

Impact of Various Dietary Protein Sources on Growth Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings and on Water Quality

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Abstract

Aquaculture industry provides proteins and unsaturated fatty acids worldwide. Fresh water aquaculture is considered as the most prominent type of aquaculture in the world. Nile tilapia aquaculture is favored because it can adopt to wide range of environments and can tolerate various abiotic stresses. However, the success and profitability of aquaculture is significantly affected by type of feeds used in aquaculture system. An experiment was conducted to assess this important aspect of aquaculture. Nile tilapia fingerlings were fed on three different protein sources to assess the effects of these experimental diets on growth of fingerlings and on water quality. Three protein sources were freshwater shrimp meal (FSM), black soldier fly larvae (BSF) and soybean meal (SBM). Fingerlings were divided into groups based on their respective experimental diets and were arranged according to CRD with three replicates. Each replicate contained 30 fingerlings. Data was recorded for growth attributes of fingerlings and for water quality attributes. Analysis of data shows that BSF caused 32% increase in final weight of fingerlings followed by FSM, which caused 24% increase as compared to controlled. Net weight gain was also significantly enhanced by experimental diets. BSF caused 88% increase in weight gain as compared to controlled. BSF also increased 43% survival rate of Nile tilapia fingerlings as compared to fingerlings fed with controlled diet. Water quality was not much affected by all diets. Water quality parameters like DO, pH, temperature, ammonia and phosphorus all were in optimum range for tilapia culture. However, BSF provided 136% more phosphorus as compared to controlled. This study reveals the beneficial effects of BSF larvae protein source for Nile tilapia culture. So, from these findings, it can be suggested to partially replace fish meal with BSF larvae meal.

Keywords: Aquaculture, Nile tilapia, Sustainable fish meal, Alternative protein Sources, Black soldier fly.

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1. INTRODUCTION

Aquaculture industry provides proteins and unsaturated fatty acids worldwide. Production of this industry in 2016 was reported as 80 million tons [1]. Fishes are crucial part of food chain; they are at tertiary level of food chain. Dietary composition of fishes such as minerals, proteins, vitamins and unsaturated fatty acids makes them an excellent choice as a food. According to American Heart Association, daily needs of omega-3-fatty acids can be fulfilled by eating fish twice in a week [2]. Availability of fish feed is the most important factor in aquaculture. Commercially available aqua feeds are mixture of many food ingredients which provide essential nutrients to aquatic organisms [3].

Aquaculture provides high quality proteins, which perform important role in combating world hunger. As human population is increasing at exponential rate, so it can be expected there will be huge demand of aquaculture products in future [4]. World Review of Aquaculture reported that importance and demand of aquaculture products is increasing [5]. Increase in human population propelled this industry to fulfill their diet needs.

In aquaculture, *O. niloticus* is widely cultured and preferred over other species. This specie is adopted to various environmental stresses [6]. Its growth rate is also fast and is tolerant to various diseases. *O. niloticus*

has the ability to adopt and utilize newly manufactured diets, can survive on less feeds and can also reproduce in captivity [7]. Nile tilapia can easily adopt to various abiotic stresses such as low oxygen levels, pH, temperature and salinity as compared to most freshwater species, which cant adopt to these abiotic stresses [8]. About 90% fishes are reared according to semi-intensive culture system. Recirculating aquaculture system (RAS), tanks and fish cages are also methods of fish rearing [9]. Availability of finances, technology, land and other resources play important role in the choice of culture. While choosing a culture the most important factor is to consider the efficiency and performance of that system [10].

