

**EVALUATION OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*)
LARVAE MEAL AS PARTIAL OR TOTAL REPLACEMENT OF MARINE
FISH MEAL IN PRACTICAL DIETS FOR TIGER SHRIMP (*PENAEUS
MONODON*)**

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Technology degree in Biotechnology.

by

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DEPARTMENT OF BIOTECHNOLOGY

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INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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APRIL– 2021

DECLARATION

We S ROHIT KUMAR (37230071) and M VENKATESAN (37230093) hereby declare that the Project Report entitled “Evaluation of black soldier fly (*Hermetia Illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for tiger shrimp (*Penaeus monodon*)” done by us under the guidance of **Dr. R THYAGARAJAN** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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SIGNATURE OF THE CANDIDATE

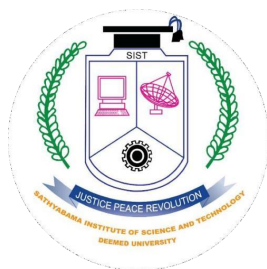
ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. Wilson Aruni**, Pro – vice – chancellor, **Dr. V Ramesh Kumar** and **Mr. G Narendra Kumar** Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. R THYAGARAJAN** for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology, Sathyabama Institute of Science and Technology who were helpful in many ways for the completion of the project.



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of S Rohit Kumar (37230071) and M Venkatesan (37230093) and who carried out the project entitled "Evaluation of black soldier fly (*Hermetia Illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for tiger shrimp (*Penaeus monodon*)" under our supervision from January 2021 to March 2021

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ABSTRACT

Black soldier larvae or BSFL are gaining popularity as a replacement protein source for aquaculture feed formulation. The utilization of BSFL as a protein replacement is evaluated in the present study. Five diets (1,2,3,4,5) were formulated containing graded levels of BSFL substituted from fish meal to meet the nutrient requirements of juvenile tiger shrimp. Diet 1 is formulated as a high-quality control containing no amount of BSFL meal. Diets 2, 3, 4, 5 are formulated with protein replacements levels of 25%, 50%, 75%, 100% from fish meal to BSFL. The inclusion rates of BSFL meal are 8%, 17%, 26% and 35% respectively. The Diets were fed to juvenile tiger shrimp (10g) stocked into 5 saltwater (29ppt) 20l aquaria and the shrimp were stocked at 6 shrimp per aquarium for a period of 30 days. The final weight of each batch is recorded and used to calculate the growth parameters of the shrimp from each batch, respectively. Growth parameters and biochemical composition of the final batches were analyzed. The batch of shrimp fed with diet 1 showed a maximum response and the performance declined with the increase of inclusion of BSFL. The batch fed with Diet 2 showed a similar response to the batch fed with diet 1. The data showed experiment indicates that the inclusion of BSFL is possible if limited within 8-15% for maximum growth performance of tiger shrimp. Further limitations must be overcome for maximum inclusion of BSFL.

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LIST OF ABBREVIATION

S.NO	ABBREVIATION	FULL FORM
1.	BSFL	Black soldier fly larvae
2.	FM	Fish Meal
3.	SYBM	Soybean Meal
4.	FCR	Feed Conversion Ratio
5.	SGR	Specific Growth Ratio
6.	FIG	Figure
7.	UV	Ultra Violet
8.	ppt	Parts Per thousand
9.	°C	Degree Celcius
10.	g	Grams
11.	Kg	Kilograms
12.	Cm	Centi meter
13.	l	Liter
14.	mm	Millimeter
15.	NaOH	Sodium hydroxide
16.	H ₂ SO ₄	Sulphuric acid
17	i.e	That is

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CHAPTER 1. INTRODUCTION

Tiger shrimp (*Penaeus monodon*) commonly known as black tiger shrimp or giant tiger shrimp is a marine shrimp species that are widely caught and reared for food.

1.1 TAXONOMIC AND BIOLOGICAL FEATURES

Penaeus monodon is found at depths from 0 to 110 m, inhabiting rock bottom mud and sand. In its natural range, *P. monodon* can tolerate water temperatures of 18–34°C and salinities of 5–45 ppt (Maheswarudu, G.;2016). *Penaeus monodon* generally appears to pick muddy mangrove channels. *Penaeus monodon* are generally dark-colored, with the carapace and abdomen transversely banded with black and white. The life cycle of *P. monodon* has an offshore planktonic larval phase of about 14 to 20 days, benthic postlarval and juvenile phase of over 6 months (33 g); a coastal subadult phase of 5 to 6 months (60 g) continued by an inshore and offshore ocean adult and spawning phase (60 to 261 g) (Motoh, H ;1981).

1.2 TIGER SHRIMP PRODUCTION

Tiger shrimp (*Penaeus monodon*) is an important commercial aquaculture commodity that is the second most widely cultured prawn species in the world. Tiger shrimp rearing makes up nearly 50% of cultured shrimp production. (Khedkar, Gulab Dattarao;2012). Total aquaculture production of *Penaeus monodon* increased gradually from 21,000 tons in 1981 to 200,000 tons in 1988; then it sharply increased to nearly 500,000 tons with a value of USD 3.2 billion in 1993. Since then, production has been quite variable, ranging from a low of 480,000 tons in 1997 to a high of 676,000 tons in 2001(thefishsite.com; 2010). Tiger shrimp is popular to culture due its tolerance to salinity and very quick growth rate. However, they are very vulnerable to fungal, viral, and bacterial infections easily.

Advantages of *P. monodon* production: - It attains a large size at a relatively short period of time. It is the fastest growing of all shrimp tested for commercial production. Tiger shrimp juveniles of 3 cm long initially have been grown to a size of 75 to 100 g in only five to six months. Due to its large size, it brings a higher selling price to the farmer. At peak seasons it can sell over Rs. 450 per kg in India. Tiger shrimp can tolerate a good range of salinity, 0.2 to 70 ppt and may tolerate temperatures up to a minimum of 37.5°C It

grows rapid rate when fed either with animal or vegetable protein. (TNAU agritech portal; 2020). Hence, fish farmers find feeding high protein artificial diets to prawns to be profitable albeit operational cost, which is especially thanks to feed, increases by 50-60% over that of the traditional rearing method. Therefore, the need to develop an economical and biologically effective diet for prawns is increasing day after day. (Alava, V. R., & Lim, C;1983)

1.3 FISH MEAL IN TIGER SHRIMP PRODUCTION

Fish meal is used as the primary source of protein for feed formulation of all types of aquaculture production due to its favorable nutrient profile, palatability, and relatively high digestibility. Fish meal is a source of high-quality protein with a well-balanced essential and nonessential amino acids profile (Tacon et al; 2008). In sight of its favorable characteristics, aquaculture production has continued to extend the demand for fish products during the last 50 years. The rapidly growing world of aquaculture and overfishing has led to high demand for organic. However, increasing use of forage fish for FM is unsustainable and, because an estimated additional 37.4 million plenty of aquafeeds are going to be required by 2025, alternative protein sources are needed (Katheline Hua et al; 2019).



