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Efficacy of Insect Feed as Protein Source in Aqua Feed and its Impact on Growth Performance of Fish

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ABSTRACT

This study aimed to evaluate the impact of using insect-based fish feed on the growth of *Ctenopharyngodon idella* (grass carp). A meal in powder form was produced using the larvae of blowflies. The mixture contained either 20% or 40% larvae, combined with conventional ingredients. The fish fingerlings were divided into three groups and fed with this formulated feed for 10 weeks, while ensuring that the physio-chemical parameters were within the optimum range. Various growth parameters such as mortality rate, feed conversion ratio (FCR), specific growth rate (SGR), length, and weight were measured. The results showed that fish diet based on insects greatly accelerated growth, with the group fed with 40% maggot meal growing to the largest size and length. Similarly, the group fed with 20% maggot meal also demonstrated an increased growth rate. Whereas, FCR was the highest in the group fed with 40% maggot meal. Additionally, the groups fed with maggot meal had a lower mortality rate. This study investigated the use of prepared aquatic feeds containing meals from five different insect species. It was concluded that although insect-based diets show promise as an alternative to inexpensive and unappetizing fish meal, further experimentation with different compositions of insect-based diets is needed in order to achieve optimal results. It is likely that the extensive raising and processing of insects for use as a component of fish feed would benefit aquaculture's viability and profitability in the years to come.

Keywords: aqua feed, blow fly, diet, feed conversion ratio (FCR), insect feed, maggot meal, growth parameters, specific growth rate (SGR)

1. INTRODUCTION

Fish is important for a variety of reasons, such as its nutritional value, economic significance, and cultural symbolism. The consumption of fish has been linked to a lower risk of cardiovascular diseases, cognitive decline, and depression. In terms of economic significance, the global fish and seafood industry is valued at over $150 billion annually and provides employment to millions of people, worldwide [1]. Fish also plays an important symbolic role in many cultures and is often associated with

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religious and spiritual practices. Fish was commonly worked into patterns in Ancient Egypt and also acquired mythological significance in Ancient Greece and Rome. Fish was also incorporated into Christianity as a religious symbol, while artists in China and Japan similarly used fish images, symbolically. In some areas, fishing is a way of life and an important aspect of local traditions and customs. Several studies have emphasized the significance of sustainable fishing methods to maintain fish populations and safeguard marine ecosystems.

Overfishing and other unsustainable practices have led to a decline in fish populations and created disruption in marine ecosystems, highlighting the need for more responsible management of fish stocks. The single species in the genus Ctenopharyngodon is grass carp, often known as the white amur or *Ctenopharyngodon idella* [2]. It is the biggest fish in the Cyprinidae family. It is a highly significant species for freshwater aquaculture and its worldwide production amounted to 5,537,794 tons in 2014 [3].

Fish meal (high-protein feed ingredient made from ground-up fish + insect feed) is a popular choice due to its abundant nutritive components, such as easily digestible energy, essential fatty acids, vitamins, and minerals that offer significant benefits [4]. As a result, the demand for fish meal and oil is ever increasing, which drives up their prices [5]. On the other hand, the demand of sea food is increasing because of the rising living standards and increasing human population. Consequently, the number of wild fish and crustaceans is decreasing day by day. In order to cope with this problem, the development of aquaculture is essential [5, 6].

Making fish feed for aquaculture, which is vital for the fishing sector, involves ensuring that there is a steady supply of fish meal. However, due to the increasing demand for farmed fish, the use of wild fish as a primary source of fish meal is causing more pressure on wild fish stocks, resulting in a decrease in their population [5]. The rising cost of aqua feed, which comprises meat meal, fish meal, and soybean meal and represents 60%-70% of the total production cost in aquaculture, is impeding the progress of the aquaculture industry. Fish meal is the primary protein source used in aqua feed [7]. The cost of fish feed remains significant, ranging from 40% to 60% of the total production expense in aquaculture. Any rise in the cost of fish feed can lead to an increase in the overall production costs of aquafarming [8, 9].

