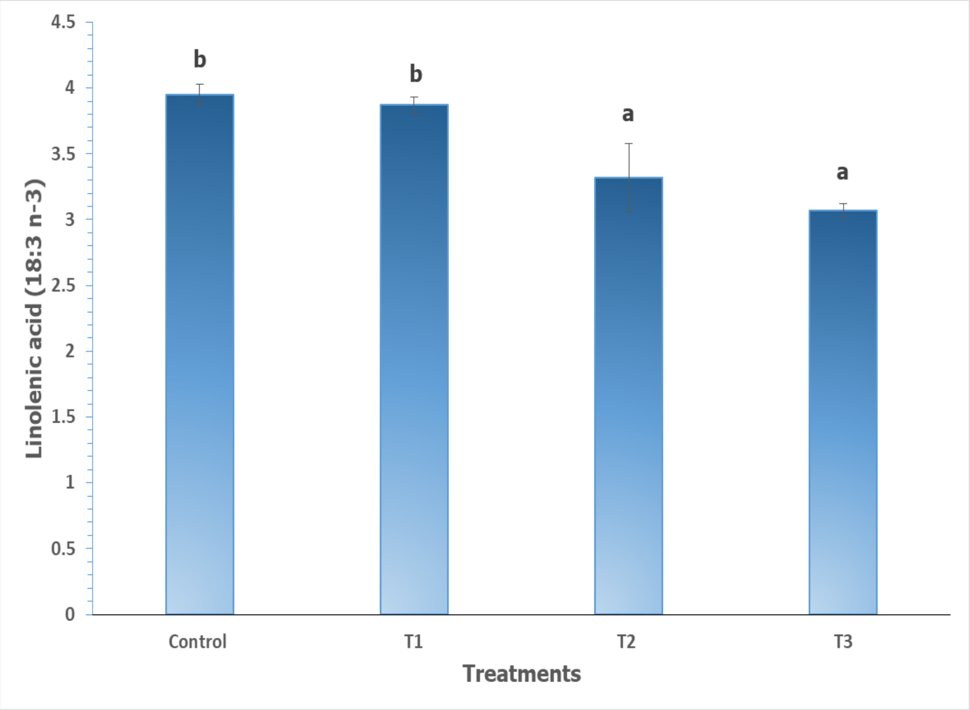


Figure 10: Linolenic acid (18:3, n-3) composition in the muscles of juvenile *Labeo rohita* fed various experimental feeds