Fig.1.1 Image of processed fish meal

Typically, the inclusion of FM in shrimp diets is 20–50% of the total diet formulation, which results in higher diet and production costs. Diet costs can account for between 50 and

80% of a producer's operational costs, and thus directly influences producer profitability. Fish meal is an unsustainable resource of limited availability which constitutes as the most expensive component in formulating shrimp feeds. To obtain a profitable aquaculture harvest shrimp, nutrition feed needs to be managed to obtain healthy shrimp and keeping the production cost at the minimum.

1.4 ALTERNATE PROTEIN SOURCES

Beyond plant-based ingredients, insect-based meals have great potential to be a major source of protein for aquafeeds over subsequent 10–20 years. (Katheline Hua; et al 2019) Many studies are done to utilize alternative protein sources for shrimp production such as soybean meal, krill meal, peanut meal, animal proteins, pea meal (Bautista-Teruel M et al; 2003) and plant protein (Delbert & Barrows et al 2007) with varying success rates. Black soldier fly larvae (*Hermetia illucens*) is an insect with relatively high protein as a larvae. The feasibility of black soldier fly larvae (BSFL) as a feed containing relatively high protein was studied by various research publications for various animals.



Fig 1.2 Image of processed soybean meal

1.5 BSFL MEAL AS PROTEIN SOURCE

The utilization of insect-based feed ingredients is gaining traction among fish-feed producers, scientists, and policymakers. The black soldier fly, BSF (*Hermetia illucens*) occurring in tropical and subtropical countries everywhere the planet proved to be a perfect candidate (Bondary et al. 1981; St-Hilaire et al. 2007). BSF can be reared on a wide range of organic waste material. The developing stages of the fly can use this waste material and reduce the volume of organic substrates by up to 50% and producing biomass with a crude protein content of about 42% and a crude lipid content of up to 35 % (Sheppard et al., 1994, Stamer et al. 2007). The suitability of the BSF meal as a protein source in feedstuff had been proven in poultry and pigs and also in warm water fish species (i.e., catfish, tilapia and turbot) (Bondari, Sheppard, 1981; Hale, 1973; Kroeckel, 2012).



Fig.1.3 Image of dry Black soldier fly larvae (Hermetia illucens)

Black soldier fly larvae meal is chosen as an alternative feed ingredient due to its relatively high protein content. The inclusion of BSFL meal in tiger shrimp feed will reduce the cost of production of feed and will lead to the replacement of fish meal in the feed. The production of BSFL meal is inexpensive, requires no special labor and easy to setup compared to the production of fish meal. BSFL production also carries lots of benefits such as vermicompost as a by-product and easy to setup in rural areas.

CHAPTER-2 LITERATURE SURVEY

Year	Author name and Journal name	Inference
1996	Oseni M. Millimena Methionine requirement of juvenile tiger <i>Penaeus monodon</i> fabricius	Amino acid requirement for tiger shrimp at several stages is observed.
2009	Shuyonchi, Beeping Tan Growth and feed efficiency of juvenile <i>Litopenaeus vannamei</i> fed formulated diets containing different levels of poultry by product meal	The feeding requirement and the feasibility of a substitute protein for Pacific white shrimp was analysed and understood.
2019	A M Parra Flores, J T Ponce Palafox Feeding behaviour and ingestion rate of juvenile shrimp of the genus <i>Penaeus</i>	The feeding behaviour, feeding pattern, ingestion methods and rates were analyzed and understood.
2016	Maheswarudu, G. Experimental culture of black tiger shrimp <i>Penaeus monodon</i> Fabricius, 1798 in open sea floating cage	The growth and behaviour of tiger shrimp when provided with formulated pellets
2014	Mohanty, Rajeeb. (2014). Water Budgeting in Black Tiger Shrimp <i>Penaeus monodon</i> Culture Using Different Water and Feed Management Systems.	Diet formulations and nutrient requirement of tiger shrimp at different stages and body weight.
2016	Varun C. Cummins Jr Evaluation of black soldier fly larvae meal as a partial or total replacement of marine fish meal	BSFL meal can be utilized as a protein source for Pacific shrimp and technical guideline for the experiment.
2014	Stamer, A.; Wessels, S.; Neidigk, R. and Hoerstgen-Schwark, G. Black Soldier Fly (<i>Hermetia illucens</i>) larvae-meal as an example for a new feed ingredients' class in aquaculture diets	Nutritional composition of BSF larvae and applications of BSFL as protein source for aquaculture feed.
1981	Bondari K., Sheppard D.C. (1981) Soldier fly larvae as feed in commercial fish production. Aquaculture	Applications and utilization of BSFL as a protein source for various aquaculture feed formulations.

2003	Myrna N Bautista-Teruel, Perla S Eusebio, Timothy P Welsh, Utilization of feed pea, <i>Pisum sativum</i> , meal as a protein source in practical diets for juvenile tiger shrimp, <i>Penaeus monodon</i>	Technical guidance of how to replace protein source for feed formulation for tiger shrimp feed.
1983	Alava, V. R., & Lim, C.. The quantitative dietary protein requirements of <i>Penaeus monodon</i> juveniles in a controlled environment	The dietary protein requirement of tiger shrimp at different stages were used as a reference for feed formulation.
2019	Saher, Noor & Noor, Syeda. Evaluation of Protein (Nutritional Property) in some Shrimp Species Found in Coastal Waters of Pakistan by Three Different Methods.	Methods to estimate the whole-body composition of shrimp were derived using the publication as a technical guide.

CHAPTER 3 - Aim and Objectives

3.1 AIM: -

To check the feasibility of BSFL meal as a protein source for Juvenile Tiger shrimp (*Penaeus monodon*) by replacing Fish meal with BSFL gradually.

3.2 OBJECTIVES: -

To identify the optimum percentage of BSFL meal as dietary protein source in Tiger shrimp feed formulation to produce results comparable to existing diets containing FM.

Various growth parameters are placed to check for the objectives: -

- FCR (Feed conversion Ratio)
- Weight gain percentage
- Survival ratio, Body composition
- Feed intake
- SGR (Specific Growth Rate).

To measure the whole-body composition of final batches of juvenile tiger shrimp to evaluate the performance of each diet compared to the diet containing no BSFL.

CHAPTER 4. MATERIALS AND METHODS

4.1 MATERIALS REQUIRED: -

4.1.1 Ingredients required for feed formulation.

- Fish meal (FM)
- Soybean meal (SYBM)
- Wheat
- Wheat gluten
- Fish oil
- Soybean oil
- Lecithin
- Mineral mix
- Vitamin mix
- Dicalcium phosphate
- Dipotassium phosphate
- Magnesium sulphate
- Cholesterol

4.1.2 Apparatus and instruments used for experiment setup, feeding, feed processing: -

- Weighing scale (0.1-2000g)
- Containers
- 20 l polyethylene containers
- 4-inch air stones
- Aquarium air pump (Venus)
- Aquarium purifier
- Fish net
- Aquarium pipes
- Aquarium siphon
- Polyethylene mesh screen

- Homemade feeding tray
- Commercial mixer (Preethi mixer)
- 2mm standard mesh sieve
- Pellet extruder mill with 2mm disc attachment

4.1.3 Apparatus used for water quality testing: -

- Lifesonic™ master test kit (pH: Ammonia: Nitrate: Nitrite)
- Digital temperature probe
- Refractometer (Atago A-412)

4.1.4 Apparatus used for nutritional and biochemical analysis: -

- Hot plate
- Furnace
- Desiccator
- Petri plates
- Hot air oven
- Moisture dish
- Silica cruse
- Burette
- 1ml pipette
- Conical flask
- Water bath
- Soxhlet apparatus
- Kjeldahl apparatus
- Autoclave

4.1.5 Chemicals used for nutritional and biochemical analysis:-

- Distilled water
- Sodium sulphate
- Copper sulphate
- NaOH

- 1N H₂SO₄

4.1.6 Software and analytical tools used: -

- Microsoft excel.