Feed almost costs 50% of total cost of fish production, which makes it the most expensive factor of aquaculture [11]. Feed selection for aquaculture depends upon accessibility, availability of finances, technology available to former, species and culture system [12]. Some farmers on small scale ponds utilize locally prepared feeds, which they manufacture by mixing easily available local ingredients to reduce fish production cost. These locally prepared feeds lack important nutrients, which results in reduced growth and yield of fishes [13]. This reduction in growth occurs due to high concentration of unrefined fiber and inadequate proteins in locally prepared feed [14]. For these reasons, farmers who have finance resources rely on expensive feeds. Poor handling and improper storage of feed result in spoilage which leads to losses. These spoiled feeds can also cause mortalities of fish, especially those feeds which have produced aflatoxin [15].

Few animal and plant protein sources for tilapia diet have been assessed. Blood meal, hydrolyzed feathers, insect meal, bone meal are few animal sources assessed for tilapia diet. Larvae of Black Soldier Fly (BSF) use as a protein source is one of the most prominent sources in aquaculture diets [16], because BSF larvae can feed on many sources and provide high quality nutrients from organic waste [17]. Additionally, BSF filter food substrates and destroy harmful bacteria

in meal [18]. Inoculation of BSF in aquaculture diets significantly enhance fish yield and reduce production cost. Some additional efforts had been made to assess plant sources in tilapia diet. Oilseed crops such as cotton seeds meal, peanut soyabean and sunflower are plant sources assessed for tilapia diet. High nutritional composition of soybean makes it important source of protein for aquaculture. But its low concentrations of lipids and high concentrations of carbohydrates can cause problems for some fish species [19]. Some aquatic sources such as freshwater shrimp can also be utilized as a protein source in aquaculture [20].

Water quality is also an important factor, as it can cause significant effects on fish growth. Water quality is determined by many factors including type of fishes and type of fish meal used [21]. These above-mentioned issues of aquaculture need to be addressed to provide quality aquaculture products for millions of people. Therefore, the current experiment was conducted to examine the effects of fish feeds (prepared from various sources) on growth of Nile tilapia and on water quality.

2. METHODOLOGY

2.1. Experimental Design

An experiment was conducted in 2023 to examine the effects of various protein sources on Nile tilapia fingerlings and on water quality of aquarium at the research area of fisheries, UAF, Pakistan. This experiment was conducted according to CRD with three replicates. Duration of this study was 10 weeks. Nile tilapia fingerlings were obtained from government fish seed hatchery. Three experimental diets obtained from Black Soldier Fly (BSF), Freshwater Shrimp Meal (FSM) and Soybean Meal (SBM) were prepared at fish nutrition laboratory and a commercially available diet was used as control. Analysis of nutrient composition of these diets were also performed at fish nutrition laboratory as shown in Table-1. All these diets (three experimental and one control) were replicated thrice and there were 30 Nile tilapia fingerlings in each replicate.

Table-1: Ingredients Composition (%) in Experimental Diets

Ingredients	FSM diet	SBM diet	BSF diet
Wheat	28.20	28.20	28.20
Maize	18.50	07.50	04.10
Palm Oil	05.90	06.60	02.30
BSF	00.00	00.00	45.00
SBM	00.00	45.00	00.00
FSM	45.00	10.00	17.90
Salt	00.20	00.20	00.20
DCP	01.70	01.70	01.70
L-Lysine	00.10	00.10	00.10
DL-Methionine	00.10	00.40	00.20
Vitamin-Mineral	00.30	00.30	00.30
Total	100	100	100

2.2. Supply and Maintenance of Fingerlings

Nile tilapia fingerlings were purchased from government fish seed hatchery. For first two weeks, these fingerlings were placed under laboratory conditions for acclimatization. When these fingerlings were fully acclimatized then these fingerlings weighing 8-9 grams were shifted to their respective study groups based on experimental diets. For each aquarium 30 fingerlings were selected randomly, and each aquarium represents a replicate of experimental diet. Fingerlings were fed with their respective experimental diet thrice a day (09:00, 13:00 and 17:00).

