Dietary Probiotic (*Bifilac*) Supplementation in the Reproductive Performance of White Molly, *Poecilia sphenops*

Chithra, P.

(12 PZO 001)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Zoology

March, 2014

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31.3.14
Signature of the Head of the Department

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Signature of the Guide

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1. INTRODUCTION

Aquaculture has made significant advances in years in the production of a wide range of aquatic organisms, both for human consumption and as ornamental species (Balcazar *et al*., 2006 and Kesarcodi *et al*., 2008). It has become an economic activity of great importance around the world. Aquaculture is developing, expanding and intensifying in most regions of the India. Aquaculture is the fastest growing food producing sector, already accounting for 36 per cent of world fisheries.

Aquaculture's contribution to world food production, raw materials for industrial and pharmaceutical use and aquatic organisms for stocking or ornamental trade has increased in recent decades. The shortage of natural resources such as fresh water and land has led to intensification of aquaculture system (Piedrahita, 2003). However this condition may also increase the incidences of disease in fish farms due to deteriorated water quality (Losordo, *et al*. 1999). The demand for animal protein for human consumption is currently on the rise and is largely supplied from terrestrial farm animals. However, aquaculture is an increasingly important option in animal protein production.

Ornamental fish keeping is one of the most popular hobbies in the developed countries of the world and is gaining popularity in many developing market of the world too. India's share in global ornamental fish trade is negligible and at present the ornamental fish export from India is dominated by the wild caught species. Ornamental fish culture in India involves breeding of exotic species and this activity is a small segment of the fisheries sector.

The commercial production of ornamental tropical fish is gaining momentum as a global component of international trade fisheries and aquaculture development. The last four decades have witnessed considerable growth and diversification in the international trade of ornamental fish. The live bearing category of the ornamental fish are the most popular of all ornamental fish with the fish hobbyists and entrepreneurs because of the fact that they are brightly coloured, accept all kinds of food and breed prolifically to produce living free swimming young ones.

Three of the top 10 species of ornamental fish imported into the USA are *Viviparous* c*yprinodontids* of the family *Poeciliidae*. In India *Poeciliids* account for fifty per cent of the market share in ornamental fish (Mahapatra *et al*., 2000 and Ramachandran, 2002). The live bearing *Poeciliab latipinna* (Lesueur), commonly known as the sailfin molly is a popular ornamental fish bred commercially in many countries including tropical India (Ghosh *et al*., 2003 and Ramachandran, 2002).

About 95 per cent of the ornamental fish produced in the United States originate from Florida, with most production facilities centered around Tampa (Hillsborough and Polk Countries) and Miami for both airport accessibility and historical reasons.

In aquaculture industry, several probiotic species were used, including *Saccharomyces* spp. (Surawicz *et al*., 1989), *Lactobacillus acidopilus* (Venkat *et al.,* 2004), *B. subtilis* (Kumar *et al*., 2006, Ghosh *et al*., 2007 and Keysami *et al*., 2007) and mixed cultures (Lessard and Brisson, 1987).

The term “probiotic” comes from Greek pro and bios meaning “profile” (Schrezenmcir and De Vrese, 2001) having different meanings over the years. In 1905, Dr. Elie Metchnikoff was the first to describe the positive role played by some bacteria among farmers who consumed pathogen-containing milk and that “reliance on gut microbes for food makes it possible to take steps to change the flora of our bodies and to replace harmful microbes by beneficial microbes” (Metchnikoff, 1907). The term probiotic are also called “probiotika”. It was used to describe substances produced by a microorganism that prolong the logarithmic growth phase in other species. It was described as an agent which has the opposite function of antibiotics. Later, Sperti (1971) modified the concept of “tissue extracts that stimulate microbial growth”.

For many years, studies focused on microorganisms characteristic from intestinal microbiota, and the term “probiotic” was mainly restricted to gram-positive lactic acid bacteria (Verschuere and Rombaut, 2000) particularly representative of the genera *Bifidobacterium*, *Lactobacillus* and *Streptococcus* (Hernandez and Sainz *et al*., 1996). In contrast to terrestrial animals, gastrointestinal microbiota of aquatic species is particularly dependent on the external environment due to the flow of water passing through the digestive tract. Thus, the majority of bacteria are transient in the intestine, due to constant intake of water and food, together with microorganisms present in them.

Although in the gastrointestinal tract (GIT) of aquatic animals have been reported potentially pathogenic bacteria such as *Salmonella*, *Listeria* and *Escherichia coli*, probiotic bacteria and other microorganisms have also been identified. These include gram-positive bacteria such as *Bacillus*, *Carnobacterium*, *Enterococcus* and several species of *Lactobacillus*, gram-negative, facultative anaerobic such as *Vibrio* and *Psedudomonas* as well as certain fungi, yeasts and algae of the genera *Debaryomyces*, *Saccharomyces* and *Tetraselmis* respectively (Leukes and Kaiser, 2002). Due to the increasing interest of probiotics in aquaculture (Moriarty, 1998) proposed extending the definition of these to “living microbial additives that benefit the health of hydrobionts and therefore increase productivity”.

A more general and common concept of probiotic is “one or more microorganisms with beneficial effects for the host, able to persist in the digestive tract because of its tolerance to acid and bile salts” (Irianto and Austin, 2002). Although the use of probiotics in aquaculture is relatively recent, interest in them has increased due to their potential in disease control (Wang and Lin, 2008).

Probiotics have been used in aquaculture to increase the growth of cultivated species, in reality it is not known whether these products increase the appetite, or if, by their nature, improve digestibility. Some people are inclined to think that it could be both factors ; furthermore, it would be important to determine whether probiotics actually taste good for aquaculture species" (Irianto and Austin, 2008). According to Balcazar *et al*. (2006) probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so as probiotics constantly added to fish cultures, they adhere to the intestinal mucosa of them, developing and exercising their multiple benefits. This also depends on factors such as hydrobionts species, body temperature, enzyme levels, genetic resistance and water quality.

Probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extracellular enzymes such as proteases, amylases and lipases as well as provide growth factors such as vitamins, fatty acid and amino acids (Vendrell and Muzquiz, 2006). Therefore nutrients are adsorbed more efficiently when the feed is supplemented with probiotics (Goda and Kabir, 2006).

Aquaculture species have high nutritional requirements, thus reproductive capacity depends on appropriate concentrations of lipids, proteins, fatty acids, vitamin C and E and carotenoids. Furthermore, the relationship of these components influences reproduction in various processes such as fertility, fertilization, birth and development of fry. At present, for most cultured fish species, there are commercially available "brood stock diets" that just are larger sized diets. In practice, many fish hatcheries improve the nutrition of their brood stock by feeding them solely on fresh fish byproducts or in combination with commercial diets.

Among livebearers, the mollies (*Piecilia sphenops*) are a very popular group of ornamental fish species due to existence of the variety of body colours and fact that they are easy to breed and keep (Dernekbasi *et al*., 2010). *Poecilia* species demonstrate viviparous strategy with female storing transferred sperms within the ovary followed by internal egg fertilization and hatching of young ones (Chong *et al*., 2004).

In guppies (*Poecilia reticulata* and *P. sphenops*) and swordtail (*Xiphophorushelleri* and *X. maculates*) the effect of incorporating *Bacillus subtilis*, isolated from the intestine of *Cirrhinus mrigala* in to their diet has been evaluated. The results show an increase in the length and weight of the ornamental fishes as well as the specific activity of proteases and amylases in the digestive tract (Sinha and Ahu, 2008). According to Moriarty (1998) *Bacillus* secretes a wide range of exo-enzymes that complement the activities of the fish and increases enzymatic digestion. In fact, the bacteria isolated from the digestive tract of aquatic animals have shown chitinases, proteases, cellulases, lipase and trypsin (Leukes and Kaiser, 2006).