In recent years, there has been an increasing interest in using insects as a source of protein for animal feed, including fish feed. Insects have several advantages over traditional protein sources such as fish meal, including their higher protein content, lower production costs, and a smaller environmental footprint. Previous research showed that replacing a portion of fish meal in fish feed with insect meal can maintain growth performance and improve the feed conversion ratio (FCR) of fish species, such as tilapia, trout, and salmon. Insects commonly used in fish feed include black soldier fly larvae, mealworms, and cricket. According to a study published in the Journal of Insects as Food and Feed, replacing up to 50% of fish meal with black soldier fly larvae meal in the diet of rainbow trout resulted in significant differences in growth performance and feed utilization, as compared to a control diet containing only fish meal. Similarly, a study published in the journal Aquaculture Nutrition found that replacing up to 25% of fish meal with mealworm meal in the diet of Nile tilapia resulted in similar growth performance and feed utilization, as compared to a control diet containing only fish meal. These studies suggest that insect meal can be a viable alternative to fish meal in fish feed. While the use of insect meal in fish feed is still in its early stages, it shows promise as a sustainable alternative to traditional protein sources. Further research is needed to optimize the use of insect meal in fish feed and to investigate the potential impact on the nutritional quality and safety of the resulting fish products. Given the increasing need for protein and sustainable food production, incorporating insect meal into fish feed could potentially be significant to address these challenges.

One of the main challenges facing the world today is the increasing demand for protein as the global population continues to grow. The second challenge that can be addressed by incorporating insect meal into fish feed is sustainable food production [10]. Fish feed should be replaced with alternative sources of protein to achieve better health and growth of fish, this would also help to further the development of aquaculture industry [4]. Insects are also the best source of proteins and used by both human beings and animals as food. Basically, insects are the most diverse group of animals in the world [11]. They are also an important source of alternative proteins in aquaculture [12]. They comprise the feed of omnivorous and carnivorous fishes [13] and other farm animals. Several insect species are important components of diet for larvae and fingerlings during their brood. All insect species live in open water but only one genus *Halobate*, occurring in two genera namely *Hermatobates* and *Halovelia*, live in tide pool marine environments and coral reef [14].

In fishes, nutritional protein ranges between 9.3% and 76% and fat content ranges between 7.9% and 40% [15]. Fatty acid and amino acid contents would be affected by the variations in these contents. Insects can also be produced in farms to increase their rate of growth and ease in management [16]. This study was designed to evaluate the effect of insects on fish and to find the savory of blowfly larvae as a protein source (along with commercially available conventional feed ingredients) in the laboratory and its implementation on an industrial scale. The objective of the study was to develop an inexpensive feed that can enhance the growth and well-being of locally farmed fish.

2. MATERIAL AND METHODS

2.1. Production of Maggots

In the Zoology Laboratory of the Government Sadiq College Women University, a temporary maggotry was formed consisting of three plastic buckets. There was plant manure at the base of each bucket and the smaller second bucket contained poultry meat waste. A collection pipe with two endpoints was affixed to a smaller bucket and a collecting box. After one week, the maggots of 8-10mm length were manually harvested, while a bucket filled with water and rotten poultry waste was left for two weeks to collect surface maggots. The collected maggots were dried at 70°C in an incubator for three hours, ground in a mortar and pestle, and used to prepare the feed.

2.2. Feed Formulation

In maggotry, maggots were produced. From the local market of Bahawalpur, rice polish, wheat bran, yellow corn, and premixes were purchased. By using the grounded maggots and the above ingredients, three diets were produced.

Equal amounts of dry wheat bran, dry rice polish, and dry yellow corn were combined with maggot meal and oil in a bowl. A small quantity of water was added to obtain a stiff mixture, which was then compressed using a hand pelleting machine to form pellets. The resulting feed was placed in airtight containers, labeled, and stored at 20°C in a refrigerator. Three groups were created based on feed formulation.

Group A: Diet with 40% insect feed.
Group B: Diet with 20% insect feed.
Group C: Control group with no maggot meal.

The detail of the formulated feed is given in Table 1.

Table 1. Feed Formula under Different Treatments

Feed Ingredients	Treatment A	Treatment B	Control Group (with-out treatment)
Rice polish	20 %	20 %	20 %
Wheat bran	20 %	20 %	20 %
Premixes	20 %	20 %	20 %
Insect Feed	20 %	40 %	0 %
Fats	20 %	0 %	20 %
Soya bean	20 %	10 %	40 %
Yellow corn	20 %	10 %	20 %

2.3. Fish Growth under Different Treatments

The experimental species for this study was fish and monoculture was maintained. Fingerlings of 5-6 inches were procured from the public fish seed hatchery in Bahawalpur and acclimatized in the laboratory for 15 days without feeding. Three independent aquariums, each measuring 20 by 40 inches and holding 15 fish per 70 liters of water, were used for the three-month experiment. Three treatment groups were used in the study. Group 1 received 40% insect feed content, Group 2 received 20% insect feed content, and Group 3 received 0% insect feed content. The aquariums were filled with tap water. Temperature, hardness, alkalinity, pH, and oxygen demand were the physio-chemical factors that were tracked regularly. The fish were fed twice daily or around 3% of their weight in wet matter. Their weight, fork length, and overall length were also measured.