4.2 DIET FORMULATION (Mohanty, Rajeeb. et al; 2014)

The Black soldier fly larvae used in this experiment were sourced from a commercial manufacturer (Protyn, India) was processed into a slurry. The slurry was solvent extracted to remove additional lipid content from the wet mass by 2:1 (v/v) ethyl-alcohol extraction two times and decanted by sun drying for 24 hours. The dried mass was then grinded into fine powder using a commercial mixer grinder (Preethi mixer) and stored until needed.



Fig.4.1 Image of BSFL grinded into slurry for extraction

Other major ingredients in the feed include Fish meal, soybean meal, wheat were all grinded for 10 minutes each to obtain a finely grinded powder for meal preparation. Diets were formulated with known nutrient and energy requirements of juvenile tiger shrimp(10g) (FAO,2003) (Alava, V. R., & Lim, C. et al; 1983).



Fig 4.2 Image of BSFL drying after processing and solvent extraction.

Other major ingredients in the feed include Fish meal, soybean meal, wheat were all grinded for 10 minutes each to obtain a finely grinded powder for meal preparation. Diets were formulated with known nutrient and energy requirements of juvenile tiger shrimp(10g) (FAO,2003) (Alava, V. R., & Lim, C. et al; 1983).

Table 4.1 Composition (g/100g) of 5 diets fed to juvenile tiger shrimp for 30 days.

Ingredients Per 100g	Diet 1 0%BSFL (g)	Diet 2 8%BSFL (g)	Diet 3 17%BSFL (g)	Diet 4 26%BSFL (g)	Diet 5 35%BSFL (g)
Fish Meal	25.0	18.7	12.5	6.2	0
BSFL	0	8.8	17.6	26.4	35.5
Soybean meal	23.0	23.0	23.0	23.0	23.0
Wheat	38.0	35.4	32.9	30.3	27.7
Gluten	5.0	5.0	5.0	5.0	5.0
Fish oil	1.0	1.5	2.0	2.0	3.0
Soybean oil	3.5	2.5	1.0	1.0	0
Lecithin	0.5	0.5	0.5	0.5	0.5
Mineral Mix	0.1	0.1	0.1	0.1	0.1
Vitamin Mix	0.4	0.4	0.4	0.4	0.4
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5
Magnesium sulphate	0.6	0.6	0.6	0.6	0.6
Dipotassium phosphate	0.8	0.8	0.8	0.8	0.8
Cholesterol	0.3	0.3	0.3	0.3	0.3

Diet 1 was formulated with 0% inclusion of BSFL meal as a control diet similar to commercially available diet to compare and observe the performance of diets 2-5. Diet 1 was formulated with 25% fish meal (FM) and 23% soybean meal (SBM). Diets 2,3,4,5 were formulated to gradually replace FM with BSFL at 25%,50%,75%,100% respectively which gives the inclusion rate of BSFL in the diets as 8%,17%,26%,35% respectively.

Other than the major ingredients many additives, binders, micronutrients are added to all the diets. The property of each of these additives are: - gluten is a necessary binder for the formulated feed, Fish oil and soybean oil is added in gradual levels because it makes the feed more palatable for the shrimp and acts as a source of lipids. Lecithin has become a staple for the feed formulation industry as a stable binder for the feed.



Fig 4.3 Image of dry ingredients mixed for each diet.

Vitamin and mineral mix is added at small quantities to provide the feed with required vitamins (Biotin, B12, folacin, niacin, myo-inositol) and minerals (zinc, manganese, iodine).

Dry ingredients were weighed in a weighing scale and mixed together for 30 mins in a commercially available mixer. Tap water was added to the slurry to obtain a 35% moisture level. The diets were double extruded through a 2-mm die to form strands and then air-dried. After drying, diets were ground into pellets of appropriate size using a disk mill from a feed manufacturer. Diets were then sieved (2-mm opening mesh and 0.5-mm mesh) using a standard test sieve (Best sieve, India). After sieving, a combination of soybean oil and fish oil that had previously been mixed using the formulation was added to the diets until all pellets were uniformly coated. Diets were stored in labeled plastic containers until fed. Proximate analysis for each formulated feed pellets is done to estimate the quantity of protein, fat, moisture, ash and carbohydrates in each diet respectively.

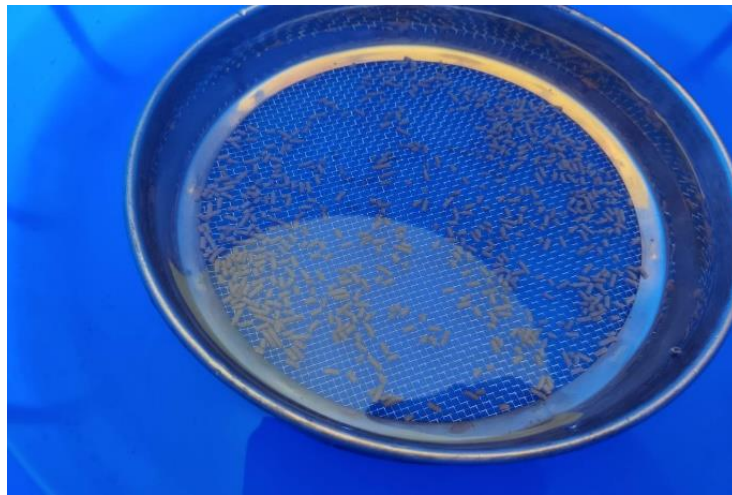


Fig 4.4 Image of pellet stability being evaluated.

The pellets were measured to be at 2-2.2mm in diameter and 2-3mm in length which was then tested for stability in water. The stability of the pellets in water was evaluated using a pellet stability test where 10 g of the dry feed was distributed on a 2mm mesh sieve. The apparatus was then lowered into static seawater(30ppt) approximately 10 cm deep for 60 mins and dried. The dried solids were then weighed to check if any solids leached onto the water. The pellets must be stable under saline waters for the duration of each feed for the shrimps. (Webster et al., 1994)

4.3 SYSTEM SETUP AND WATER QUALITY

The feeding trial was conducted in an indoor aquaria setup consisting of 5 polyethylene 20 l containers. The experiment was conducted at the first week of February and concluded at the first week of March. The containers were uv sterilized and washed with distilled water. Each of the containers were fitted with 4-inch air-stones connected to air pumps (Venus air pump) to provide dissolved oxygen in the water, the containers were also fitted with aquarium purifiers (generic aquarium purifiers) to maintain the cleanliness of the system to provide a complete aquaria setup. The water utilized in the experiment was purified sea water using activated carbon filtration, dechlorinated, and brought up to 29 ppt. Each of the aquaria was filled with purified seawater and fitted with air stones and purifiers. The outflow from the purifiers was connected to a separate drainage.