The studies related to the role of probiotic on the reproductive performance of ornamental fishes are very spotty and meager and hence the present study was undertaken in order to find out the **“DIETARY PROBIOTIC (*Bifilac*) SUPPLEMENTATION IN THE REPRODUCTIVE PERFORMANCE OF WHITE MOLLY (*Poecilia sphenops*)”.**

The objectives of the present study are

1. To formulate four different types of fish feeds using probiotics (*Bifilac*) in different concentration with basal diet (control, T_1, T_2, T_3 and T_4).
2. To analyse and compare the proximate composition such as protein, carbohydrate and fat content in the control and four different prepared feed.
3. To find out the growth rate (length and weight) of white molly, *Poecilia sphenops* grown in the control and four different treatments during 15, 30, 45 and 60 days of the experiment.
4. To find out the relative fecundity of white molly, *P. sphenops* in the control and four different treatments.
5. To find out the fry survival of white molly, *P. sphenops* during different days of the experiment in the control and four different treatments.
6. To find out the length and weight of the fry of white molly, *P. sphenops* during different days of the experiment in the control and four different treatments.
7. To analyse the dead fry of white molly, *P. sphenops* during different days of the experiment in the control and four different treatments.
8. To estimate the water content, protein, carbohydrate and fat in the white molly, *P. sphenops* grown in the control and four different treatments before and after the experiment and the results were subjected to statistical analysis to recommend the best treatment.

2. REVIEW OF LITERATURE

The available literature pertaining to the present study entitled **"Dietary Probiotic (*Bifilac*) Supplementation in the Reproductive Performance of White Molly (*Poecilia sphenops*)"** was reviewed and presented in this chapter.

Re *et al*. (2000) reported the presence of probiotic bacteria in the digestive tracts of fish is subjects several factors such as their ability to adhere to the surface of the intestinal epithelium and the production of substance that antagonize pathogenic microorganisms. Riquelme *et al*. (2000) studied the use of probiotics microorganisms that promote the welfare of the host they inhabit by improving its digestion and immuno response as well as by inhabiting the growth of pathogenic microorganisms.

Izquierdo *et al*. (2001) observed lipid, protein, fatty acid profile, vitamin E, C and carotenoids as major nutrients influencing various production processes such as fecundity, fertilization, hatching and larval development. Kruger *et al*. (2001) obtained a large number of fry *Poeciliid. X. hellerii* were maintained on a flake diet supplemented with live daphnia. Molly is popular ornamental fish bred commercially in many countries including tropical India were demonstrated by Ramachandran (2002).

Iso-Osmotic salinities minimize osmo regulatory stress and associated energy coasts there by increasing the energy available for growth and survival was studied by Binachini (2002). Irianto and Austin (2002) reported probiotics must be considered as potentially useful for the control of fish diseases. Probiotic bacteria in the fish intestine enhances host enzymes secretion by the superior maturation of fish intestine secretary cells were observed by Tovar *et al*. (2002).

Saccharomyces cerevisae yeast is a natural feed additive, which positively influences the non specific immune responses of many aquaculture species were described by Thanardkit *et al*. (2002). Tena-sempere and Huhtaniemi (2003) studied the reproductive functions and are regulated by the three glands, hypothalamus, pituitary and gonads. Pathogen resistance has been observed in studies testing both gram-positive and gram-negative bacteria species along with increased growth described by Rengpipat *et al*. (2003).

Venkat *et al*. (2004) observed that in aquaculture industries several probiotics species were used including *Saccharomyces* spp. and *Lactobacillus acidopilus*. Chong *et al*. (2004) demonstrate *Poecilia* species viviparous strategy with female storing transferred sperms within the ovary by internal egg fertilization and hatching of young ones.

Wang *et al*. (2005) studied the commercially probiotics products are used by adding in the food or water containing aquatic species. Fernandez *et al*. (2006) observed that the reproduction is gated the state of body energy reserve and is sensitive to different metabolic cues. Haroun *et al*. (2006) studied the probiotic product was shown to increase growth performance in tilapia. Wache *et al*. (2006) analysed the larval rearing depends on the foot that is really consumed efficiently digested and provides the required nutrients support good growth and health.

The nucleic acid as well as mono oligosaccharids are capable to enhance the immune responses were studied by Abdel-Tawwab *et al*. (2006). Vasagam *et al*. (2007) studied dietary effect on fry production and growth performance of sail fin molly, *Poecilia litipinna*, in salt water. Panigrahi *et al*. (2007) have reported on the immune system modulation of rainbow trout fed on entero faccium diet. Probiotics tested in fish have been selected due to their efficiency in humans and livestock were reported by Azad and Ai-Marzoak (2008). Lauzon *et al*. (2008) investigated the probiotic were selected from the natural microbiota of cod larva culture their growth characteristics and inhibition of fish pathogens.

Ghosh *et al*. (2008) observed that the health and nutrition are two important aspects in the ornamental fish. Effect of probiotics on growth may occur through several mechanisms including increasing nutrients availability for absorption and activity of digestive enzymes in gastro intestinal tract were studied by Chat and Coyne (2008).

Soltan *et al*. (2008) investigated the effect of probiotics and some spices as feed additives on the performance and behaviour of the *Nile tilapia*, *Oreochromis nilaticus*. Dernekbasiet *et al*. (2010) reported the black mollies *(Poecilia sphenops*) are very popular group of ornamental fish species due to existence of the variety of body colours and fact that they are easy to breed and keep.

Dharmaraj and Dhevendran (2010) evaluated the efficacy of *Streptomyces* as a probiotic feed for the growth of ornamental fish, *Xiphophorusheleri*. Hernandez *et al*. (2010) analyzed the effect of the commercial probiotics *Lactobacillus casei* on the growth and protein content of skin mucus and stress resistance of juveniles of the porthole live bearer *Poecilia graeilis* (*Poecilidae*).

The effect of the probiotic *Lactobacillus rhamnosus* as a feed additive on zebra fish fecundity were studied by Giaechini *et al*. (2010). Effect of dietary (fatty acid and protein) content during spawning season on fertility eggs and larval quality of common porgy was studied by Abrenoueh *et al*. (2010).

Probiotic use in the fish farming industry, studies concerning their effects on vertebrate reproduction are very scarce. Only a few studies have investigated the beneficial effects of probiotics dietary supplementation on the reproductive performance (GSI, fecundity, fry production, BW and TL of fry) of four live bearing ornamental species *Poecilia reticulata* (*P. sphenops*) and *Xiphophorus hellerii* (*X. maculatus*). Their results have been complemented by the findings obtained by Gioacchini *et al*. (2010).

Helene *et al*. (2010) reported the impact of probiotic intervention on microbial load and performance of Atlantic cod (*Gadus morhua* L.) juveniles. Lombardo *et al*. (2011) observed that probiotic based nutritional effects on killifish reproduction. Lenger (2011) studied the culture of edible fish, dietary requirements and feeding of ornamental fish. Mansa and Allah (2011) studied the effect of dietary vitamin C on reproductive performance of a fresh water ornamental species the platy *(Xiphophorous maculatus*).

Protozoa reproduce by simple division a single organism can multiply into hundreds of new parasites reported by Kanani *et al*. (2012). Nekoubin *et al*. (2012) studied the effect of symbiotic (biominimbd) on fecundity and reproduction factors of zebra fish (daniorerio). Dosta *et al*. (2012) isolated and identified the 16 sr DNA, bacteria with probiotic capabilities from the digestive tract of *Pterophyllum scalare* and evaluate their ability to adhere the intestinal epithelium using immuno histo chemical techniques and bacteriological analysis.