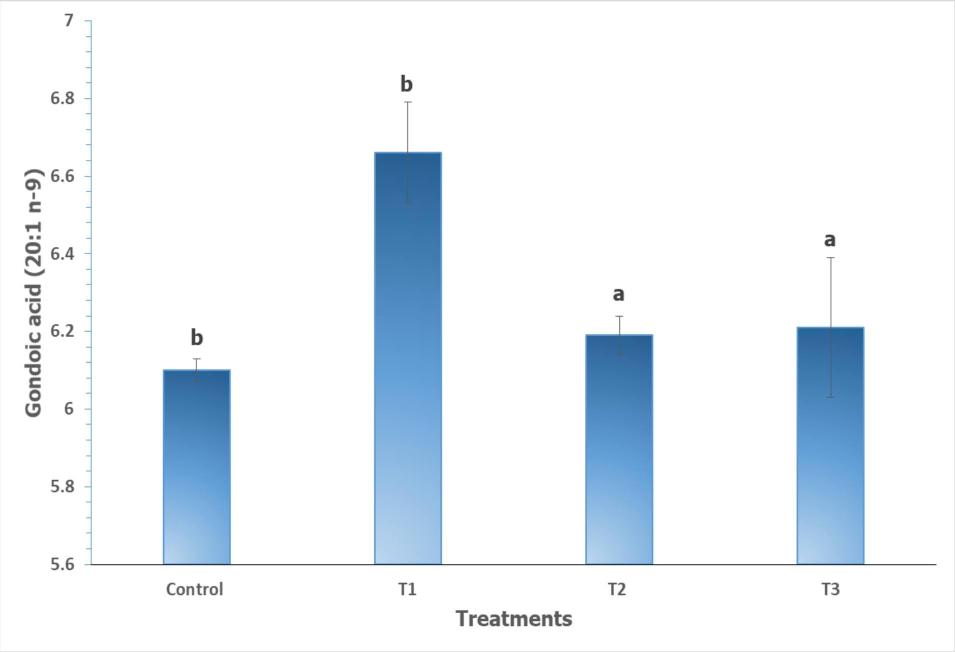


Figure 11: Gondoic acid (20:1, n-9) composition in the muscles of juvenile *Labeo rohita* fed various experimental feeds

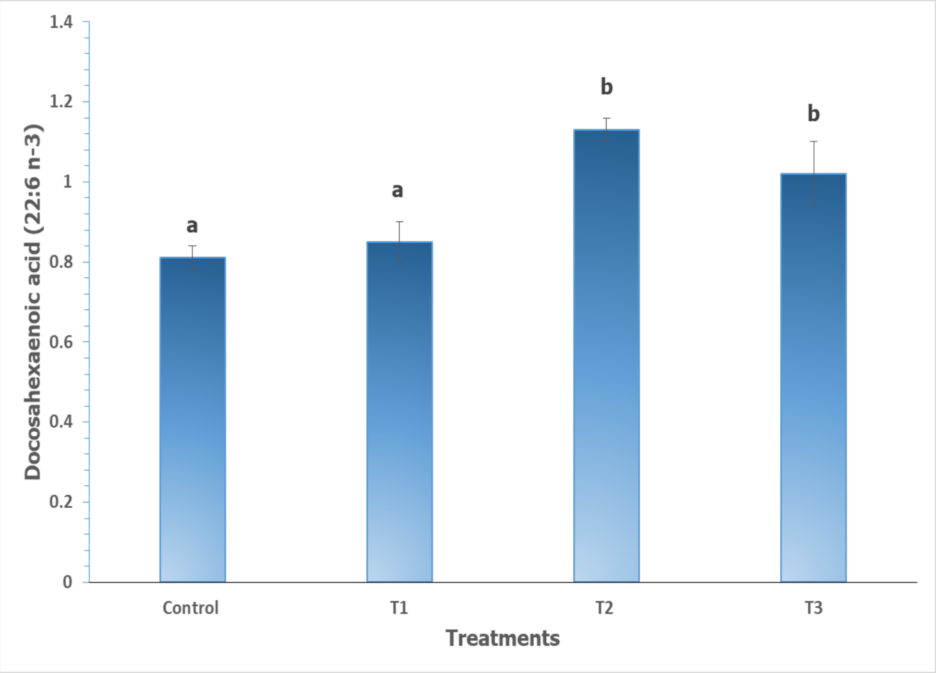


Figure 12: Docosahexaenoic acid (22:6, n-3) composition in the muscles of juvenile *Labeo rohita* fed various experimental feeds