2.4. Protein Anaylsis (%) Diet and Faeces of Fish Species

At the conclusion of the experiment, the fecal samples from each aquarium were collected using siphoning pipes and transferred to plastic vials. To preserve them until analysis, they were stored in a freezer at a temperature of 20°C. Kjeldhal method was used to analyze the fecal samples. [17]. In order to determine the percentage of crude protein in a sample of fish feed, 0.1 g of the feed was subjected to digestion using a BUCHI labortechnik K-435 digester and 12ml of concentrated H_2SO_4 for one hour at 400°C in a fume hood. Afterward, an automatic rapid steam distillation machine was used to digest the sample with NaOH for a duration of five minutes. The amount of protein in the sample was then determined by multiplying the percentage of nitrogen present by a factor of 6.25.

2.5. Parameters

Using precise formulas, growth performance measures such as body weight gain, feed conversion ratio (FCR), feed consumption, and survival rate were calculated [18]. For instance, by deducting the fish's initial weight (W1) from its final weight, it is possible to calculate the weight gain that occurred during a feeding period (W2). To calculate the specific growth rate (SGR) of fish as percentage per day, the formula SGR = 100 (ln W2-lnW1)/T can be used, where T represents the number of days in the feeding period. Furthermore, the percentage of fish that survived can be calculated by multiplying the result by 100 and dividing it by the total number of fish that were stocked.

2.6. Utilization of Feed

To determine the feed utilization of a fish population, the feed conservation ratio (FCR) can be calculated using the formula FCR = Feed intake/Weight gain [19].

2.7. Physico-chemical Parameters

Digital meters namely HANNA HI-8053, HI-8733, HI-8520, and HI-9146 were used to measure the test media's water temperature, electrical conductivity, pH, and dissolved oxygen twice a day. Using the aforementioned techniques, total hardness, total ammonia, chlorides, sodium, and potassium concentrations were measured for each test medium [20]. During each fish's trial, the water's pH, temperature, and total hardness were kept constant at 7.25, 30°C, and 225mgl-^{1}, respectively. To adjust the pH level of the testing medium, NaOH and HCl were added to increase or decrease it. Water temperature was kept at 30°C by an automated heater.

2.8. Statistical Analysis

The statistical analysis involved examining the growth parameters, metal bioaccumulation, and water chemistry of the tests. This was done using methods outlined by Steel and Torrie [21]. The researchers used Tukey's/Student Newman-Keuls tests and the Micro-Computer Factorial Experiment (RCBD) to determine the statistical differences between the variables. Furthermore, correlation and regression analyses were carried out to find any relationship among the multiple factors recorded in the study.

3. RESULTS

The experiment lasted from April 2022 to June 2022, that is, for a period of 10 weeks. Maggot meal was used as a source of proteins and three feeds were formulated for the analysis of the growth rate of fish fingerlings. In Zoology Laboratory of the Government Sadiq College Women University, maggot meal was produced in 3 plastic buckets. Grounded maggots and conventional ingredients were used for the formulation of three diets and three groups were made, namely Group 1 (40% insect feed), Group 2 (20% insect feed), and Group 3 (control). The diet given to them was equal to 3% of their wet body weight. Physiochemical measurements were taken and body length and weight along with fork length were recorded on a weekly basis. By using Kieldhal method [17], the percentage of protein of experimental diets and faeces were measured.

3.1. Physico-chemical Calculations

The water was kept constant at 30°C, 7.25 pH, and 225mgl-1 total hardness throughout the duration of growth tests. Table 2 displays the average values of various parameters, including water temperature, pH, total hardness, total ammonia, dissolved oxygen, carbon dioxide, electrical conductivity, sodium, potassium, calcium, and magnesium, measured and recorded throughout the development trials.