Martinez *et al*. (2012) studied the growth of aquaculture as an industry has accelerated over the past decades this has resulted in environmental damages and low productivity of various crops. The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics in aquaculture practices. The probiotics can improve the digestibility of nutrients, increase tolerance to stress and encourage reproduction. Commercial probiotic products prepared from various bacterial species such as *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp. and the yeast *Saccharomyces cerevisiae* among others and their use is regulated by careful management recommendations.

Karimzadeh *et al*. (2013) reported effects of different levels of immunogen on growth performance, intestinal bacteria colonization and survival rate in Rutilus kutum larvae. Sahandi *et al*. (2013) studied the effect of feed with probiotic blend on the growth performance and infection resistance of the guppy *(Poecilia reticulata*).

Safari *et al*. (2013) studied the effects of probiotic, *Pediococcusacidilactici* in the diet on some biological indices of Oscar astronautsocellatus.

S. cerevisae contains various immune stimulating compounds such as β glucans, which are most commonly found in the cell wall of yeasts are generally considered as the main factor for the immunological mechanism observed by Tukmechi and Bandboni (2013).

3. MATERIALS AND METHODS

Experimental Animal

White molly, *P. sphenops* is an omnivorous ornamental fish was chosen for the present study as an experimental animal (Figure – 1). It belongs to the genus *Poecilia*, known under the common name molly. It is sometimes called short-finned molly or common molly. It inhabits in fresh water live-bearer and successfully establishes in a variety of environmental conditions. It can grow to a maximum length of 3 inches.

Procurement and Acclimatization

About 4 months old juveniles of white molly, *P. sphenops* were purchased from a commercial fish farm (Benna Fish Farm) in Coimbatore. The collected fishes were transported to laboratory in a polythene bag with oxygen. *P. sphenops* having a length of 3 cm approximately were selected for the study. The chosen fishes were acclimatized to the laboratory conditions for a period of 15 days (Figure – 2). The fishes were kept in water tub and the water was changed daily in order to maintain sufficient amount of oxygen and to get rid of toxic ammonia in the trough. They were fed with pellet diet regularly.

Probiotics

A commercial probiotic (*Bifilac*) has been selected for the present investigation. The *Bifilac* tablet contains mixture of bacterial culture including

Streptococcus faecalis (30 million), *Clostridium butyricum* (2 million), *Bacillus mesentericus* (1 million) and *Lactic acid bacillus* (50 million).

Probiotics Feed Preparation

Probiotic (*Bifilac*) tablet is used in the present study as a supplementary feed for the preparation of fish feed. The feed ingredients used in the fish feed were rice bran, ground nut oil cake and probiotic (*Bifilac*) tablet. The feeds were prepared in different combinations (Figure – 3). The selected probiotic (*Bifilac*) tablet was supplemented at the levels of 0.5, 1.0, 1.5 and 2.0 gm to 100 gm of basal diet. The basal diet was prepared by using rice bran and oil cake. Experimental diets were prepared by mixing the rice bran, oil cake and *Bifilac* tablet in different proportion and four different treatments were prepared. The different treatments selected for the present investigation includes :

Treatment	Basal Diet	Supplement Probiotics
Control	100 g	-
T_1	99.5 g	0.5 g
T_2	99 g	1 g
T_3	98.5 g	1.5 g
T_4	98 g	2 g

The pellets were prepared by mixing together the required quantities of finely powdered ingredients. The mixture was cooked well by adding *Bifilac* tablet with required quantities of hot water to form soft dough. Then the dough was fed through an extruder having a perforated disc with 0.8 cm diameter holes. The moist noodles were dried in hot sun and were broken into pieces of approximately 0.5 cm in length. These pellets were packed in an air tight polythene bags to avoid fungal infection. The proximate composition such as protein, carbohydrate and lipid were analysed in the control feed and four different feed and is used for the present study.

Feeding Trials

The experiments were carried out for 60 days. The feed trials were carried out in three replication (Figure – 4). Each trough contains 10 individuals in the control and four different treatments.

During experimental period *P. sphenops* in control and four different treatments were fed with five per cent of their body weight. The rational feed was given once a day. Biochemical composition such as protein, carbohydrate and fat were calculated in the prepared fish feeds. The length (cm) and weight (gm) of the fishes grown in the control and four different treatments before and after the experiment were evaluated and the results were subjected to two way ANOVA.

Parameters Analysed

The reproductive performance like relative fecundity, fry survival, (total live fry after time / total fry production x 100), dead fry and weight and length of fry were found out and subjected to two way ANOVA.

Biochemical Composition

Biochemical composition such as protein, carbohydrate and fat were analysed in control feed and four different feeds.

White molly, *P. sphenops* were brought to the laboratory to estimate the biochemical composition. Biochemical composition such as water content, protein, carbohydrate and fat were analysed before the experimental period and after 60 days of the experiment.

(1) Water Content

Water content was calculated in the fish of *P. sphenops* in the control and in the fishes grown in four different treatments. The dissected fish were placed in separate vials and dried in hot air oven for 24 hours at 80°C until attaining constant weight.

The weight difference between wet and dried body fish elucidate the water content present in the particular fish and its percentage was calculated.

(2) Protein

Protein was estimated by adopting the method of Lowry *et al*. (1957) in the feed prepared for control having rice bran and oil cake alone and in the feed prepared for four different treatments having rice bran, oil cake and probiotic (*Bifilac*) in different ratios. Protein was also estimated in *P. sphenops* grown in the control and in four different treatments before and after the experiment.

Principle

The blue colour developed by the reduction of phosphomolybdic – phosphor tungstic components in the folin-ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured by Lowry *et al*. (1957) method.

Reagents

Reagent A

Two per cent sodium carbonate in 0.1 N sodium hydroxide W/V (2 gm / 100 ml).

Reagent B

0.5 per cent copper sulphate in 1.35 per cent sodium potassium tartarate. This was prepared just before use.

Reagent C

50 ml of reagent A is mixed with 1 ml of reagent B.

1N Folin Reagent

Folin-Ciocalteau reagent was diluted with equal volume of glass distilled water.

Standard Solution

25 mg of bovine serum albumin was dissolved in 100 ml of 0.1 N sodium hydroxide using 100 ml standard flask.

5 per cent Trichloro Acetic Acid

5 gm of trichloro acetic acid in 100 ml distilled water.

Procedure

Standard Preparation

For plotting the standard curve a set of standard solution was taken in a series of test tubes. The volume of each test tube was made up to 1 ml with distilled water. 5 ml of alkaline copper reagent was added, mixed and allowed to stand for 10 minutes at room temperature. 0.5 ml of 1N Folin-Ciocalteau reagent was then added to each tube and shaken well the blue colour developed was read at 720 nm after 30 minutes along with the reagent blank in a colorimeter.

A standard graph for this was plotted with corresponding OD values on Y axis and standard concentration on X axis.

Sample Preparation

100 mg of feed (control, T_1, T_2, T_3 and T_4) was homogenized with 1 ml of 0.9 per cent sodium chloride solution, 1 ml of 5 per cent trichloro acetic acid was added, and then centrifuged at 8000 rpm for 20 minutes. The precipitate dissolved in 1 ml of 0.1 N sodium hydroxide. 0.1 ml of this aliquot was taken and made up to a final volume of 1 ml with distilled water.

The same procedure as described for the standard was followed. A set of standard were run along with each set of samples.

100 mg of *P. sphenops* in the control fish and also in the T_1, T_2, T_3 and T_4 fed fishes were taken and the same procedure described for the feed samples was followed.

The amount of protein present in the aliquot of the sample was calculated by referring to the standard curve obtained. The protein concentration was expressed in percentage.