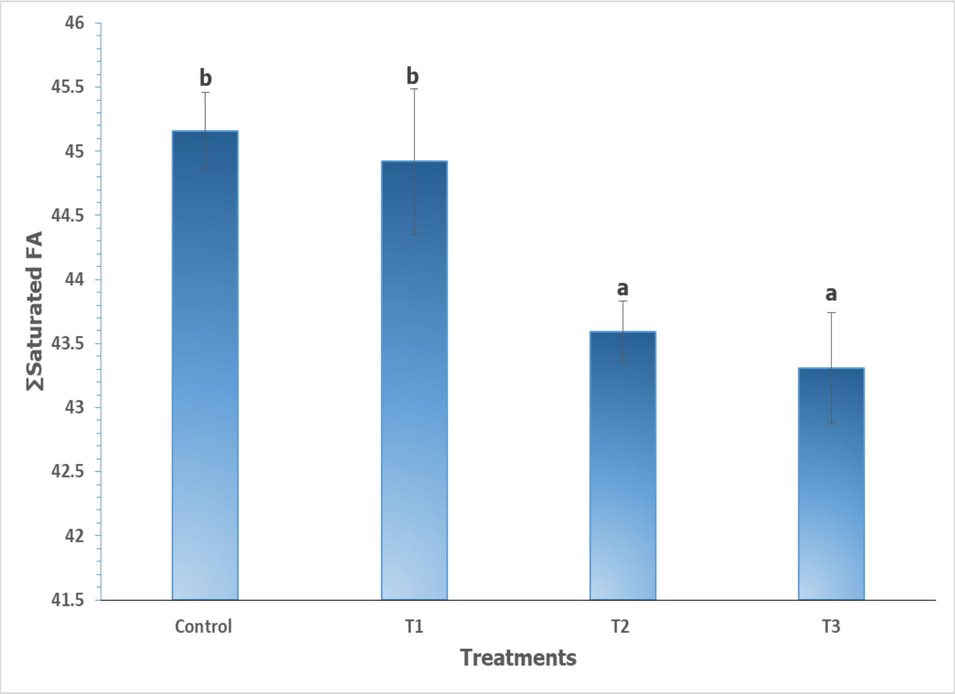


Figure 13: Saturated Fatty acid composition in the muscles of juvenile *Labeo rohita* fed various experimental feeds.

5. DISCUSSION

5.1. Water quality parameters

Dissolved oxygen, temperature, total hardness, nitrate, ammonia, pH, and nitrite were among the water quality indicators that were found to be in the ideal range for carp rearing in this analysis (Mohapatra et al., 2012; Debnath et al., 2007). Even so, there was no significant ($P > 0.05$) difference in the water quality parameters among the experimental groups. These suggest that the water quality parameters did not affect the other measured parameters among the treatment groups.

5.2. Proximate analysis of ingredients

In the present study, the proximate analysis of all the ingredients were supported by the previous studies of Mohanta et al. (2016), NRC (2011), Aniebo et al. (2008), and Salem et al. (2008). The nutritional contents of all the ingredients used in the study was supported by these authors and found suitable to be used for the formulation of the present study.

5.3. Proximate composition of the experimental diets

All the experimental diets prepared were isonitrogenous and isolipidic in nature. The nutritional contents of the experimental groups were suitable for the fingerlings of *Labeo rohita* and were prepared as per the nutritional requirement studies of Sahoo et al. (2020), Rahimnejad et al. (2019), Wang et al. (2017), and Ngoc et al. (2016).

5.4. Nutrient efficiency and growth performance

The dietary soybean meal, silkworm pupae meal, housefly maggots meal, and earthworm meal had a significant ($P < 0.05$) effect on final body weight (FBW), feed conversion ratio (FCR), percent weight gains (WG %), protein efficiency ratio (PER), and specific growth rate (SGR) at the completion of the feeding experiment. The final body weight, weight gain percentage, and specific growth rate were significantly higher in silkworm pupae meal than in housefly meal. Although, the growth performance of earthworm meal-fed the fish was comparable with those of soybean meal-based control diet without any ill effect. The fish fed with silkworm pupae meal and housefly meal showed the lowest FCR. The values of the protein conversion ratio also showed similar trends to the growth performance indices. The findings of the present study were supported by many researchers in different fish species using silkworm pupae meal, housefly meal, and earthworm meal as alternative fish feed ingredients.