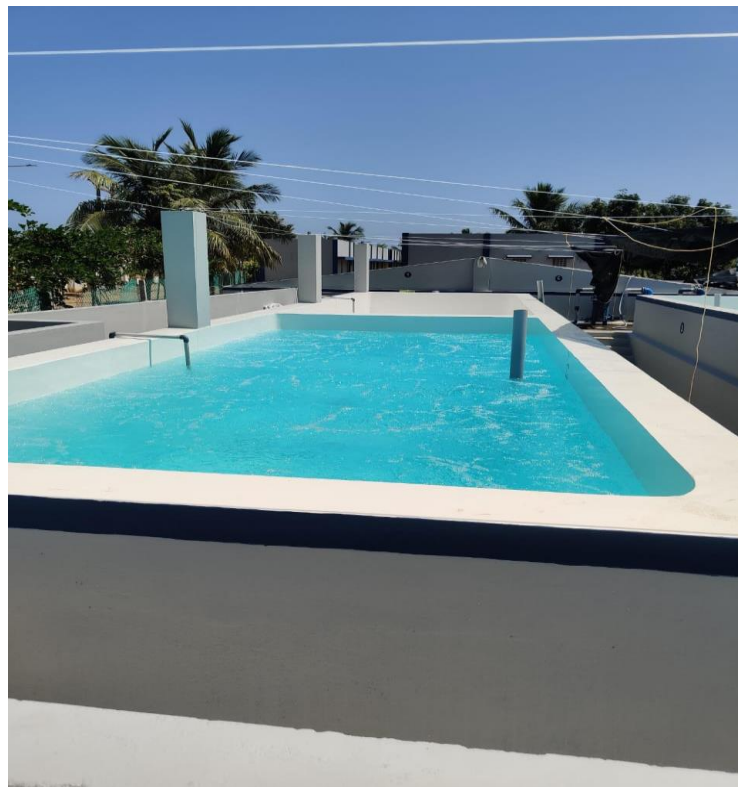


Fig 4.5 Image of seawater being purified using activated carbon filtration.

During the experiment, the water from each aquarium was replaced every week by slowly siphoning off the water in the system and replaced with purified salt water (29ppt) slowly. The aquaria were covered with a polyethylene mesh to prevent the shrimp from jumping.



Fig 4.6 Image of water being slowly replaced using an aquarium siphon.

All aquaria were siphoned and cleaned daily to remove any uneaten food, feces, or moults. The moults were removed from each aquarium using an aquarium fish net and discarded. The system had a photoperiod of 12h:12h light: dark cycle.



Fig 4.7 Image of moults being removed from aquaria using a fish net.

The Water quality parameters such as salinity, pH, temperature, total ammonia levels, nitrite and nitrate levels were monitored every 7 days to maintain the integrity of the system.

4.4 SHRIMP STOCKING AND FEEDING TRIAL.

The juvenile tiger shrimp (10g) used in this experiment was sourced from a grow out pond in Marakkanam, Tamil Nadu, India. The shrimp were then acclimated to the experimental setup for 7 days by slowly replacing the water with purified water (29ppt). The shrimp were fed a commercially available tiger shrimp diet (type: Grower) two times a day for the duration of the acclimation period as a conditioning diet.



Fig 4.8 Image of the juvenile tiger shrimp during the acclimation period.

After the acclimation period the shrimp were weighed to identify the mean initial weight of the juvenile tiger shrimp and the shrimp were randomly stocked into the aquaria at a density of 6 shrimp/aquaria. Biochemical analyses were done on the initial batch of the

shrimp to identify the quantity of crude protein, moisture, crude lipid, ash and carbohydrates.



Fig 4.9 Image of Initial weight of juvenile tiger shrimp utilized for this experiment.



Fig 4.10 Image of experiment setup after 7 days from the start of the experiment.

The juvenile tiger shrimp were fed with diets 1-5 accordingly using a homemade stainless steel feed tray at different intervals of the day. The shrimp were fed at 0830, 1130, 1430,

1530 for a period of 30 days. The feed intake for each shrimp from each batch was recorded.



Fig 4.11 Images of feed containing gradual levels of BSFL being weighed and fed to each respective batch. (top left: diet 1, top right: diet 2(8%BSFL), middle: diet 3(17% BSFL), bottom left (26%BSFL), bottom right (35% BSFL)).

The feed was weighed each time and about 3-5% of body weight of the juvenile tiger shrimp was fed to each batch every time. The feed left on the tray after 60 mins of feeding was observed and the total feed intake was observed for the duration of the experiment.



Fig 4.12 Image of Feed being placed into the setup using a mesh feed tray.



Fig 4.13 Image of experiment setup after 14 days from the start of the experiment.

The amount of feed placed in the tray, approximate uneaten feed was recorded every day.

4.5 PHYSICAL AND CHEMICAL ANALYSIS

4.5.1 Water quality parameters

The water quality parameters are used to identify the condition of water present in each of the aquaria by testing the pH, Temperature, Salinity, Total ammonium nitrogen levels, Nitrate levels and nitrite levels. The water quality parameters were tested every 7 days since the beginning of the experiment.

The temperature in each aquaria is measured using a digital thermal probe (29-31°C) and maintained at a favorable range.



Fig 4.14 Image of Temperature being measured using a digit thermal probe.

The salinity was measured using a refractometer (Atago A-412) which showed 29ppt which was maintained throughout the experiment. Other water quality parameters such as pH, Total Ammonium levels, nitrate levels, nitrite levels are analyzed using a commercially available water quality test kit (Lifesonic™ Master water test kit). The results

obtained from the test kit were then observed as approximate values. Water from each aquarium was tested by following the instructions given in the test kit.

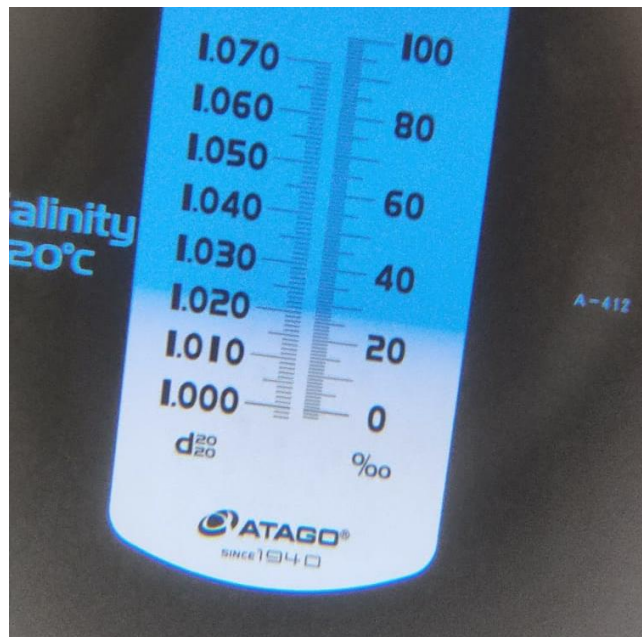


Fig 4.15 Image of salinity of purified seawater being tested(29ppt) at 20°C using a refractometer.