(3) Carbohydrate

The method of Hedge and Hofreiter (1962) was employed to estimate carbohydrate. The carbohydrate was estimated in the control feed and four different feeds. In the fishes also estimated before and after the experiment in the control and four different treatments.

Reagent

5 per cent trichloro-acetic acid.

Anthrone Reagent

50 ml of anthrone and 1 gm of thiourea dissolved in 100 ml of 66 per cent sulphuric acid.

Standard Solution

100 mg of glucose in 100 ml distilled water.

Procedure

Standard Preparation

For plotting the standard curve, a set of standards were run 0.1, 0.2, 0.3, . . ., 1 ml in a series of test tubes. The volume in each test tube were made upto 1 ml with distilled water. 5 ml of anthrone reagent was added. A blank containing 1 ml of distilled water and 5 ml of anthrone reagent was also kept.

Standard Procedure

100 mg of feed (control, T_1, T_2, T_3 and T_4) and also fish, *P. sphenops* grown in the control and four different treatments were homogenized in 1 ml of 0.9 per cent sodium chloride solution. 1 ml of 5 per cent trichloro acetic acid was added to 1 ml of each extract. The homogenate was centrifuged at 8000 rpm for 20 minutes. To 1 ml of the supernatant 5 ml of anthrone reagent was added. The series of test tubes were kept in boiling water bath for 10 to 15 minutes and then cooled in dark. After 30 minutes the OD value was read at 620 nm in colorimeter.

(4) Fat

Total fat was resoluted by gravimetric method using chloroform methanol mixture (3 : 1) (Folch *et al*., 1957) of (*P. sphenops*) in the control feed and in four different feeds prepared for the present study. Fat is also estimated in the fishes grown in the control and four different treatments before and after the experiment.

Reagents

Chloroform methanol mixture.

Procedure

100 mg of feed (control, T_1, T_2, T_3 and T_4) were weighed and ground well with 5 ml of chloroform methanol mixture. In the same way of fish *P. sphenops* are also ground well with 5 ml of chloroform methanol mixture.The homogenated was centrifuged, taken in a small weighed beaker and the beaker was placed inside a large beaker and filled with water along the sides and kept overnight in hot air oven without any disturbance. In between the methanol with dissolved protein layer and chloroform with dissolved fat a white precipitate was formed. The methanol layer was removed without disturbing the chloroform layer. The chloroform was evaporated in an oven at about 60°C. The beaker was weighed and the difference between the final and initial weight of the beaker gives the lipid content of the feed and fish.

Statistical Analysis

The estimated protein, carbohydrate and fat is in the control and four different feeds were subjected to ANOVA – two-way analysis ($P < 0.05$) of variance. Similarly, the water content, protein, carbohydrate and fat analysed in the control fish and the fishes grown in the four different treatments before and after the experiments were also subjected to statistical analysis to find out the significance.

FIGURE 1

LIVE BEARING ORNAMENTAL FISH, WHITE MOLLY, *P. sphenops*

FIGURE 2

ACCLIMATIZATION OF WHITE MOLLY, *P. sphenops* IN THE LABORATORY CONDITION

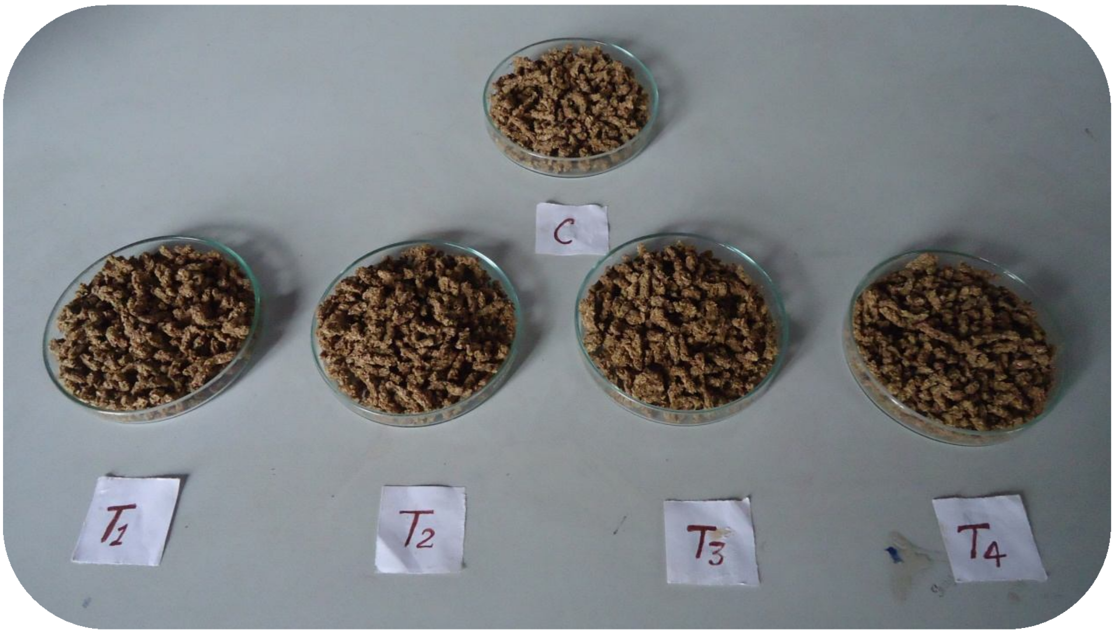

FIGURE 3

FISH FEED PREPARED FROM RICE BRAN, OIL CAKE AND PROBIOTIC (*Bifilac*) IN DIFFERENT COMBINATIONS

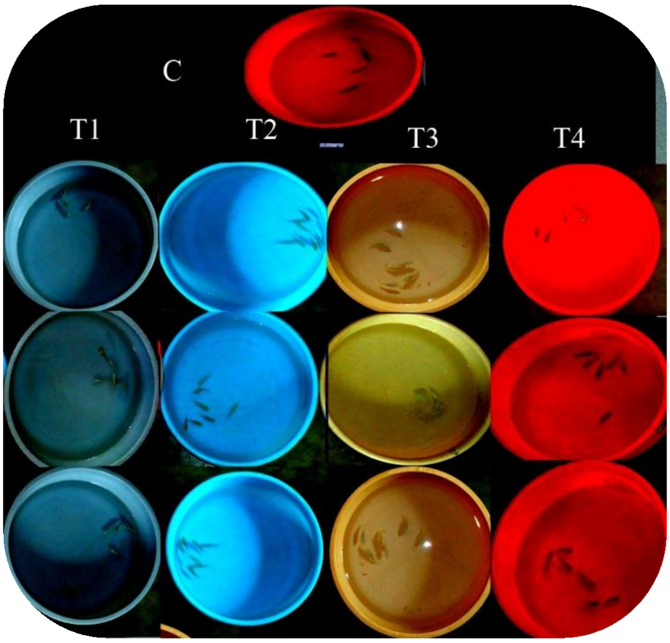

FIGURE 4

EXPERIMENTAL SETUP SHOWS WHITE MOLLY, *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

4. RESULTS

The results of the present investigation on the **"Dietary Probiotic Supplementation in Reproductive Performance of Female White Molly, *Poecilia sphenops*"** are presented and discussed in this chapter.

A. GROWTH PARAMETERS (WEIGHT AND LENGTH) IN *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

(i) Weight of Spawning Female

Weight (gm) of *P. sphenops* during different days of the experiments in the control and four different treatments is shown in Table 1 and Figure 5.

During 15 days of the experiment the weight was maximum in T_1 (4.00 gm) and T_3 (4.00 gm) followed by T_4 (3.80 gm) and T_2 (3.50 gm). The minimum weight (3.30 gm) was observed in the control fish fed with rice bran and oil cake in the ratio of 1 : 1. During 30 days of the experiment, the weight was maximum in T_3 (4.40 gm) followed by T_1 (4.30 gm), T_4 (4 gm) and T_2 (4 gm). The minimum weight (3.68 gm) was recorded in the control.