Table 2. Physico-chemical Variables during Trails

Physico-chemical Variables	20 %	40 %	Control
T (^{O}C)	29.42±0.49	29.52±0.40	30.02±0.26
Ph	7.00±0.09	29.52±0.41	7.17±0.05
Hardness (mgL$^{-1)}$	214.25±2.86	29.52±0.42	221.67±3.67
NH3 (mgL-^{1}))	1.85±0.08	29.52±0.43	1.55±0.26
DO_2(mgL-$^{1)}$	5.97±0.33	29.52±0.44	6.28±0.37
Co_2 (mgL^{-1})	1.25±0.11	29.52±0.45	1.39±0.20
EC(mgL^{-1})	2.88±0.05	29.52±0.46	2.97±0.05
Na (mgL^{-1})	289.92±7.36	29.52±0.47	293..42±5.86
K (mgL^{-1})	7.85±0.20	29.52±0.48	7.84±0.21
Ca (mgL^{-1})	15.11±0.22	29.52±0.49	15.20±0.30
Mn(mgL^{-1})	40.39±0.24	29.52±0.50	40.38±0.23

3.2. Growth Performance Parameters

Fish fingerlings in all the aquariums actively consumed diet of all ratios (40%, 20%, and 0%). There was an increase in the growth of fish. The growth performance of fingerlings fed with diets (20% and 40%) during the whole study is given in the following graphs.

3.3. Measurement of Fish Growth Parameters during 10 Weeks of Trial

To estimate the growth performance of fingerlings, growth parameters (weight, total length, fork length) of all the groups (40%
, 20%, control) were measured and observed on a weekly basis.

3.4. Increase in weight

At the start of the experiment, the initial weight of fish was 14.03±7.44g in 40% maggoty diet group. The final average weight of fish after the experiment was 16.77±7.29g, with an average maximal increment of 0.39g during 10 weeks. The average initial weight of fish was 14.62±7.97g in 20% maggoty diet group. The final average weight of fish after the experiment was 26.61±8.63g, with an average maximal increment of 1.76g during 10 weeks. The average initial weight of fish was 14.78±7.45g in control group on maggoty diet. The final average weight of fish after the experiment was 29.14±8.34g, with an average maximal increment of 1.32g during 10 weeks **(Figure 1 and 2).**

3.5. Increment in Total Length

At the start of the experiment, the average length of fish was 8.38±1.27cm in 40% maggoty diet group. The final average length of fish after the experiment was 19.89±7.91cm, with an average maximal increment of 0.39cm during 10 weeks. At the start of the experiment, the average length of fish was 8.82±1.80cm in 20% maggoty diet group. The final average length of fish after the experiment was 23.83±7.55cm, with an average maximal increment of 1.76cm during 10 weeks. At the start of the experiment, the average length of fish was 10.23±5.91cm in control group. The final average length of fish after the experiment was 25.69±5.78cm, with an average maximal increment of 1.64 cm during 10 weeks **(Figure 3 and 4).**

3.6. Increment in Fork Length

At the start of the experiment, the average length of fish was 1.65±2.36cm in 40% maggoty diet group. The final average length of fish fork after the experiment was 3.86±0.92cm, with an average maximal increment of 0.35cm during 10 weeks. At the start of the experiment, the average length of fish was 1.68±1.78cm in 20% maggoty diet group. The final average length of fish fork after the experiment was 4.82±1.52cm, with an average maximal increment of 0.71cm during 10 weeks. At the start of the experiment, the average length of fish fork was 1.62±1.79cm in control group. The final average length of fish fork after the experiment was 5.28±1.76cm, with an average maximal increment of 0.95cm during 10 weeks (Figure 5 and 6).

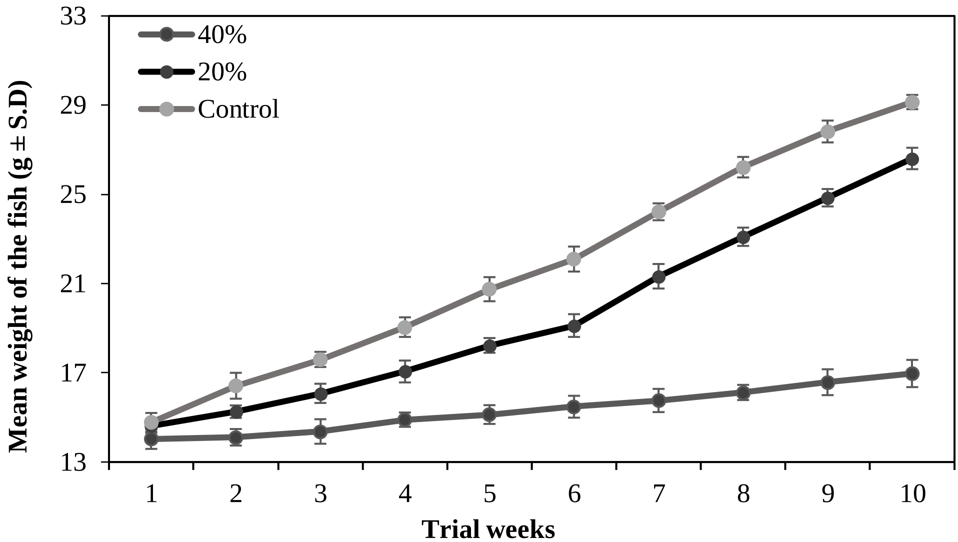

Figure 1. Mean weight (g ± S.D) of Fish at The Start of Each Week for A Period Of 10 Weeks