2.3. Monitoring of Water Quality in Aquaria

Throughout the experiment, water quality parameters such as dissolved oxygen, temperature, and pH were closely monitored using a multi-parameter meter. Objective of this monitoring was to ensure that values of these parameters lie within moderate ranges for Nile tilapia. Indophenol blue spectrophotometric method was employed to determine the levels of ammonium nitrogen by using a spectrophotometer as outlined by Li *et al.*, [14]. Then these values were used to calculate the total ammonia content. Electric aerator pump equipped with a suitable filter was used for uniform aeration to maintain optimal water conditions in each aquarium. To ensure efficient functioning, two times routine removal and cleaning of the filters were done daily. Furthermore, siphoning of solid organic waste, including unconsumed feed and fish excreta that had settled at the bottom of each aquarium, was carried out at least twice a day. In order to manage water quality effectively, two-thirds of the experimental water volume were exchanged with fresh, water three times a week.

2.4. Data Collection

For the purpose of data collection of fingerlings growth, after ten days samples of fingerlings were collected randomly. Final weight of fingerlings was measured with the help of weighing scale. When experiment was completed, number of fishes was counted to determine the survival rate of fingerlings. At the end of experiment water quality parameters (temperature, dissolved oxygen, pH and ammonia) were also measured with the help of multi-parameter meter. While phosphorus level of water samples of experimental diets were determined at water quality laboratory UAF.

2.5. Evaluation of Growth Parameters

Effects of experimental diets on growth parameters such as relative growth (RG) and specific growth rate (SGR) were measured according to Lugert method [22]. RG, SGR, feed conversion ratio (FCR) and survival rate were measured according to following formulae:

$$RG \% = \text{Weight gain} \times 100 / \text{Initial weight}$$

Where,

$$SGR \%d^{-1} = (\log \text{ Final weight} - \log \text{ Initial weight}) \times 100 / \text{Experimental period}$$

$$FCR = \text{Quantity of feed consumed} / \text{Weight gain}$$

$$\text{Survival} = \text{Final count of fish} \times 100 / \text{Initial count of fish}$$

2.6. Data Analysis

During a 10-week study, the growth performance of Nile tilapia in aquaria was examined with three experimental diets obtained from three different protein sources. After 10 days, a random sample of fingerlings was chosen for weight measurements. The weight data collected was organized in a sheet using MS Excel software for analysis. ANOVA was performed by using Statistix 8.1 software. The significance level was set at $p < 0.05$ to determine statistical significance. Tucky test was used to check significant or non-significant differences among mean values of parameters. Correlation among water quality parameters was measured by using R-Studio. Correlation matrix among water quality parameters was also drawn with the help of R-Studio.

3. RESULTS

3.1. Growth Attributes of *O. niloticus* Fingerlings

Analysis of recorded data of growth parameters showed that no significant differences were found in initial weight of fingerlings across all treatments as all fingerlings purchased from fish hatchery had uniform weight. But the growth of Nile tilapia was significantly increased by different protein sources meal, as the experiment progresses. Table-2 shows that final weights of fingerlings were not uniform for all treatments. T3 (Black Soldier Fly) showed the highest growth of fingerlings and provided highest final weight as compared to T1, T2 and T0. T0 (controlled) did not perform good for growth of fingerlings, final weight and weight gain were the lowest of controlled feed as compared to other three protein sources feed (Table-2). Significance value ($p < 0.005$) for weight gain influenced by various diets was extremely small, which shows that influence of diet on weight gain was significant. Mean values in Table-2 shows that T3 provided most prominent results for net weight gain followed by T1 and T2. While controlled diet performed poorly for net weight gain. Specific growth rate (SGR) and relative growth (RG) showed prominent differences. Highest values of SGR and RG were reported in fingerlings fed with T3. While controlled diet provided lowest values of SGR and RG. Feed conversion ratio (FCR) was the only growth parameter which was reported high in fingerlings fed with controlled diet. At the end of experiment fish numbers were counted in each aquarium to analyze the survival rate of fingerlings. Results showed that survival rate of fingerlings was highest under T3 experimental diet. These results shows that Black soldier fly can become a promising protein source in aquaculture diet.