Fig 4.16 Image of Water quality parameter test kit (Lifesonic™ Master test kit)

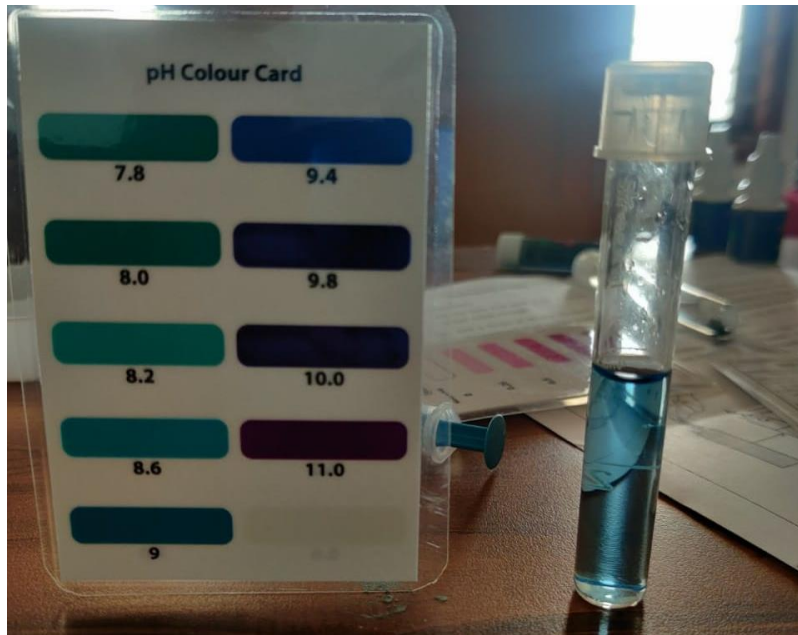


Fig 4.17 Image of pH being tested.



Fig 4.18 Image of water quality parameters being tested (Left to right: - pH test, Ammonia test, Nitrite test, and nitrate test)

From the tests, the range of water quality parameters measured in the system for the entire duration was optimum for the survival and growth of juvenile tiger shrimp and approximated range: Temperature (29-30°C), pH (8-8.2), salinity (29ppt). The total

ammonia levels, nitrate levels and nitrite levels were closer to zero and hence considered ideal.

4.5.2 Nutritional analysis of formulated diets

Nutritional analysis was done for the formulated diets i.e., Diet 1-5 containing gradual levels of BSFL to quantify the amount of crude protein, crude fat, ash moisture, and carbohydrate present in each diet. The following methods were utilized for the nutritional analysis tests:

Each diet 1-5 was finely grinded into powder which was used as the sample for each diet containing gradual levels of BSFL inclusion.

Ash test: Weight of the silica cruse is noted and 5grams of sample is taken in a silica cruse weight of the silica cruse along with the sample is noted and kept in a hot plate for the complete burning of samples. Then the sample in silica cruse is kept in a muffle furnace for 16 hours. And the ash present in the cruse is weighed.

$$\% \text{ash} = \frac{(\text{weight of cruse with sample} - \text{empty cruse weight})}{(\text{ash weight} - \text{empty cruse weight})} * 100$$

Moisture test: Weight about 5 grams of sample in a moisture dish. Place the dish in the hot air oven for 4 hours at 105°C. Cool in a desiccator and weighed. calculate the percentage of moisture.

$$\% \text{moisture} = 100(w_1 - w_2) / (w_1 - w)$$

W-weight of empty dish

W1-weight of dish with sample before drying.

W2-weight of dish with sample after drying.

Fat test (Soxhlet method): Weight about 5 grams of sample in a round bottom flask and kept for fat extraction in Soxhlet apparatus.

$$\% \text{ fat} = \frac{\text{weigh of empty flask}}{\text{Weight of flask with fat}}$$

Protein test (kjeldahl method): 2gram of the sample, 10gram of sodium sulphate, 0.4 gram of copper sulphate, 25ml of H₂SO₄ was taken in a round bottom flask and kept for protein digestion for 1 hour. By using kjeldahl method protein percentage is determined.

Protein% = (blank sample) * 0.14 * N * 6.25

Sample weight * 0.1

Carbohydrates test: Carbohydrates% = 100 - protein% - moisture% - fat% - ash%

4.5.3 Whole-body composition of initial and final shrimp batches

After the conclusion of the experiment, the final batches of shrimp fed with gradual levels of BSFL (Diet 1-5) were harvested by chill killing the shrimp in an ice bath immediately. The harvested shrimp were then labeled for whole-body composition analyses.



Fig 3.19 Image of harvesting the final batches of juvenile tiger shrimp fed with formulated diets containing graded levels of BSFL.

The tiger shrimp were cut into small pieces with a sharp knife and the shells and offal were removed then dried at 75°C for 24 hours. Dried samples were grounded and powdered (Saher, Noor, Syeda, et al; 2019)

The whole-body composition of the initial shrimp batch and final shrimp batches fed with formulated diets containing gradual levels of BSFL inclusion is conducted using standard AOAC methods given in 3.5.2.

4.5.4 Growth response parameters

The growth response parameters are certain values that evaluate the performance obtained from the feed. There are various growth parameters that specifically observe an aspect of feed relating to the growth. The parameters that are observed in this experiment are: -

- Final individual weight (g/shrimp) = W_f
- Percent weight gain (%) = $[(W_f - W_i) \times 100] / W_i$
where W_i = initial individual weight (g/shrimp);
- Specific growth rate (SGR) = $(\ln W_f - \ln W_i \times 100) / t$
where t is time in days;
- Feed intake (g/shrimp);
- Feed conversion ratio (FCR) = dry feed intake (g)/final weight gain (g);

The data observed for each parameter for each batch of juvenile tiger shrimp fed with formulated diets with gradual levels of BSFL inclusion was input into an excel sheet where the values are used to plot graphs and observe the results.

The graphs are then analyzed to compare the results with the control diet (diet 1) and the optimum percentage of inclusion of BSFL is identified.

CHAPTER 5 – RESULTS AND DISCUSSION

5.1 DIETARY COMPOSITION

The composition of diets were formulated in accordance with known dietary requirements of the shrimp. The relatively lower protein content of BSFL (40-50%) compared to FM (55-60%) was taken into consideration while replacing FM with BSFL at 25%,50%,75%,100% for diets 2,3,4,5 respectively.

Table 5.1 Nutrient composition of formulated feed containing graded levels of BSFL on a dry matter basis(g/100g)

Diets	Protein (g)	Fat (g)	Moisture (g)	Ash (g)	Carbohydrates(g)
Diet 1(0%BSFL)	38.15	10.25	8.34	10.07	33.19
Diet 2(8%BSFL)	37.03	10.38	8.42	10.18	33.99
Diet 3(17%BSFL)	36.94	10.59	8.46	9.35	34.66
Diet 4(26%BSFL)	36.19	10.93	8.35	8.72	35.81
Diet 5(35%BSFL)	35.36	12.47	8.68	8.26	35.24

All the Parameters do not differ greatly with the inclusion of BSFL at gradual levels. Although the protein levels of the diets gradually decrease by increasing the percentage of inclusion of BSFL to the feed, the difference is negligible. Overall estimation of the nutrient composition yields an average results of; 35-38% protein, 10-12% fat, 8% Moisture, 8-10% Ash and 33-35% Carbohydrates. The obtained values fit perfectly in the required nutritional values required for feeding Juvenile Tiger shrimp(10g). (FAO,2003) (Alava, V. R., & Lim, C. et al; 1983). The fat value in diet 5(35%BSFL) exceeds the expected quantity and shows an increase of 2% then what is required for the juvenile tiger shrimp(10g). The excess of fat in diet 5 may be caused by the amount of lipids present in BSFL and complete substitution of FM by BSFL.