During 45 days of the experiment highest weight was recorded in T_1 (4.70 gm) followed by fishes in T_3 (4.60 gm) fed with rice bran and oil cake 98.5 gm and probiotic (Bifilac) 1.5 gm and fishes in T_4 (4.50 gm) fed with rice bran and oil cake 98 gm and probiotic (bifilac) 2 gm and fishes grown in T_2 (4.50 gm) fed with rice bran and oil cake 99 gm and probiotic (Bifilac) 1 gm. The minimum weight (3.90 gm) was noticed in the control fishes.

During 60 days of the experiment the weight was maximum in T_2 (5.20 gm) followed by T_1 (5.10 gm), T_3 (5 gm) and T_4 (4.80 gm) respectively. The minimum weight (4 gm) was observed in control fishes.

ii) Length of Spawning Female

Length (cm) of *P. sphenops* during different days of the experiment in the control and four different treatments is given in Table 2 and Figure 6.

During 15 days of the experiment maximum length was noted in T_2 (4.80 cm) followed by T_4 (4.50 cm), T_1 (4.40 cm) and T_3 (4.30 cm). Minimum length (4.20 cm) was recorded in the control fishes. During 30 days of the experiment, the maximum length was observed in T_2 (5 cm) followed by T_4 (4.80 cm), T_1 (4.80 cm) and T_3 (4.60 cm). The minimum length (4.50 cm) was recorded in the control fish.

During 45 days of the experiment the highest length was noticed in T_2 (5.30 cm) followed by T_1 (5.10 cm) and T_4 (5 cm). The minimum length was noticed in control (4.80 cm) and T_3 (4.80 cm).

During 60 days of the experiment the maximum length (5.50 cm) was noticed in T_2 fish fed with rice bran and oil cake 99 g and probiotic (Bifilac) 1 g, followed by T_1 (5.30 cm), T_4 (5.20 cm) and T_3 (5.10 cm). The minimum length was noticed in control (4.90 cm).

The length of *P. sphenops* in four different treatments during 15, 30, 45 and 60 days of the experiment were subjected to statistical analysis and found to be significant.

iii) Relative Fecundity

Relative fecundity in *P. sphenops* in the control and four different treatment is shown in Table 3 and Figure 7.

Relative fecundity was maximum in T_2 (75.14) followed by T_3 (18.01), T_4 (14.50) and T_1 (12.10) respectively. The minimum relative fecundity was noticed in the control fish (7.32).

iv) Fry Survival

Fry survival in *P. sphenops* during different days of the experiments in the control and four different treatments is shown in Table 4 and Figure 8.

During 15 days of the experiment the fry survival was maximum in T_2 (28.00) followed by T_3 (24.33), T_4 (22.67) and T_1 (20.33). The minimum fry survival of 15.33 was observed in the control fish fed with rice bran and oil cake in the ratio of 1 : 1. During 30 days of experiment, the fry survival was maximum in T_2 (35.33) followed by T_3 (28.00), T_4 (26.33) and T_1 (24.67). The lowest fry survival (18.33) was noticed in the fishes grown in control feed.

During 45 days of the experiment highest fry survival was recorded in T_2 (34.00) followed by fishes in T_3 (29.67) fed with rice bran and oil cake 98.5 gm and probiotic (Bifilac) 1.5 gm and fishes in T_4 (27.00) fed with rice bran and oil cake 98 gm and probiotic (Bifilac) 2 gm and fishes in T_1 (27.00) fed with rice bran and oil cake 99.5 gm and probiotic (Bifilac) 0.5 gm. The lowest fry survival (21.00) was noticed in the control fish.

During 60 days of the experiment the fry survival was maximum in T_2 (36.67) followed by T_3 (31.00), T_4 (29.00) and T_1 (26.00) respectively. The minimum fry survival was observed in control fishes (22.00).

Among the four different treatments and the control analysed the fry survival during different days of the experiment showed maximum value in T_2 fishes fed with rice bran and oil cake 99 gm and probiotic (Bifilac) 1 gm. The values were found to be statistically significant.

v) Fry Growth Rate (Weight)

a) Weight

Fry growth rate in *P. sphenops* during different days of the experiment in the control and four different treatments is presented in Table 5 and Figure 9.

During 15 days of the experiment, the fry weight (gm) was maximum in T_2 (1.30 gm) followed by T_3 (1.15 gm), T_4 (1.08 gm) and T_1 (0.90 gm). The minimum fry weight was noticed in control (0.83 gm) fishes. During 30 days of the experiment the fry weight was 1.32 gm in T_2, 1.16 gm in T_3, 1.06 gm in T_4, 0.90 gm in T_1 and 0.87 gm in the fishes grown in the control.

During 45 days of the experiment the maximum fry weight was observed in T_2 (1.34 gm) followed by T_3 (1.18 gm), T_4 (1.08 gm) and T_1 (0.95 gm). The minimum fry weight was estimated in control (0.92 gm). In 60 days of the experiment the fry weight was recorded as 1.35 gm, 1.20 gm, 1.08 gm, 0.99 gm and 0.94 gm in T_2, T_3, T_4, T_1 and control respectively.

Among the four different treatments and control the fry weight during 15, 30, 45, and 60 days experiment, the maximum value was recorded in the fishes grown in T_2.

vi) Fry Growth Rate (Length)

Fry length in *P. sphenops* during different days of the experiment in the control and four different treatments is recorded and given in Table 6 and Figure 10.

During 15 days of the experiment the fry length was maximum in T_2 (1.34 mm) followed by T_3 (1.14 mm), T_4 (1.11 mm) and T_1 (1.06 mm). The minimum fry length was noticed in control (1.03 mm). During 30 days of the experiment maximum fry length was recorded in T_2 (1.37 mm) followed by T_3 (1.16 mm), T_4 (1.14 mm), T_1 (1.07 mm) and control (1.03 mm) respectively.

During 45 days of the experiment the fry length was highest in T_2 and lowest in control (1.04 mm). In T_3, T_4 and T_1 the length was (1.17 mm), (1.14 mm) and (1.08 mm) respectively. During 60 days of the experiment the length was maximum in T_2 (1.39 mm) followed by T_3 (1.17 mm), T_4 (1.14 mm) and T_1 (1.11 mm). The minimum length was observed in control (1.04 mm)

vii) Dead Fry

Dead fry in *P. sphenops* during different days of the experiment in the control and four different treatments is shown in Table 7 and Figure 11.

Dead fry was observed only in control (4.00), T_1 (2.10) and T_4 (2.00).

Growth rate (weight and length) fry and dead fry observed during different days of the experiment were subjected to two way ANOVA and found statistically significant.

B. BIOCHEMICAL COMPOSITION (PROTEIN, CARBOHYDRATE AND FAT) IN THE CONTROL AND FOUR DIFFERENT FEEDS

The protein, carbohydrate and fat contents in the control and four different feeds is shown in Table 8 and Figure 12.

i) Protein

The protein content in control and four different feeds is shown in Table 8 and Figure 12.

The protein content was maximum in T_2 feed (32.06) followed by T_3 (35.02), T_4 (34.02) and T_1 (32.76). Minimum protein content was noticed in control feed (32.03) prepared from 50 gm rice bran and 50 gm oil cake in 1 : 1 ratio.

The protein content was maximum in T_2 feed (37.06) when compared to other feeds.

ii) Carbohydrate

The carbohydrate content in control and four different feeds is shown in Table 8 and Figure 12.

The highest value of carbohydrate content was estimated in T_2 (13.06) feed prepared from rice bran and oil cake 99 g and probiotic (Bifilac) 1 g, followed by T_3 (12.75), T_4 (12.57) and T_1 (12.27). The lowest value was noticed in control feed (12.03).