Table-2: Mean Values \pm SE of Growth Performance of Nile tilapia under different Experimental Diets

Treatments	Controlled (T0)	Freshwater Shrimp Meal (T1)	Soybean Meal (T2)	Black Soldier Fly (T3)
Initial Weight (g)	8.523 \pm 0.01	8.518 \pm 0.007	8.527 \pm 0.005	8.539 \pm 0.015
Final Weight (g)	13.37 \pm 0.11	16.57 \pm 0.09	15.71 \pm 0.07	17.64 \pm 0.08
Weight Gain (g)	4.85 \pm 0.03	8.15 \pm 0.011	7.18 \pm 0.014	9.1 \pm 0.012
SGR (%)	1.96 \pm 0.0009	2.89 \pm 0.0012	2.65 \pm 0.0008	3.15 \pm 0.0018
RGR (%)	56.91 \pm 0.09	95.68 \pm 0.13	84.2 \pm 0.07	106.57 \pm 0.19
FCR	2.91 \pm 0.011	1.72 \pm 0.017	1.96 \pm 0.013	1.55 \pm 0.021
Survival Rate (%)	58.77 \pm 1.67	76.86 \pm 1.13	73.24 \pm 1.47	84.33 \pm 0.89

Figure-1 shows the percentage effect of experimental diets on growth parameters of Nile tilapia fingerlings with respect to controlled diet. Figure-1 shows that T3 caused 32% increase in final weight of fingerlings followed by T1 which caused 24% increase as compared to controlled. Net weight gain was also significantly enhanced by experimental diets. T3 caused 88% increase in weight gain as compared to controlled. SGR and RG were also significantly increased by all

three experimental diets. T3 caused 62% increase in SGR and 87% increase in RG of fingerlings as compared to their respective controlled. While FCR of fingerlings fed with all three experimental diets was less than fingerlings fed with controlled diet. Survival rate was also reported highest in fingerlings fed with T3 experimental diet. This diet increased 43% survival rate of Nile tilapia fingerlings as compared to fingerlings fed with controlled diet.