5.2 WHOLE BODY COMPOSITION OF SHRIMP

After the juvenile tiger shrimps were sourced and acclimated by feeding a maintenance diet, the experimental feeding process was started. Whole-body analysis of the shrimp was done at the beginning and at the end of the experiment.

Table 5.2 Whole-body composition of initial juvenile tiger shrimp and final juvenile tiger shrimp fed with diets containing graded levels of BSFL (%)

Diets	Moisture %	Protein %	Lipid %	Ash %	Carbohydrates %
Initial	76.55	18.87	0.97	2.48	1.13
Diet1(0%BSFL)	67.22	25.87	4.78	0.99	1.14
Diet2(8%BSFL)	66.94	25.74	4.76	0.97	1.48
Diet3(17%BSFL)	68.34	24.88	4.71	0.93	1.14
Diet4(26%BSFL)	69.36	23.95	4.65	0.95	1.15
Diet5(35%BSFL)	71.48	22.04	4.54	0.95	1.15

The initial batch of juvenile tiger shrimp was set as a reference to check the increase of the amount of protein and lipid. The quantity of protein and lipids gradually decrease when the percentage of BSFL inclusion gets higher. The protein levels of juvenile tiger shrimp fed with diet 1(0%BSFL) and diet 2(8%BSFL) show a similar range of output and slowly decrease in diet 3(17%BSFL), diet 4(26%BSFL), diet 5(35%BSFL). This pattern is also showcased in the lipid levels of juvenile tiger shrimp fed with formulated diets containing gradual increase of BSFL inclusion.

The moisture levels of the final batch increase with the increase of BSFL inclusion. The carbohydrates and ash percentage are roughly similar in all diets.

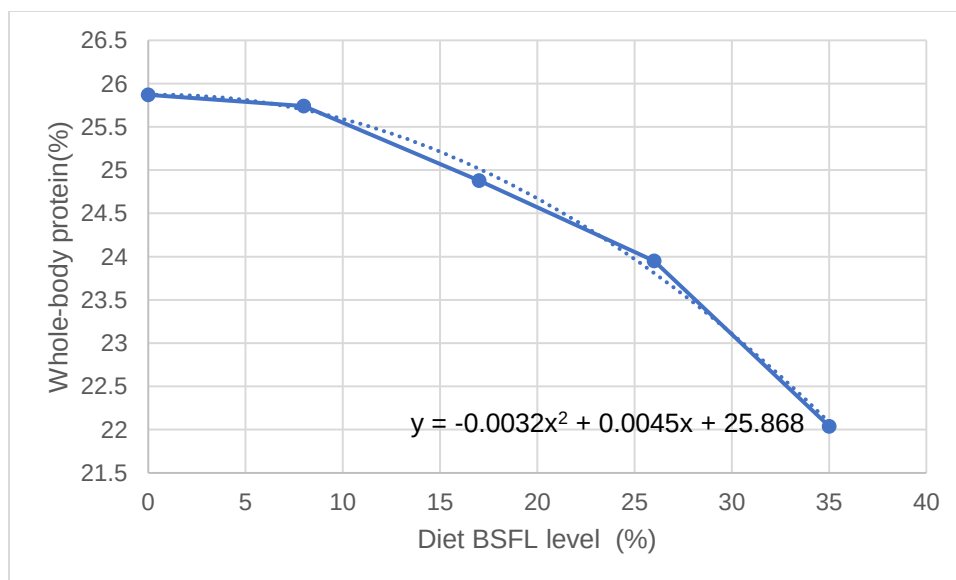


Fig 5.1 Graph of whole-body protein percentage in juvenile tiger shrimps fed with 5 diets with graded levels of BSFL for 30 days.

The graph plotted of whole-body protein levels of juvenile tiger shrimp against percentage of BSFL inclusion in the diet shows the gradual decrease of protein with the increase of BSFL in diets. However, the differences between Diet 1(0%BSFL) and Diet 2(8%BSFL) is very low and can be considered negligible.

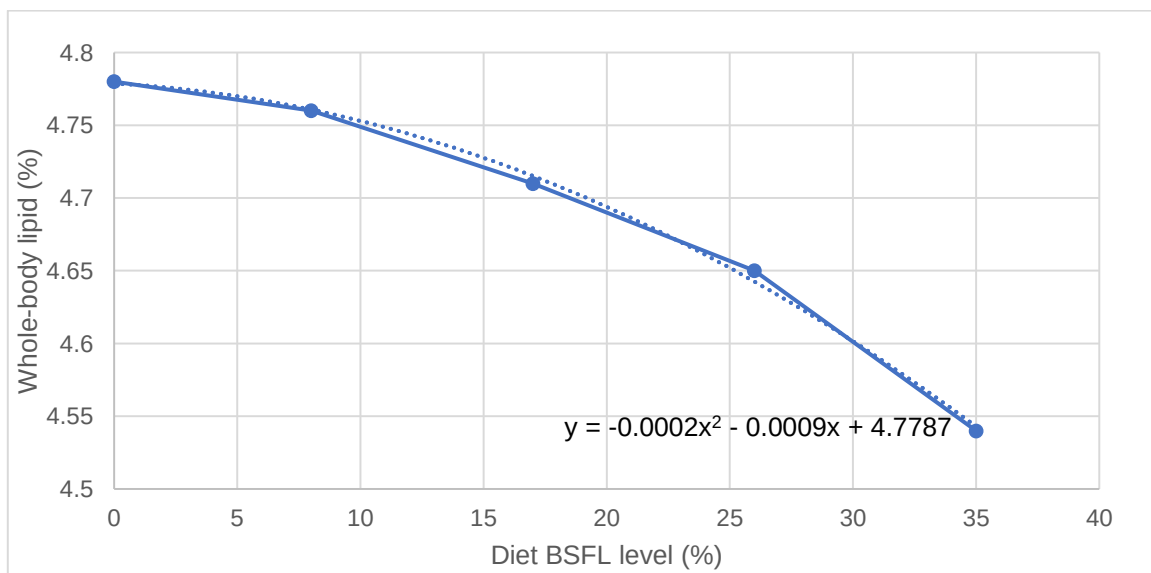


Fig 5.2 Graph of whole-body lipid percentage in juvenile tiger shrimps fed with diets with graded levels of BSFL for 30 days.

5.3 GROWTH PERFORMANCE OF SHRIMP

In terms of survival, the survival percentage of the juvenile tiger shrimps fed with diets containing graded levels of BSFL did not vary as all the test subjects in all batches remained alive throughout the duration of the experiment. Feed intake among treatments did not differ very much but the feed intake of the shrimps from diet 3(17%) and diet 4 (26%) showed a slight increase in feed intake, the slight difference may be present due to irregular shrimp sizes present in the respective setups.

Table 5.3 Growth parameters response of juvenile tiger shrimp (10g) fed 5 diets with graded levels of BSFL for 30 days.