The carbohydrate content was maximum in T_2 feed (13.06) and minimum (12.03) in control feed.

iii) Fat

The fat content in control and four different feeds is given in Table 8 and Figure 12.

The fat content was maximum in T_2 (15.02) followed by T_3 (14.81), T_4 (14.66) and T_1 (14.41). The minimum value was observed in control (14.03) feed prepared from 50 g rice bran and 50 g oil cake.

The fat content was maximum in T_2 feed when compared to other feeds.

C. BIOCHEMICAL COMPOSITION IN WHITE MOLLY, *P. sphenops*

i) Water Content

Water content (%) of *P. sphenops* in the control and four different treatments before and after the experiment is shown in Table 9 and Figure 13.

The water content of *P. sphenops* before the experiment was maximum in T_2 (57.87 per cent) followed by T_3 (56.03 per cent), T_4 (54.09 per cent) and T_1 (52.08 per cent). The minimum water content was noticed in control (48.37 per cent).

After the experimental period water content was highest in T_2 (70.05 per cent) followed by T_3 (68.01 per cent), T_4 (63.04 per cent) and T_1 (59.02 per cent) respectively. The lowest water content was analysed in the control (54.08 per cent). The water content of *P. sphenops* in the four different treatments before and after the experiment was found to be statistically significant when compared to control.

ii) Protein

The protein content of *P. sphenops* in the control and four different treatments before and after the experiments is shown in Table 10 and Figure 14.

The highest value of protein content of *P. sphenops* before the experiment was noticed as 28.88 gm in T_2 followed by 27.91 gm in T_3, 27.11 gm in T_4 and 26.90 gm in T_1 respectively. The lowest value of 26.3 gm was noticed in control. After the experimental period, the protein content was maximum in T_2 (36.47 gm) followed by T_3 (34.10 gm), T_4 (32.40 gm) and T_1 (31.79 gm). The lowest protein content was analysed in control (29.73 gm).

The protein content of *P. sphenops* in the four different treatments before and after the experiment was found to be statistically significant when compared to control.

iii) Carbohydrate

The carbohydrate content of *P. sphenops* in the control and four different treatments before and after the experiment is shown in Table 11 and Figure 15.

The highest value of carbohydrate was recorded in T_2 (1.30 gm) before the experimental period followed by T_3 (1.27 gm), T_4 (1.23 gm) and T_1 (1.20 gm). The lowest value was noticed in control (1.18 gm). After the experimental period, the maximum level of carbohydrate was noticed in T_2 (1.70 gm) fish fed with rice bran and oil cake 99 gm and probiotic (Bifilac) 1 gm, followed by T_3 (1.62 gm), T_4 (1.56 gm) and T_1 (1.46 gm) respectively. The minimum value (1.40 gm) was noticed in control fish.

The carbohydrate content was found to be maximum in *P. sphenops* grown in T_2 treatment when compared to other treatments.

iv) Fat

The fat content of *P. sphenops* in the control and four different treatments before and after the experiment is shown in Table 12 and Figure 16.

The fat content of *P. sphenops* before the experiment was maximum in T_2 (2.41 gm) followed by T_3 (2.37 gm), T_4 (2.30 gm) and T_1 (2.27 gm). The minimum fat content (2.20 gm) was recorded in control fishes. After the experimental period the highest value of fat content was analysed in T_2 (2.86 gm) followed by T_3 (2.72 gm), T_4 (2.60 gm) and T_1 (2.51 gm) respectively. The minimum fat content was observed in control fishes (2.43 gm).

The fat content was found to be maximum in T_2 fishes when compared to other treatments and control.

TABLE 1

WEIGHT (gm) OF SPAWNING FEMALE WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	Number of days during experiment			
	15	30	45	60
Control	3.30 ± 0.20	3.68 ± 0.16	3.90 ± 0.20	4.00 ± 0.17
T_1	4.00 ± 0.10	4.30 ± 0.12	4.70 ± 0.17	5.10 ± 0.10
T_2	3.50 ± 0.15	4.00 ± 0.16	4.50 ± 0.18	5.20 ± 0.15
T_3	4.00 ± 0.12	4.40 ± 0.14	4.60 ± 0.14	5.00 ± 0.13
T_4	3.80 ± 0.11	4.00 ± 0.10	4.50 ± 0.19	4.80 ± 0.13
SEd	0.12181			
CD ($p < 0.05$)	0.24618			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 2

LENGTH (cm) OF SPAWNING FEMALE WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	Number of days during experiment			
	15	**30**	**45**	**60**
Control	4.20 ± 0.10	4.50 ± 0.18	4.80 ± 0.15	4.90 ± 0.12
T_1	4.40 ± 0.20	4.80 ± 0.13	5.10 ± 0.10	5.30 ± 0.19
T_2	4.80 ± 0.12	5.00 ± 0.12	5.30 ± 0.17	5.50 ± 0.09
T_3	4.30 ± 0.14	4.60 ± 0.17	4.80 ± 0.17	5.10 ± 0.10
T_4	4.50 ± 0.16	4.80 ± 0.11	5.00 ± 0.20	5.20 ± 0.13
SEd	0.11974			
CD ($p < 0.05$)	0.24200			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 3

RELATIVE FECUNDITY OF WHITE MOLLY, *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TRETAMENTS

Treatments	**Relative Fecundity**
Control	7.32
T_1	12.10
T_2	75.14
T_3	18.01
T_4	14.50

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 4

FRY SURVIVAL OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	**Number of days during experiment**			
	15	**30**	**45**	**60**
Control	15.33 ± 0.51	18.33 ± 0.22	21.00 ± 0.12	22.00 ± 0.38
T_1	20.33 ± 0.54	24.67 ± 0.36	28.00 ± 0.52	26.00 ± 0.36
T_2	28.00 ± 0.24	35.33 ± 0.32	34.00 ± 0.43	36.67 ± 0.39
T_3	24.33 ± 0.13	28.00 ± 0.25	29.67 ± 0.38	31.00 ± 0.20
T_4	22.67 ± 0.26	26.33 ± 0.23	27.00 ± 0.83	29.00 ± 0.40
SEd	0.4781			
CD ($p < 0.05$)	0.6592			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 5

FRY WEIGHT (gm) OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	Number of days during experiment			
	15	30	45	60
Control	0.83 ± 0.03	0.87 ± 0.06	0.92 ± 0.02	0.94 ± 0.04
T_1	0.90 ± 0.05	0.90 ± 0.08	0.95 ± 0.03	0.99 ± 0.10
T_2	1.30 ± 0.02	1.32 ± 0.06	1.34 ± 0.10	1.35 ± 0.05
T_3	1.15 ± 0.05	1.16 ± 0.06	1.18 ± 0.06	1.20 ± 0.10
T_4	1.05 ± 0.05	1.06 ± 0.06	1.08 ± 0.07	1.08 ± 0.08
SEd	0.05148			
CD ($p < 0.05$)	0.10404			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 6

FRY LENGTH (mm) OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	**Number of days during experiment**			
	15	**30**	**45**	**60**
Control	1.03 ± 0.09	1.03 ± 0.02	1.03 ± 0.02	1.04 ± 0.03
T_1	1.06 ± 0.04	1.07 ± 0.03	1.08 ± 0.07	1.11 ± 0.04
T_2	1.34 ± 0.08	1.37 ± 0.05	1.39 ± 0.07	1.39 ± 0.10
T_3	1.14 ± 0.03	1.16 ± 0.05	1.17 ± 0.08	1.17 ± 0.05
T_4	1.11 ± 0.07	1.13 ± 0.05	1.14 ± 0.03	1.14 ± 0.03
SEd	0.04581			
CD ($p < 0.05$)	0.09259			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)
Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)
Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)
Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)
Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 7