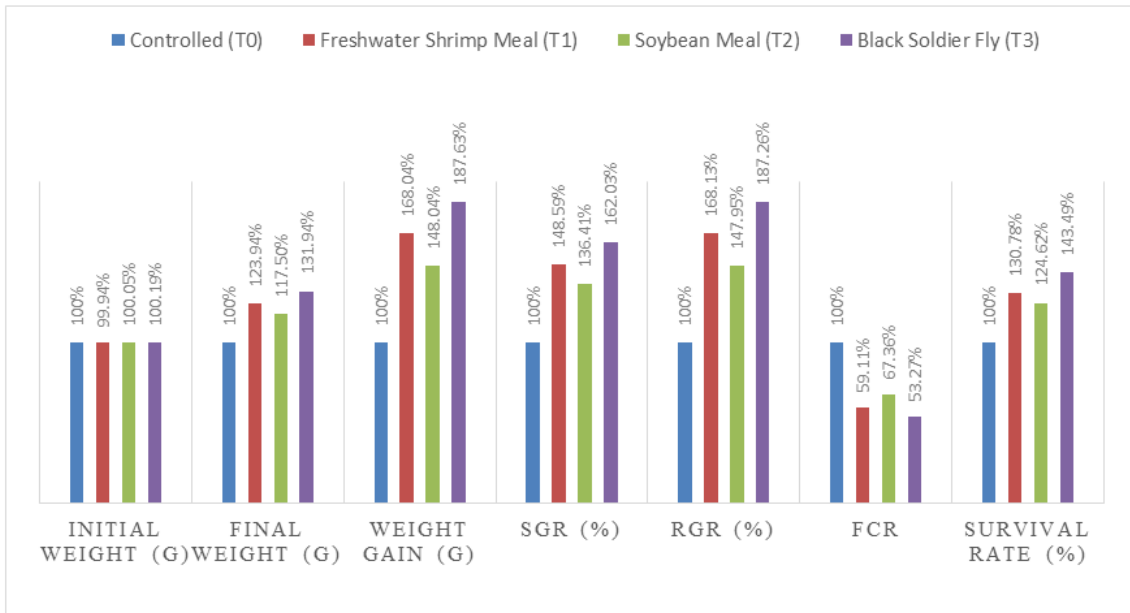


Figure-1: Graphical Representation of Percentage effects of Freshwater Shrimp Meal, Soybean Meal and Black soldier fly meal on growth attributes of Nile tilapia fingerlings

3.2. Water Quality Parameters

Data was recorded for temperature, DO, pH, ammonia and phosphorus to assess the effect of various experimental diets on water quality. Analysis of data revealed that experimental diets caused a slight increase in temperature of water as compared to controlled diet. T1 (Freshwater shrimp) caused 0.43 °C increase in temperature of water. While the effect of these experimental diets on dissolved oxygen (DO) of water showed varying results. T1 cause slight reduction in DO while T2 and T3 cause slight increase in DO of water as shown in Figure-2. pH of water was also affected by all

three experimental diets. These diets cause slight reduction in pH. T1 caused 0.22 reduction in pH of water as compared to controlled diet. Ammonia level in water was not significantly affected by these experimental diets. While phosphorus was the only parameter of water quality which was most significantly affected by all three experimental diets as shown in Figure-2. T3 experimental diet caused 136% increase in phosphorus level of water as compared to controlled diet followed by T1 diet which caused 56% increase in phosphorus level of water.