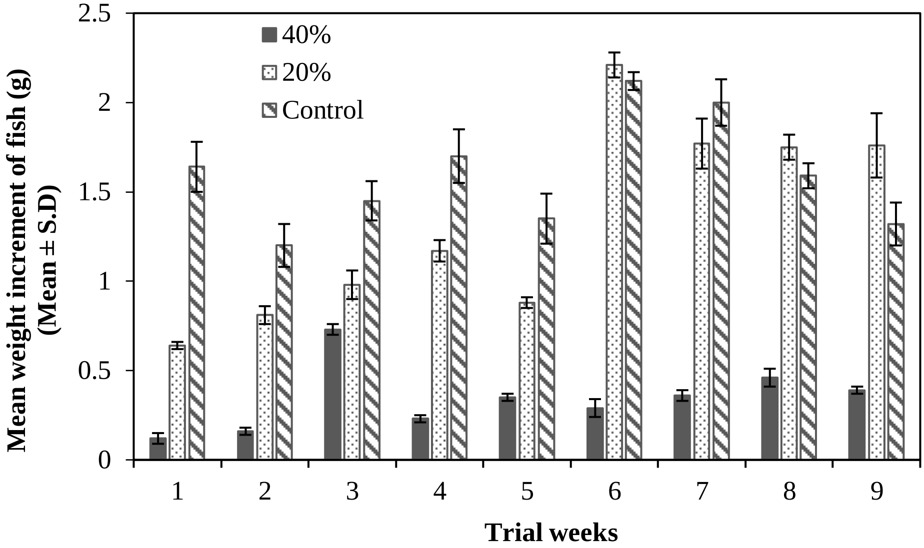

Figure 2. Mean Increase in Weight (G ± S.D) Of Fish at The End of Each Week for A Period of 10 Weeks.

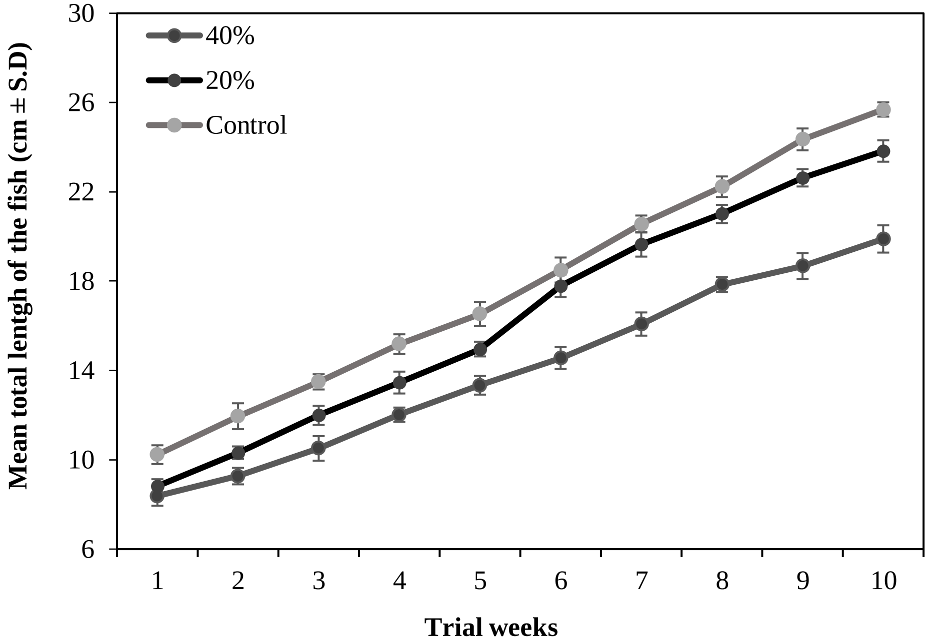

Figure 3. Mean Total Length (Cm ± S.D) of Fish at The Start Of Each Week for A Period of 10 Weeks.