DEAD FRY OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	**Number of days during experiment**			
	15	**30**	**45**	**60**
Control	4.00 ± 0.05	2.13 ± 0.56	3.14 ± 0.38	2.06 ± 0.18
T_1	2.09 ± 0.34	1.15 ± 0.38	2.10 ± 0.35	1.08 ± 0.25
T_2	-	-	-	-
T_3	-	-	-	-
T_4	1.01 ± 0.04	2.00 ± 0.10	1.15 ± 0.39	1.09 ± 0.36
SEd	0.20253			
CD ($p < 0.05$)	0.40933			

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 8

PROTEIN, CARBOHYDRATE AND FAT CONTENT (%) IN THE CONTROL AND FOUR DIFFERENT FEEDS

Treatments	**Protein (%)**	**Carbohydrate (%)**	**Fat (%)**
Control	32.03 ± 0.10	12.03 ± 0.10	14.03 ± 0.11
T_1	32.76 ± 0.84	12.27 ± 0.14	14.41 ± 0.16
T_2	37.06 ± 0.22	13.06 ± 0.13	15.02 ± 0.16
T_3	35.02 ± 0.14	12.75 ± 0.14	14.81 ± 0.05
T_4	34.02 ± 0.25	12.57 ± 0.19	14.66 ± 0.21
SEd	0.3366	0.1168	0.1197
CD ($p < 0.05$)	0.7499	0.2602	0.2667

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 9

WATER CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Water content (%)	
	Before the experiment	**After the experiment**
Control	48.37 ± 0.77	54.08 ± 0.30
T_1	52.08 ± 0.77	59.02 ± 0.68
T_2	57.87 ± 0.57	70.05 ± 0.22
T_3	56.03 ± 0.08	68.01 ± 0.10
T_4	54.09 ± 0.45	63.04 ± 0.45
SEd	0.0643	
CD ($p < 0.05$)	0.1327	

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 10

PROTEIN CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Protein content (%)	
	Before the experiment	After the experiment
Control	26.37 ± 0.20	29.73 ± 0.40
T_1	26.90 ± 0.10	31.80 ± 0.50
T_2	28.88 ± 0.40	36.47 ± 0.20
T_3	27.90 ± 0.20	34.10 ± 0.10
T_4	27.10 ± 0.50	32.40 ± 0.40
SEd	0.13501	
CD ($p < 0.05$)	0.27287	

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 11

CARBOHYDRATE CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Carbohydrate content (%)	
	Before the experiment	After the experiment
Control	1.18 ± 0.10	1.40 ± 0.30
T_1	1.20 ± 0.10	1.46 ± 0.20
T_2	1.30 ± 0.20	2.03 ± 0.76
T_3	1.27 ± 0.10	1.62 ± 0.20
T_4	1.23 ± 0.20	1.56 ± 0.30
SEd	0.13501	
CD ($p < 0.05$)	0.27287	

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

TABLE 12

FAT CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Fat content (%)	
	Before the experiment	After the experiment
Control	2.20 ± 0.20	2.43 ± 0.30
T_1	2.27 ± 0.10	2.51 ± 0.40
T_2	2.41 ± 0.30	2.86 ± 0.50
T_3	2.37 ± 0.20	2.72 ± 0.20
T_4	2.30 ± 0.20	2.60 ± 0.40
SEd	0.13501	
CD ($p < 0.05$)	0.27287	

Values are mean ± SD of three samples in each column.

Rice bran and oil cake 100 gm (Control)

Rice bran and oil cake 99.5 gm and Probiotic, *Bifilac* 0.5 gm (T_1)

Rice bran and oil cake 99 gm and Probiotic, *Bifilac* 1.0 gm (T_2)

Rice bran and oil cake 98.5 gm and Probiotic, *Bifilac* 1.5 gm (T_3)

Rice bran and oil cake 98 gm and Probiotic, *Bifilac* 2.0 gm (T_4)

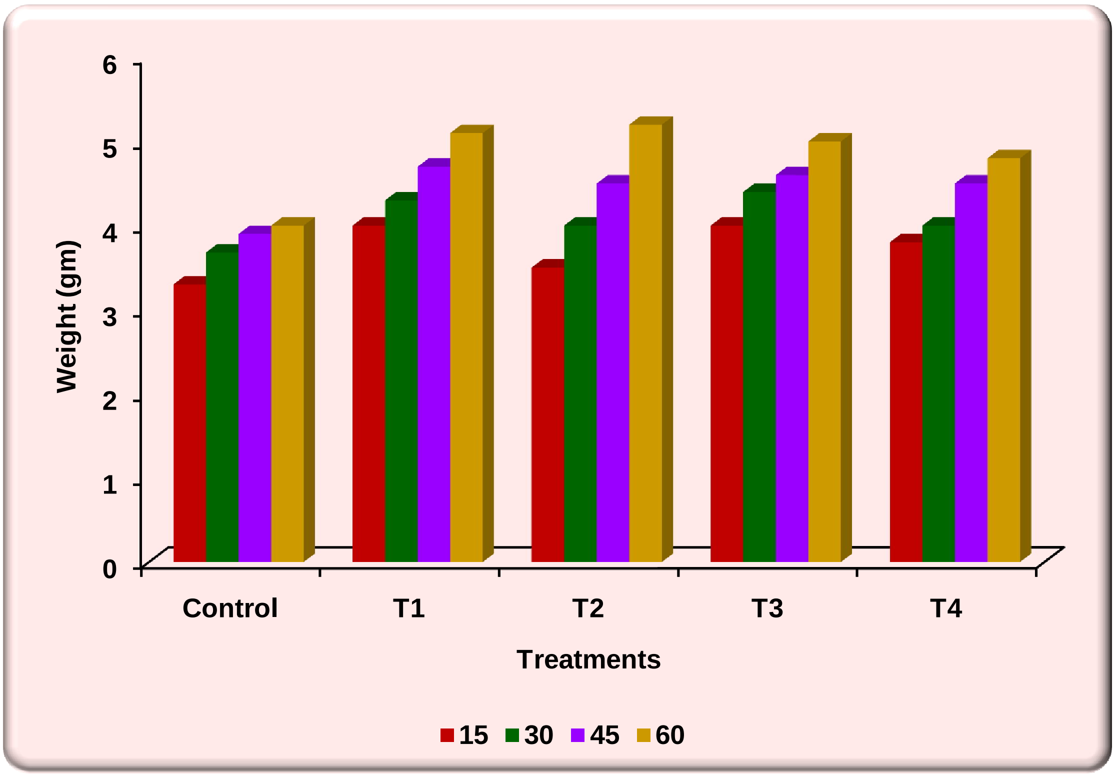

FIGURE 5

WEIGHT (gm) OF SPAWNING FEMALE WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