Diets	Total weight (g)	Final weight (g)	Feed intake (shrimp/30 days)	Final Weight gain (%)	FCR	SGR
Diet 1 (0%BSFL)	123.6	20.6	28.7	1060	2.70	2.40
Diet 2(8%BSFL)	118.7	19.7	28.2	970	2.90	2.26
Diet 3(17%BSFL)	113.3	18.8	29.4	880	3.34	2.10
Diet 4(26%BSFL)	104.7	17.4	30.1	740	4.06	1.84
Diet 5(35%BSFL)	96.1	16.1	28.5	610	4.68	1.58

Generally, the maximum responses were observed in the batch of tiger shrimp fed with diet 1(0%BSFL) although the responses of shrimp fed with diet 2 (8%BSFL) show similar to slightly lower response compared to diet 1. The performance of diet 3(17%BSFL) to diet 5 (35%BSFL) shows gradual decrease of response with the increase of BSFL inclusion in the diets.

The final weight of the juvenile tiger shrimp ranges from 20.6-16.1g. The final weight of juvenile tiger shrimps again shows that the maximum response is observed in diet 1(0%BSFL) with diet 2 (8%BSFL) showing comparable results. The decrease in final weight with the increase in BSFL inclusion with the diets.



Fig 5.3 Final weight of juvenile tiger shrimps fed with diet 1(0%BSFL)



Fig 5.4 Final weight of juvenile tiger shrimps fed with diet 2 (8%BSFL)



Fig 5.5 Final weight of juvenile tiger shrimps fed with diet 3 (17%BSFL).



Fig 5.6 Final weight of juvenile tiger shrimps fed with diet 4 (26%BSFL)



Fig 5.7 Final weight of juvenile tiger shrimps fed with diet 5 (35%BSFL)

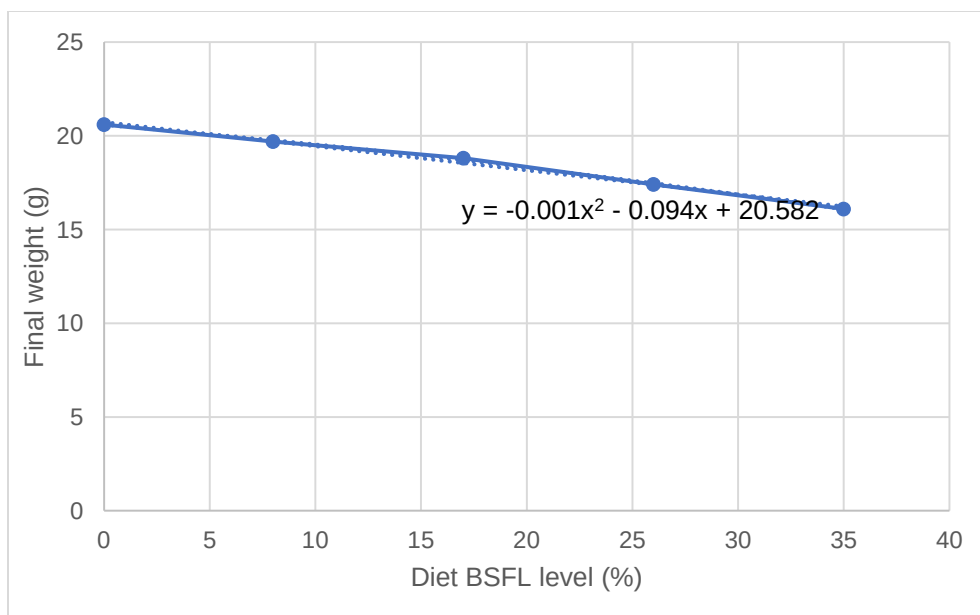


Fig 5.8 Graph of final weight (g) of juvenile tiger shrimps fed with diets with graded levels of BSFL for 30 days.

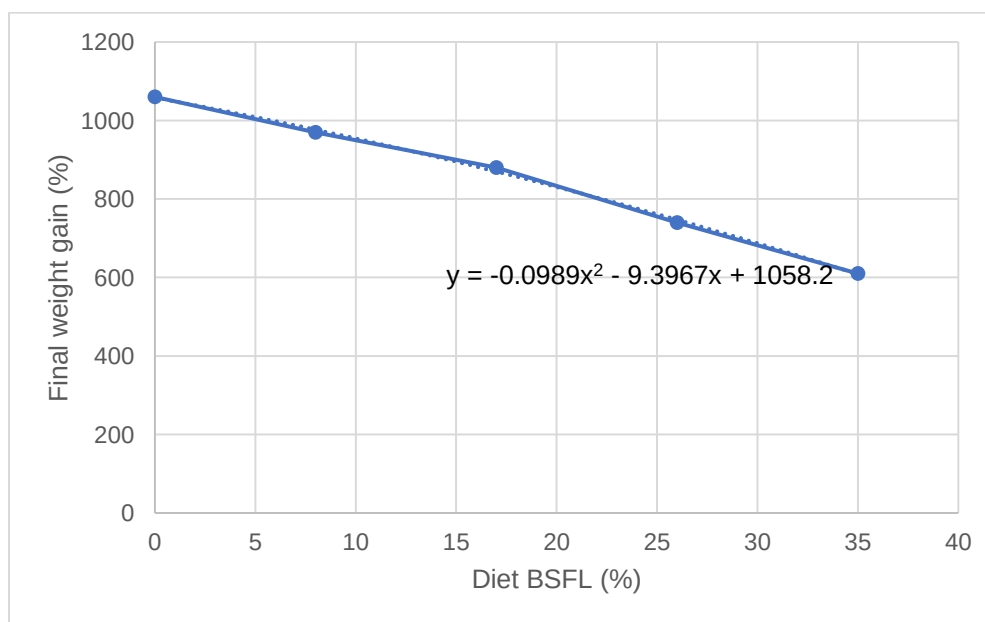


Fig 5.9 Graph of final weight gain (%) of juvenile tiger shrimps fed with diets with graded levels of BSFL for 30 days.

Generally, for FCR the lower the FCR, the higher the efficiency of the feed. The observed values from table 5.3 show that with the increase of BSFL levels in diets the FCR tends to increase, i.e., the efficiency of the feed is reducing with the increase of BSFL. The

FCR observed in the final shrimp batches ranges from 2.70-4.68. The FCR of the feed formulated must be lower to increase the efficiency of the feed. The FCR performance of diet 2 (8%BSFL) shows a similar performance to diet 1 (0%BSFL). (Table 5.3)