The best growth performance was seen in earlier research with common carp (Nandheesa et al., 2000) and rohu (Begun et al., 1994), which used up to 50% fish meal substituted with silkworm pupae. According to Karthick Raja et al. (2019), weight gain in fish fed diets containing 40% or 50% silkworm pupae in place of fishmeal was significantly lower than weight gain in fish fed diets containing 30% silkworm pupae. When the amount of silkworm pupae was increased in the diet, the growth rate was noticeably reduced. Ji et al. (2015) in Jian carp and Salem et al. (2008) in Nile tilapia found results that were similar. According to Cummins et al. (2017), this may be principally caused by the silkworm pupae's high quantities of chitin (5–15%), which are difficult for fish to digest. Further research on fish revealed that higher insect meal substitutes or replacements simultaneously raised chitin levels and impacted lipid and protein digestibility (Kroeckel et al., 2012; Longvah et al., 2011). In contrast to fish givena control diet, common carp (Gangadhar et al., 2017; Jayaram and Shetty, 1980), catla (Umalatha et al., 2018), and *Labeo firmbriatus* (Gangadhar et al., 2017) demonstrated greater protein digestibility when fish meal was replaced with 30% non-deoiled SWP. Salem et al. (2008) findings showed that silkworm pupa meal can be employed profitably in Nile tilapia feeds in place of fish meal up to 66.66% due to its favourable impacts on growth development, feed conversion effectiveness, nutrient assimilation and protein utilization. According to Sogbesan et al. (2006), 25% silkworm pupae meal in the diet of *Claries gariepinus* fingerlings induced an increase in fish growth, while levels of 50%, 75%, and 100% caused a decrease in fish growth. The same pattern was discovered by Khatun et al. (2005), who reported increasing fish development by replacing 6 to 8% of fish meal with silkworm pupae meal. However, 50–100% of fish meal protein was replaced by *Bombyx mori* in *Clarias gariepinus* without influencing growth and feed utilization, according to the study by Oso and Iwalaye (2014), which found that 25% produced the best results. Enzymatic hydrolysates of defatted silkworm pupa can substitute 50% of the fish meal in the feed of juvenile mirror carp without having a deleterious impact on development, according to Xu et al. (2018). According to Shakoori et al. (2016) findings, rainbow trout's flesh quality, growth, or survival may all be maintained for a period of 60 days while consuming silkworm pupae in proportions up to 10% of fish meal. Even in *Litopenaeus vannamei*, a species of Pacific white prawn, a diet based on silkworm pupae can replace 75% of fish without impairing growth and immunological indices (Rahimnejad et al., 2019).

In addition, despite several research studies on silkworm pupae, housefly larval meal is still rarely produced commercially or distributed locally, especially in underdeveloped nations. Because of this, the effectiveness of housefly larval meal in the manufacturing of fish feed can be enhanced through creative methods that get over the numerous drawbacks of using earthworms in the formulation of animal diets. According to Fasakin et al. (2003), fingerlings of *C. gariepinus* did not exhibit any differences in growth performance or nutrient uptake when fed a diet comprising either 32% sun-dried and defatted or oven-dried 27% defatted housefly larval feed. In line with this earlier research, Wang et al. (2016) proposed that when the dietary level was 33% or less, housefly larval meal might generate a favourable nutritional component in the diets of Nile tilapia. Even though Nile tilapia were fed diets containing up to 68% housefly larvae meal, a prior investigation of the same fish species found no evidence of impaired growth performance or nutrient utilization (Ogunji et al., 2008).

Numerous studies have documented the effectiveness of *E. fetida*, earthworm meal, in elevating fish growth performance, enhancing digestibility, improving survival, increasing reproduction, lowering feed conversion ratios, improving feed utilization efficiency, and reducing stress, either alone or in combination with other ingredients. In the study conducted by Ngoc et al. (2016), common carp showed a highly positive reaction to feeds including earthworm meal as the primary source of protein, entirely replacing fish meal in the diet. Popek et al. (1996) on *Carassius auratus* reached the lowest replacement level of 10%, whereas Kostecka and Pczka (2006) on Gupy achieved the greatest replacement level of 100%. However, Popek et al. (1996) found that 10% *E. fetida* meal quadrupled the reproductive rate of *C. auratus*. Kostecka and Pczka (2006) discovered that replacing fish food with *E. fetida* meal resulted in enhanced survivability, improved reproduction, and increased biomass in aquarium fish, *P. reticulata*. The scientists demonstrated how ecological boxes might be used in families and schools to produce *E. fetida* on a modest scale, potentially cutting costs associated with maintaining aquariums and backyard ponds with fish. Vodounnou et al. (2016) found that