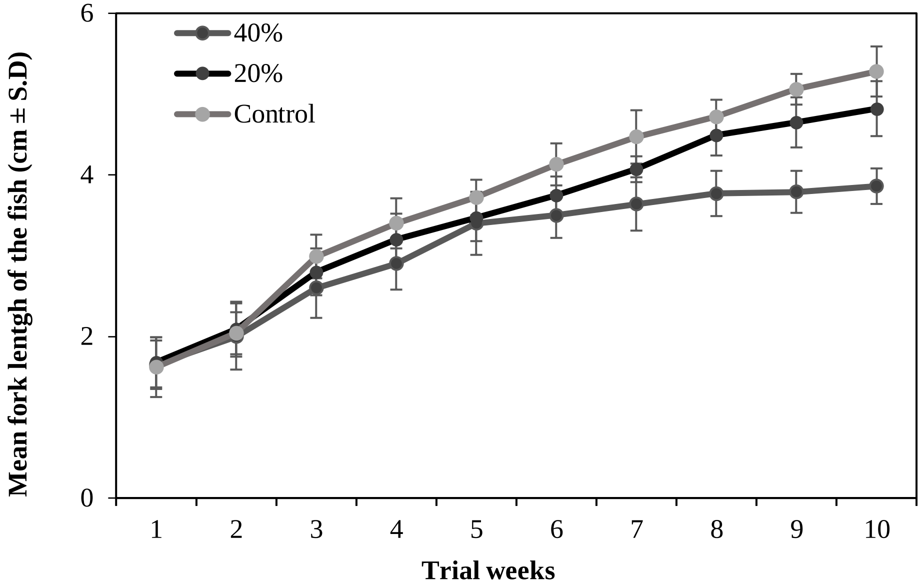

Figure 4. Mean Increase in Total Length (Cm ± S.D) of Fish at The End of Each Week For A Period Of 10 Weeks.

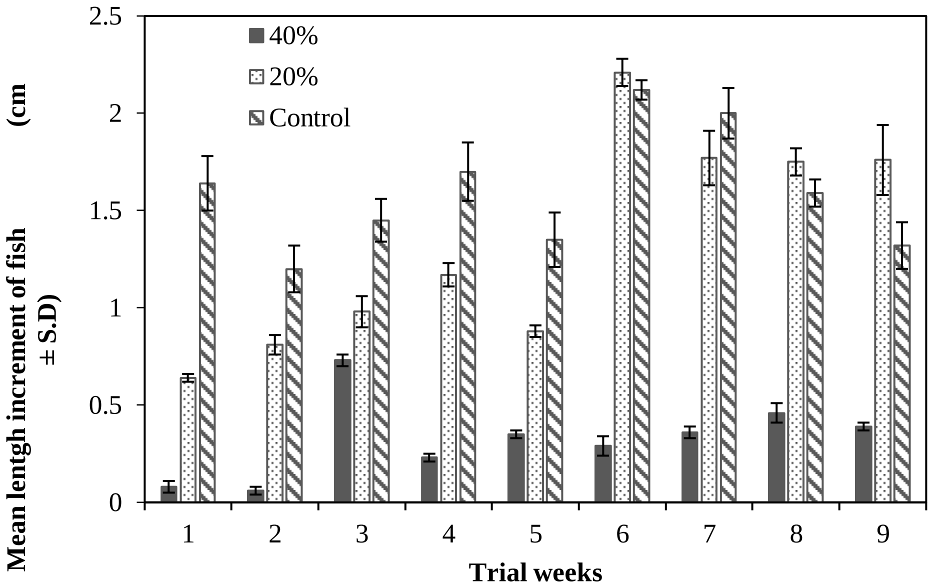

Figure 5. Mean Fork Length (Cm ± S.D) of Fish at The Start of Each Week For A Period Of 10 Weeks