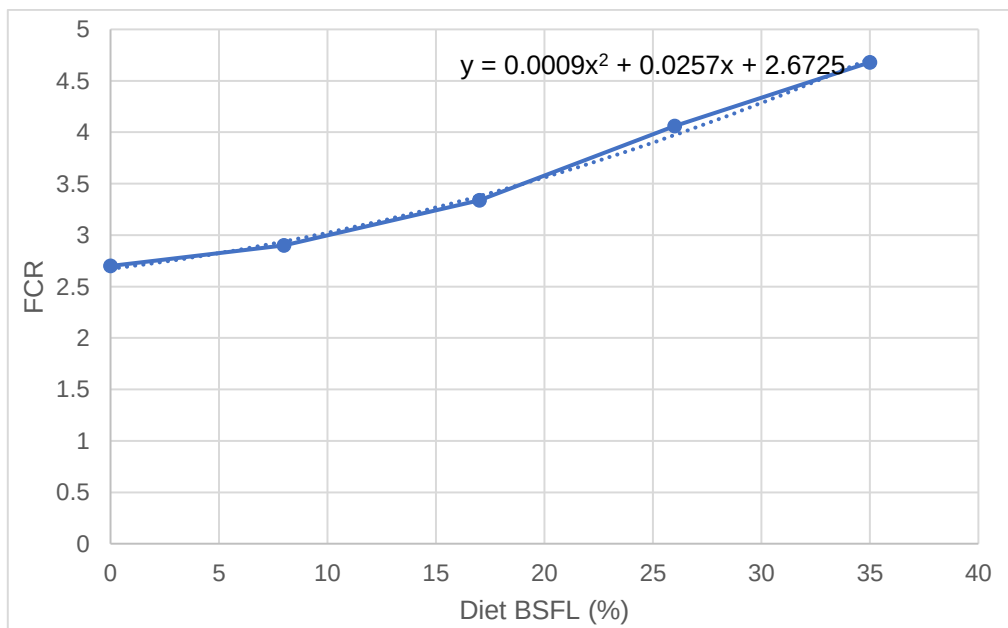


Fig 5.10 Graph of FCR performance of juvenile tiger shrimps fed with diets with graded levels of BSFL for 30 days.

The SGR or Specific Growth Rate is a growth indicator which shows the rate of growth of the shrimp fed with diets containing graded levels of BSFL. The greater the SGR (%), the greater growth over time. SGR of the final shrimp batches was observed to be in the range of 1.58%- 2.40%. The SGR also follows a similar trend where the increase of BSFL percentage in the diets leads to the decrease of SGR overall. The SGR of diet 2 (8%BSFL) shows similar performance to that of shrimps fed with diet 1 (0%BSFL). (Table 5.3)

Overall, the growth parameters observed in the experiment show a gradual trend of lowering of performance when the BSFL level in the diet increases. The performance of Diet 2 shows similar results when compared to diet 1 in all the parameters observed.

Diets 3,4 and 5 containing 17%, 26%, 35% BSFL display that the replacement of FM with BSFL above 25% significantly lowers the growth performance of the final juvenile tiger shrimp. (Table 5.3)

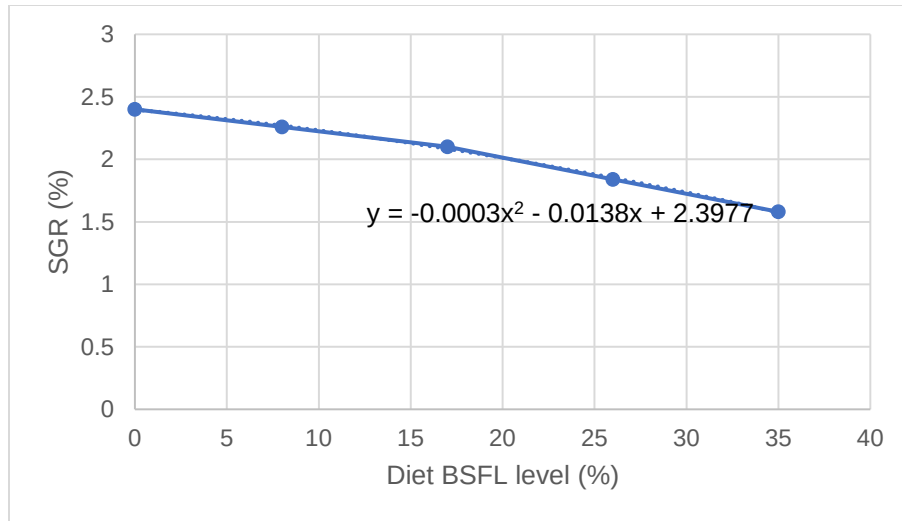


Fig 5.11 Graph of SGR performance of juvenile tiger shrimps fed with diets with graded levels of BSFL for 30 days.

CHAPTER 6: - SUMMARY AND CONCLUSIONS

This experiment is the first study to investigate the replacement of FM with BSFL meal in diets for juvenile tiger shrimp (*Penaeus monodon*). The juvenile tiger shrimp used in the experiment showed great progress and adapted to the formulated diet well. The system quality parameters such as salinity, pH, temperature, nitrate, and nitrite levels were maintained for the optimum growth of juvenile tiger shrimp. (Pushparajan, N & P., Soundarapandian. et al.,2010.)

Juvenile tiger shrimp fed with diet 1 (0%BSFL) showed the maximum performance and exhibited results that are similar or lower compared to previous studies done on Juvenile tiger shrimp (Alava, V. R., & Lim, C. et al., 1983; Pushparajan, N & P., Soundarapandian. Et al ;2010.; A M Parra Flores, J T Ponce Palafox et al., 2019)

The whole-body composition of shrimp shows that the percentage of protein and lipid decreases with the increase of BSFL in diets. Overall, the performance of shrimp fed with Diet 2 (8%BSFL) is similar to the maximum performance showed by diet 1 (0%BSFL). The decrease of available protein and lipid with the increase of BSFL in diets indicates that the inclusion rate of BSFL should be limited to 8-10%. (Fig 5.1and fig 5.2)

The growth performance of juvenile tiger shrimp fed with diets containing gradual levels of BSFL was observed using parameters such as Final weight (g), Final weight gain (%), FCR (Feed Conversion Ratio), and SGR (Specific Growth Rate) (%). The results (Table 5.3) showed similar patterns where the performance decreases with the increase of BSFL in diets.

The growth performance of juvenile tiger shrimp fed with diet 2 (8%BSFL) showed results slightly lower or similar to shrimp fed with diet 1 (0%BSFL) and follows a similar trend until the BSFL inclusion increases above 10%. All the growth parameters exhibited a similar range of performance where the inclusion of BSFL under 10-15% shows growth similar or slightly lower than shrimp fed with diets containing no BSFL.

In a similar study where pacific white leg shrimp (*Litopenaeus vannamei*) fed with diets containing gradual levels of BSFL, the composition and growth performance decreased with the increase of BSFL inclusion after a certain percentage. The diminishing growth

performance of the pacific white leg shrimp with increasing levels BSFL arises due to imbalances of essential amino acids as stated by the study (C. Cummins, D. Rawles et al., 2019) but since the present study did not analyze the amino acid compositions of the diets and the final shrimp fed with the formulated diets, the particular cause of the decline of the performance is unknown. This experiment concluded that the ideal percentage of inclusion of BSFL to produce 90-100% response is 10-17%.

The similar performance of tiger shrimp fed with a diet containing 8% BSFL and 18% FM compared to maximum performance response of shrimp fed with diet containing 0% BSFL and 25% FM shows the ideal BSFL inclusion rate to obtain similar performance is 8-15%.

In conclusion, the current results give insight into the limitations of using BSFL meal in Tiger shrimp and possibilities of expanding BSFL replacement of fishmeal in aquaculture diets. With judicious modifications to diet formulations which include BSFL meal and modifications to the substrates used for BSFL production, increased inclusion levels may be possible in diets for Tiger shrimp (*Penaeus monodon*).

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