Parachanna obscura fingerlings experienced a high SGR of 2.11 grams per day when given with *E. fetida* meal. Even though *E. fetida* has a high protein and similar nutritional qualities, most of the studies whose findings were summarised above revealed that earthworm meal can only substitute up to 50% of dietary fish meal. When *E. fetida* meals were present in amounts greater than 25%, most studied found that fish growth was inhibited. These findings were attributed to the foul-smelling coelom fluid and indigestible chitin, which are well-known to impair digestibility as well as palatability (Dedeke et al., 2013). When given *E. fetida* meal instead of fish meal, Nile tilapia (*O. niloticus*) and common carp (*C. carpio*) experienced slower SGR of 1.3 grams per day and 2 grams per day subsequently (De Chaves et al., 2015). Furthermore, in spite of many studies on earthworm meal, it is not regularly produced for sale or traded locally, especially in underdeveloped nations. As a result, creative methods can be used to get over the many drawbacks of using earthworms in the formulation of animal diets, increasing the incorporation of earthworm meal in the production of feed.

5.5. Nutritional composition of the entire fish body

Dried silkworm pupae offer nutritional value similar to fishmeal but at a much lower cost. The amount of crude protein from 52 to 72%, whereas the defatted feed can have a protein value of up to 80% in fish (Karthick et al., 2019). SWP protein contains a high concentration of important amino acids such as phenylalanine,valine and methionine. The essential amino acid content of SWP protein was compatible with the dietary requirements of fish (FAO, 2009). Silkworm pupae have notably high amounts of lysine like as 6% to 7% in 100 grams CP and cystine plus methionine about 4%. In this study, there was no significant ($P > 0.05$)difference in entire body crude protein composition between diets based on soybean meal and those based on silkworm pupae meal.Despite this, Salem et al. (2008) found no significant differences (P >

0.05) in the dry matter, CP,ash and ether extract of Nile tilapia fed diets including or without silkworm pupae meal. These findings are consistent with those of Sogbesan et al. (2006) and Nandeesha et al. (2000).

Due to its high protein content, housefly maggot's meal has been established as an alternative protein source for replacing fish meal in various fish species. The effectiveness of substituting fish meal in fish diets varies greatly depending on the ingredient. It is due to the nutritional value of alternate protein sources. According to Makkar et al. (2014), the composition of crude protein in housefly maggots meal ranges from 42.3 - 60.4%. The varied levels of protein content are commonly linked to the procedures used for processing, drying, storage, and protein measurement, as well as the substrates used for cultivating housefly larvae (Ogunji et al., 2008). The housefly utilized in this investigation was good quality. This could explain why the entire body CP of *Labeo rohita* was highest when fed a housefly maggot's meal-based diet. However, several aspects of the production process, such as dietary protein sources, might alter flesh quality (Hopkins and Mortimer, 2014). Ogunji et al. (2008) and Idowu et al. (2003) found similar results in *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Wang et al. (2016) found that a dietary housefly maggots meal did not impact on the muscle proximate composition of Nile tilapia.

Earthworms have rich nutritional resources like protein and lipids and have been proposed as a viable aqua feed element (Sogbesan and Ugwumba, 2008). Dong et al. (2010) and Tacon & Metian (2009) showed that the quality of protein of earthworm meal was comparable as fishmeal, with good quantities of the necessary amino acids. Earthworm meal, similar to fishmeal, was appropriate as a partial substitute for fishmeal, offering comparable or even advantageous effects on fish development. In the current investigation, the total body protein content of soybean meal and earthworm meal-based diets was shown to be statistically similar. Pucher et al. (2014) discovered a substantial increase in whole-body protein content in common carp fed an earthworm meal-based diet in place of a plant-based element. However, there was no significant influence of dietary earthworm meal on the common carp's body proximate composition (Ngoc et al., 2016).