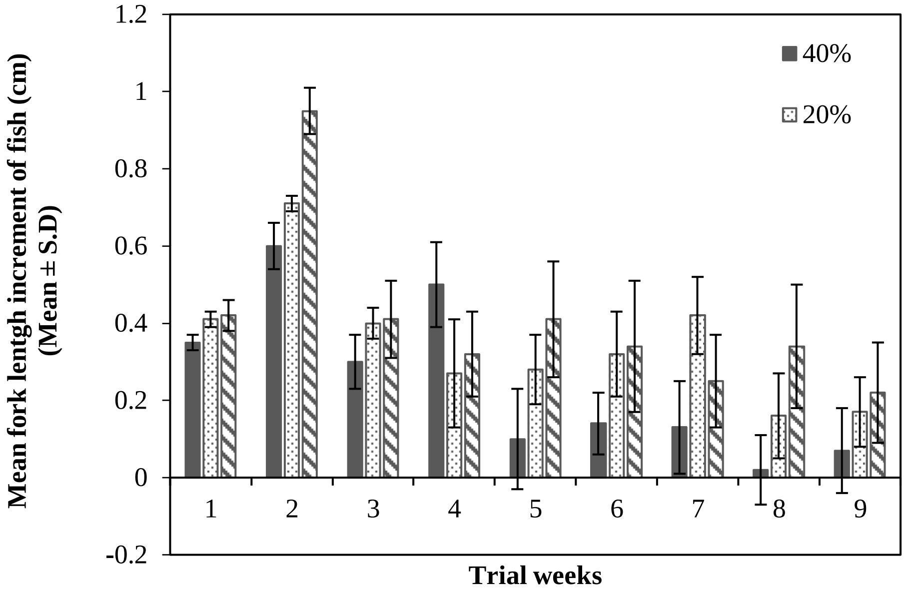

Figure 6. Mean Increase in Fork Length (cm ± S.D) of Fish at The End of Each Week for A Period Of 10 Weeks

3.7. Growth Performance Parameters of Fish in 40%, 20%, and Control Group

FCR (feed conversion ratio) is a useful metric for fish farmers as it represents the amount of feed required to increase the weight of fish to 1 kg. In this study, control group was found to have a higher FCR as compared to the two groups that received 20% and 40% maggoty meal. This finding indicates the best utilization of feed by fish. On the other hand, the group that was given 20% maggoty meal showed the highest SGR (specific growth rate) as compared to other groups (40% and control). This finding indicates that fish fed on control diet and 20% maggoty diet showed better SGR than fish fed on other diet (40%). The group that was given 40% maggoty meal showed the highest survival rate, while the group with 20% maggoty meal showed 72.29% survival rate and control group showed the lowest survival rate with 52.28%. The mortality rate of fish is gauged by the SGR of fish. The average of the fish intake of experimental diet given to them on a daily basis is mentioned below (Table 3).

3.8. Assessment of the Percentage of Crude Proteins in faeces and Fish Proteins

By Kjeldhal method, the percentage of protein in experimental feed and faeces was determined [17], as showed in Table 4. Control group contained a high amount of protein in faeces than other groups fed with insect diet. It showed a significant decrease in digestibility and consumption of diet rich in soya bean. The group that was given 40% insect meal showed more digestibility as compared to the group that was given 20% insect meal, since it showed less protein content in feces. Hence, the group that was given 40% maggoty diet was able to actively consume proteins (Table 5).

3.9. Variance in Weight (measured in grams) and Overall Length of the Fish

One-way Anova was used for to calculate the mean of weekly weight gain. The value obtained for treatment group 1 (p=0.000) showed a significant difference in weekly weight gain. The treatment groups (2 and 3) showed visible increase in growth performance. One-way Anova was used to examine the average total length of fish. The value obtained for treatment groups 1, 2, and 3 showed significant differences in total length.

Table 3. Performance of Fish *Ctenopharyngodon idella* under Different Treatments

Parameters	40 %	(20) %	Without any treatment
Initial Average Wet Weight (g)	14.03±5.44	14.62±7.95	14.78±0.46
Final Average wet Weight (g)	16.97±2.29	26.61.90±8.63	29.14±8.32
Increase in Wet Weight (g)	0.73	0.98	2.12
Average Total Length (Initial) [22]	8.38±1.27	8.82±0.90	10.23±1.92
Average Total Length (Final) [22]	19.89±0.91	23.83±1.54	25.69±1.88
Increase in Total Length [22](Initial)	0.46	1.77	1.32
Average Fork Length (Initial) [22]	1.65±2.36	1.68±1.78	1.62±1.80
Average Fork Length (Final) [22]	3.86±0.92	4.82±1.52	5.28±1.76
Increase in Fork Length [22]	0.13	0.42	0.95
Gain in Weight	1.59	6.8	2.84
Specific Growth Rate (SGR)	2.26 %	9.58 %	4.05 %
Feed Intake	30.50	58.83	82.29
Feed Conversion Ratio (FCR)	1.93	2.26	2.92
Survival Rate	79.28	72.29 %	52.28 %

Table 5. Percentage of Crude Protein in Fish Diet and Faeces

% of Protein	Fish Diet			Fish Feces		
	40 %	20 %	Control	40 %	20 %	Control
	2.81 %	5.20 %	12.77 %	0.070 %	0.19366 %	0.2812%