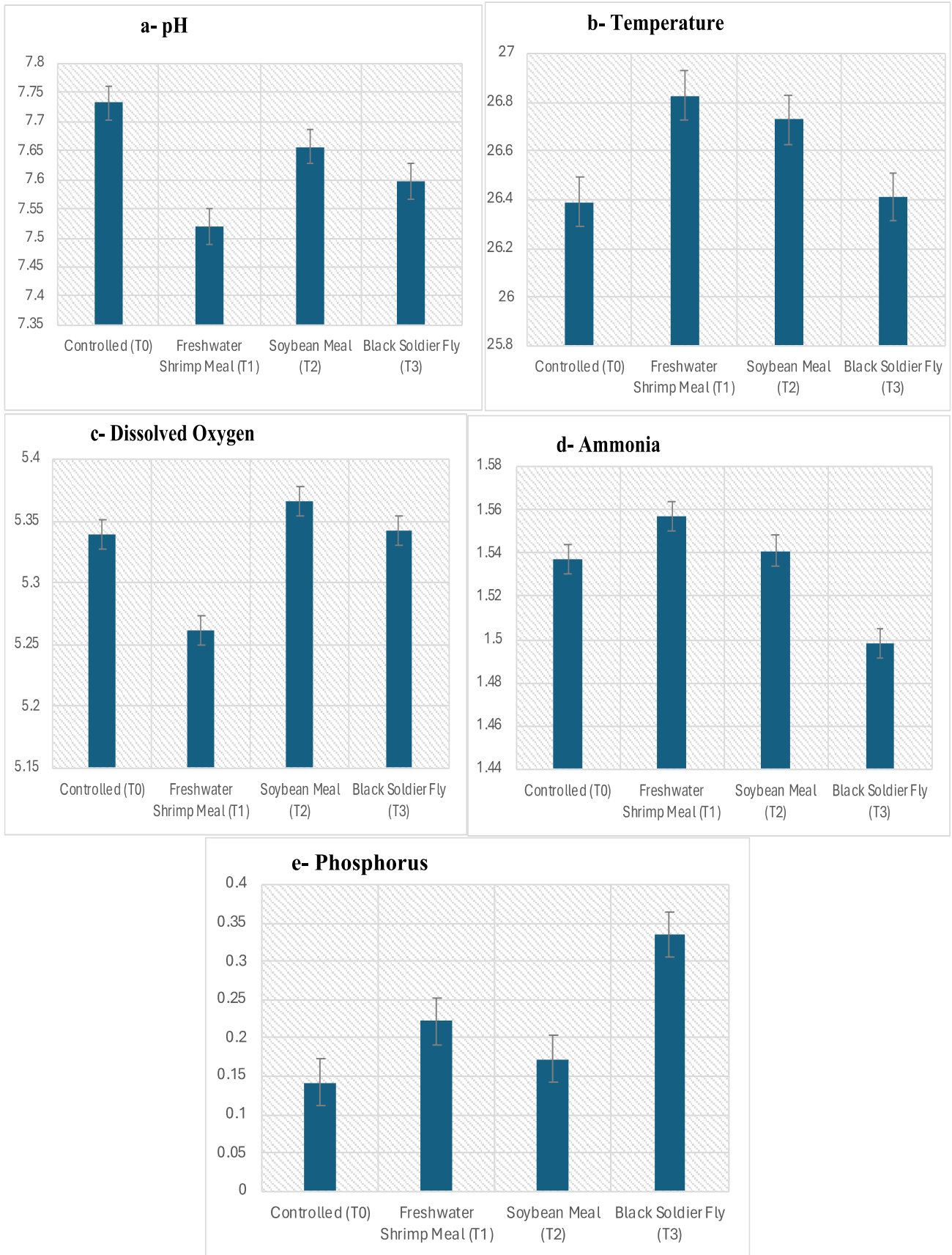


Figure-2: Graphical Representation of Mean Values of Water Quality Parameters; pH, Temperature, DO, Ammonia and Phosphorus under three different Experimental Diets

3.3. Correlation among Water Quality Parameters

Figure-3 shows the correlation matrix between water quality parameters. Strong positive and strong correlations were found among parameters of water quality. pH showed positive correlation with DO and negative correlation with temperature and phosphorus, while no correlation was found between pH and

ammonia. Temperature showed positive correlation with ammonia. DO showed positive correlation with pH as shown in Figure-3. While phosphorus didn't show positive correlation with any other parameter of water quality, but it showed highly negative correlation with ammonia.