5.6. Composition of fatty acids in fish muscle

Fatty acids, or lipids, are the primary source of stored energy in the body, which is crucial during a fast. Fatty acids are also required for the normal functioning of cellular metabolic activities. In the current study, the total saturated fatty acid content of soybean and silkworm pupae meal-based diets was substantially greater than that of housefly maggot’s meal and earthworm meal-based diets. The sum of monounsaturated and polyunsaturated fatty acids, on the other hand, was unaffected by diverse dietary intakes. Similar observation was recorded by Feng et al. (2021) and Cheng et al. (2017) in silkworm pupae-based diets; Hashizume et al. (2019) and Lin & Mui et al. (2017) in housefly larvae based diets; and Guniya et al. (2016) in earthworm meal.

6. CONCLUSION

At the end of the present research, it is evident that that incorporating silkworm pupae and housefly maggots into the diet of *Labeo rohita* may enhance growth performance and efficiency in feed consumption compared to diets based on soybean and earthworm meals. This suggests that silkworm pupae and housefly maggot meals are superior alternatives for optimizing the nutritional regimen of *Labeo rohita*.

Additionally, the study found that earthworm meal-based diets can serve as an effective substitute for soybean meal-based diets, given that they demonstrated similar performance metrics. This equivalence offers flexibility in diet formulation, providing an alternative protein source that can potentially reduce reliance on traditional soybean meal.

The insights gained from this study are valuable for the aquaculture industry, particularly in formulating and preparing cost-effective feeds for *Labeo rohita*. By integrating alternative insect-based feed ingredients, fish farmers can potentially reduce feed costs while maintaining or improving fish growth and health. These findings pave the way for more sustainable and economically viable feeding practices in aquaculture, leveraging the nutritional benefits of insect-based meals.

7. SUMMARY

Aquaculture is rapidly emerging as the fastest-growing sector in food manufacture worldwide and is essential to addressing the anticipated future gap between global demand and supply of aquatic food. However, successful aquaculture sector requires lower production costs, efficient production systems and environmental sustainability measures. Fish feed often forms 60% to 70% of the operational costs in aquaculture systems, making it one of the costliest inputs in aquaculture production systems. Hence, providing economically viable, environmentally sustainable, and nutritionally dense fish feeds is of paramount. Additionally, the inability of aquaculture to address the formidable task of bridging the expanding disparity between fish supply and demand in India can be attributed to various factors, with the absence of high-quality feeds being a significant contributor.

Soybeanmeal is the leading plant-based protein source in compound aquafeeds and is the most frequently used alternative to fishmeal in aquaculture feeds.Alongside soybean meal, other plant proteins are gaining popularity, encompassing corn products like legumes, corn gluten meal such as peas and lupins, cottonseed meal, sunflower meal, oilseed meals and protein sourced from cereal products like rice, barley and wheat. Nevertheless, several factors, including relatively low protein content, imbalances in amino acids, the presence of anti-nutritional components, and elevated crude fiber content comprising cellulose, hemicellulose, pectin, and lignin, pose constraints on the integration of plant-based ingredients into fish feeds. Due to a lack of fibre digesting enzymes such as cellulose, hemicellulose, and others, fish cannot utilise dietary fibre; also, high dietary fibre interferes with appropriate nutrient utilisation, resulting in poor performance. As a result, removing anti-nutritional factors and correcting nutrient imbalances in plant-based ingredients, particularly amino acid imbalances, with proper techniques should improve their assimilation in fish feeds. Moreover, there is a possibility to fulfil the demand for fish feed ingredients by replacing conventional protein-rich ingredients through locally available quality ingredients like silkworm pupae, earthworm and housefly maggot meal. Again, these ingredients are rich in all the essential nutrients and fulfil all the nutritional requirements of fish to achieve optimum growth. Moreover, these alternate ingredients are affordable for all fish farmers and can support the sustainable development of fish culture in a cost-effective way.