4. DISCUSSION

The current study was designed to determine the potential of fish to consume insects as food [23]. As a food source, fish are in high demand. Thus, the culturing of fish is being promoted in many countries including Thailand, Brazil, Philippines and Malaysia [24]. Fish meal is getting expensive day by day. Hence, scientists are striving to find other sources of fish food that have the same dietary effect as fish meal. Insects are used as food by fishes, poultry, and pig [25]. Maggot meal can be used to replace the entire fish feed [26]. In the current study, blow fly larvae that grow on the waste produced by animals and provide a great source of protein were used as fish feed [27]. The protein content of the maggots of *Chrysoma megacephala* is 52% as compared to vitamin B complex, trace elements, maggots of house fly (39%), and phosphorous [28]. Environmental factors were kept at an optimum range during the experiment. The quantity of dissolved oxygen was 5-7.6mg/l and the average value of pH was 7.18-7.25 in all the tanks which supported the feed utilization efficacy [29]. The results are very similar to the findings reported by other authors with a different quantity of protein in fish diet. [30] reported growth performance with 45% protein diet. Several scientists reported the same results for different types of diets for tilapia but the difference was due to different types of protein content in these diets[31].

FCR is a critical metric for assessing the efficiency of feed utilization in achieving the desired output. It enables the estimation of the amount of feed needed during the growth phase [32]. An FCR value below 1 signifies optimal food utilization and enhanced growth performance [33]. In the current study, it was observed that maggoty diet promoted fish growth, as evidenced by the best FCR values of 0.92 and 0.91 recorded for 40% and 20% treatment groups, respectively. This finding is similar to the previous report that low FCR was observed in fish fed on high protein diets. However, fish fed on maggoty diet were found to have a lower (better) FCR than those fed on commercial diets [34]. The best FCR for tilapia fry was found with negligible differences between diets containing 35% and 45% protein [35]. It was observed that fish fed on 100% maggoty diet also showed the best FCR (1.34) [36]. According to a previous research, fry tilapia fed with a 45%-CP diet achieved the best FCR. Contrarily, greater FCR was recorded by [34] for fish taking control diet which matches the current findings. So, it was concluded that the feed utilization efficiency of control diet was less as compared to maggoty diet. Control group showed less performance and less addition in weight and length as compared to 40% and 20% maggoty diet groups. There was an increase in growth and health performance of fish due to proteins. So, protein is an important diet for fishes [7].

The results indicated a higher assimilation and consumption of protein given in experimental diets. These results are in line with the results on SGR reported by Abdel-Tawwab [30]. Fish feed with a high dietary protein level had a high SGR with 2.25 (high) in treatment group 1 as the percentage of crude protein was high. SGR increased to 9.57 in treatment group 2 and 4.40 in treatment group 3, as the percentage of crude protein was low [37]. The energy level needed to control the absorbed amino acid may reduce if there is a small decrease in SGR value. Protein level and the weight of fish affected the feed utilization of fish but not their interaction. The fingerlings of tilapia showed better growth performance with a low FCR and a high SGR value [38]. Diet rich in protein is necessary for fish to gain fast growth. The results showed that the percentage range of dietary protein, that is, from 2.81% to 12.75%, is essential for fish fingerlings of tilapia to gain 0.40g/day to 0.51g/day. As fish grows, the dietary protein level decreases from the optimum percentage (27% to 37%) [39] . In this study, the decrease in protein in the feces of fish indicated an increase in protein level in the body. So, it is concluded that maggoty meal is useful for fish growth and improved health and should be used as fish diet [40].

4.1. Conclusion

The study concludes that using insect meal in fish diet can boost the absorption and utilization of proteins and other nutrients, making it a cost-effective substitute of pricier alternatives. Insect meal is considered a cheap source of protein for farmed animals, with blow fly larvae being the best among commercially available insects. However, more research is needed to improve the nutritional value of insect meals in fish feed, taking into account factors such as their chemical composition, nutrient and energy bioavailability, amino acid and fatty acid profiles, mineral content, and production processes. Although insect meals can turn biowastes into high-quality protein, precautions must be taken to ensure their safety from harmful elements. To replace fishmeal in aqua feeds, it is necessary to enact a legal framework, legislation, and risk assessment procedures. Moreover, their impact on seafood safety, quality, and societal acceptance should be investigated. Although insect meal production is expected to increase in the coming decade, it would still be insignificant in comparison to other aqua feed constituents. The sustainability, financial success, and environmental friendliness of aquaculture are expected to considerably increase with the expansion of the insect farming sector for the production of insect meal as fish feed.

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