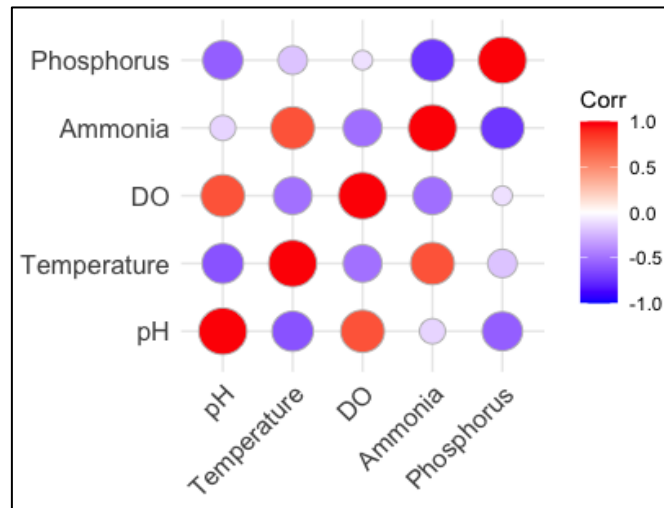


Figure-3: Correlation Matrix among Water Quality Attributes

4. DISCUSSIONS

Different protein sources diets caused significant variations in the growth performance of Nile tilapia fingerlings in this experiment. BSF larvae feed provided most significant results of weight gain, RG, SGR and also enhanced survival of fingerlings. BSF larvae reported lowest FCR as compared to other tested diets. This low FCR represents proper use of food for growth which can be attained by nutritional quality of food [23, 24]. This performance of fingerlings reveals that BSF have high digestibility, nutrient and energy content which helped fingerlings in attaining optimal growth [25]. BSF larvae possess the most favorable amino acids and high protein content, which makes it the most suitable and sustainable protein source in fish and animal feeds [26]. Villazana [27] also found similar results to our study. He found that BSF diets enhanced the growth of tilapia fish as compared to tilapia fed with other fish diets. These results suggest that BSF has the potential to replace other diets in aquaculture. Now use of insect or animal protein sources in feeds of aquaculture is approved by European Union. So, BSF larvae can be considered as an effective source in aquaculture diets [23]. It contains 42% proteins and 28% lipids, which make it suitable for aquaculture [24]. In feeds of Rainbow trout and Jian carp, it has been reported that replacing fish oil with BSF larvae oil or soybean oil has no negative effects on fish growth.

On the other hand, tilapia fingerlings fed with freshwater shrimp meal (FSM) also showed significant results. Although, these results were higher than controlled diet, but these were not than BSF diet. Shrimp

meal possess high protein content, but it lacks some essential amino acids required by tilapia for proper growth [27]. Chitin present in exoskeleton of cause reduction in nutrient absorption and utilization by fishes [28]. Seck *et al.*, [29] found that 50% shrimp meal diet provides higher growth to tilapia as compared to 50% soybean meal. Methionine plays important role in growth and protein synthesis in fishes, its concentrations are usually low in soybean [30]. So, such type of amino acids must be supplemented in diets, prepared from soybean [31]. Shaw *et al.*, [32] found that 40% soybean diet caused highest growth in tilapia as compared to other protein sources diets. However, his findings were not in correspondence with our current findings. His findings might be influenced by other growth promoting factors. It can be assumed that digestibility of soybean may be lower as compared to SBM which resulted in poor performance of tilapia as compared to other protein sources fed tilapia [33]. So, from above discussion it can be suggested that partially replace fish meal with other diets. Processing and handling of diets during formulations also affect nutrient composition of diet.

Water quality was not much affected by all diets. Water quality parameters like DO, pH, temperature, ammonia and phosphorus all were in optimum range for tilapia culture. However, BSF provided 136% more phosphorus as compared to controlled. This may be because of phosphorus rich food for BSF. BSF larvae feed on a nutrient rich wide range of organic substances such as food scraps [34]. As larvae consumes a lot of phosphorus, so larvae can retain huge concentrations of phosphorus in their body, when they

are used in fish meal, they release extra phosphorus to water [34]. In current study pH was in optimal range for tilapia culture [35]. DO was also in moderate range to support aerobic respiration and prevent stress in tilapia [35]. Water temperature remained 25 to 30°C range, which is moderate and suitable for tilapia metabolism and growth. These less differences in all water quality parameters suggests that if protein sources are handled carefully and properly, they have less effects on water quality. Maintaining appropriate water quality is crucial for growth and yield of aquaculture [36]. In current study, stable water quality can be attributed to many factors such as efficient utilization of nutrients from all tested experimental diets. Other factor may be microorganisms facilitated proper biological filtration, which is beneficial for maintaining water quality in aquaculture [37]. A study on various feed ingredients such as fishmeal, soybean meal and poultry meal were tested on Nile tilapia meal and researchers found that there were no significant impacts on all tested parameters of water quality, which ensured suitable culture for tilapia rearing [15].

CONCLUSIONS

The study demonstrates the significant influence of selecting different protein sources on the growth performance of Nile tilapia. BSF meal proved to be the most effective protein source in enhancing tilapia growth, followed by FSM meal and SBM meal. While the commercially available diet which was used as a control in this study, resulted in the lowest growth rate of tilapia fingerlings. These findings align with existing literature and case studies, highlighting the importance of preparing diets specific to the species to achieve optimal growth and feed efficiency in aquaculture. These experimental diets also didn't affect much on water quality. Except BSF diet which increased phosphorus level in water.

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