Based on the above hypothesis, the present research work was conducted to formulate low cost feeds using non-conventional ingredients from animal sources for fresh water fish (*Labeo rohita*) and to assess the growth of *Labeo rohita* using different formulated feeds. For the study, four experimental diets were designed to evaluate the potential of meal of silkworm pupae, housefly meal and earthworm meal in the feed of *Labeo rohita* in place of defatted soybean meal at 30% inclusion level. A completely randomized design has been followed to carry out the experiment. Three replication of each experimental group was arranged in the study. Different components like silkworm pupae meal, housefly meal, earthworm meal, mustard oil cake, groundnut oil cake, de-fatted soybean meal, de-oiled rice bran (DORB), wheat flour, vitamin-mineral, and sunflower oil were used for the feed preparation. The experimental diets were found as isonitrogenous and isolipidic in nature with around 35% crude protein and 6% crude lipid. The experimental was conducted in 12 uniform sized experimental tanks (300 L water volume) for 60 days. Juveniles of *L. rohita* with initial average body weight of 5.07 ± 0.01 g were used in the current experiment with a stoking density of 16 nos /tank. The conclusion of the feeding experiment, SGR, FBW, FCR, weight gain percentage, and PER were significantly different ($P < 0.05$) in the context of the experimental groups

•. The values for FBW, SGR, WG%, PER, and feed conversion efficiency (FCE) were notably higher ($P < 0.05$) in the groups fed silkworm pupae meal and housefly meal compared to the control and earthworm meal groups. However, no significant differences ($P > 0.05$) were found between the control and earthworm meal groups and between the silkworm pupae meal and housefly meal groups. Feed conversion ratio (FCR) was significantly higher in the control and earthworm meal groups whereas significantly lower in the silkworm pupae meal and housefly meal groups. Moisture, crude lipid, and total ash content of the whole body of *L. rohita* did not differ significantly ($P > 0.05$) among the experimental groups. Among the insect meal-based diets, the highest body crude protein deposition was observed in the earthworm meal-based diet, followed by the silkworm pupae meal-based diet. The total carbohydrate content was significantly ($P < 0.05$) higher in the control and earthworm meal-based diets compared to the silkworm pupae meal and housefly meal-based diets. Significant differences ($P < 0.05$) were found in the muscle fatty acid compositions, including palmitic acid (16:00), linolenic acid (18:3, n-3), gondoic acid (20:1, n-9), and docosahexaenoic acid (22:6, n-3) among the experimental groups. Additionally, the total saturated fatty acids showed significant ($P < 0.05$) differences, with higher levels in the control and silkworm pupae meal-based diets compared to the earthworm meal and housefly meal-based diets.

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9. LIST OF APPENDIX

Appendix-1

9.1 List of Conferences

1. Manju Rani, Mahendra Pratap Singh and Pawan Kumar. Comparative evaluation of Silkworm pupae, Housefly Maggots and Earthworm meals in the diet of *Labeo rohita.* 20th to 21th Feb., 2023. WBBIAGT 2023, ISBN No. 978-93-91575-87-8/ Vol-16.
2. Manju Rani, Mahendra Pratap Singh and Pawan Kumar. Effect of dietary Silkworm pupae, Housefly Maggots and Earthworm meals on the proximate and fatty acid composition *Labeo rohita.* 28th to 30th April, 2023, SCALFE 2023, ISBN No. 978-93-91872-31-1

Appendix-2

9.2 List of Publication

Manju Rani, Neeta Raj Sharma and Pawan Kumar. Comparative analysis of using housefly maggot, silkworm pupae and earthworm meal based diets in rohu, *Labeo rohita* (Hamilton, 1822), Entomon Journal. 2024. Vol. no..49(2), 203-214.