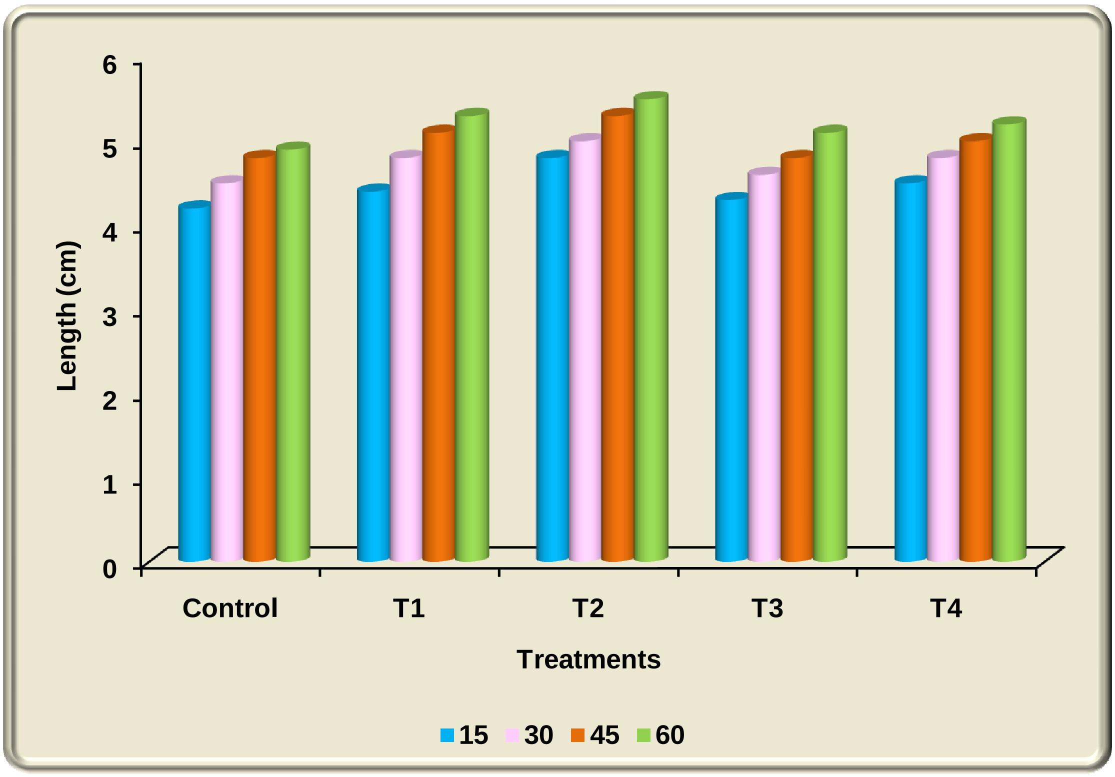

FIGURE 6

LENGTH (cm) OF SPAWNING FEMALE WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

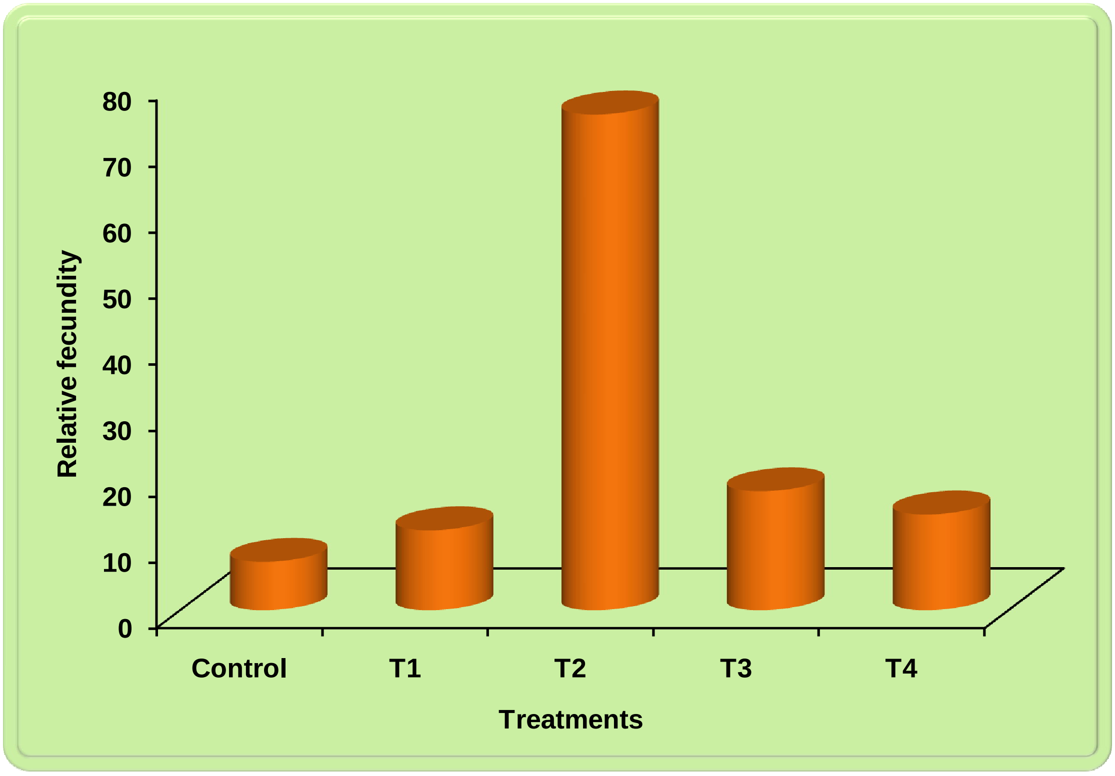

FIGURE 7

RELATIVE FECUNDITY OF WHITE MOLLY, *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

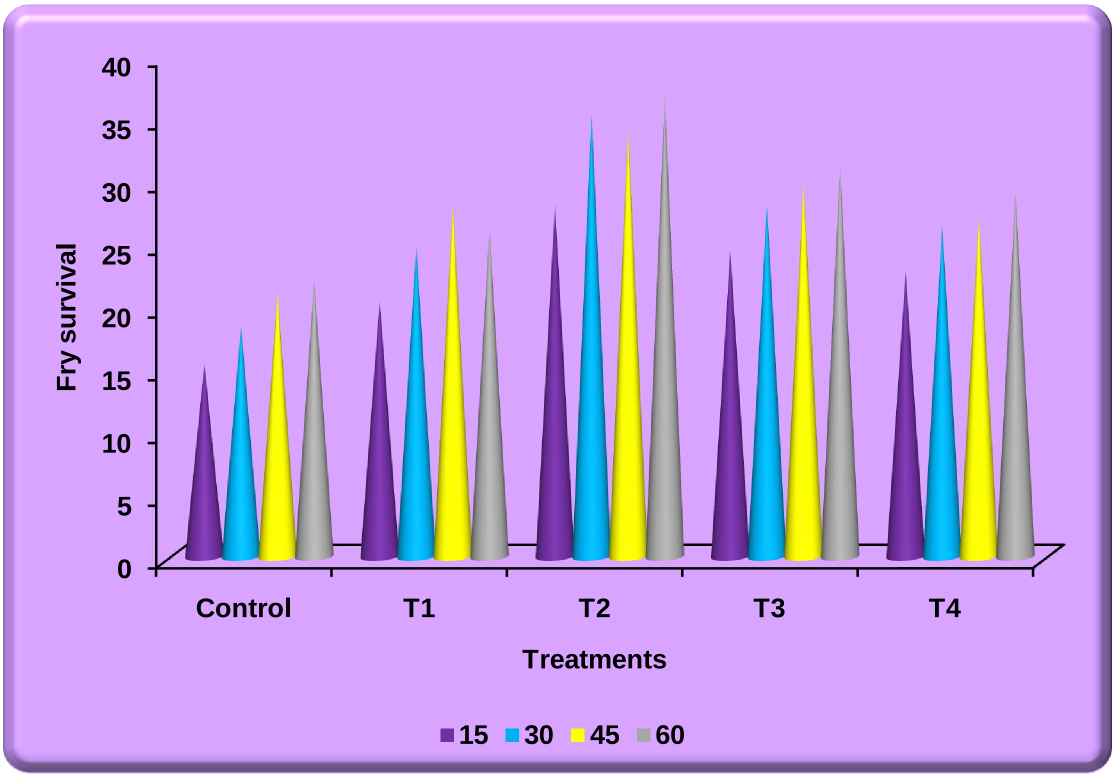

FIGURE 8

FRY SURVIVAL OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

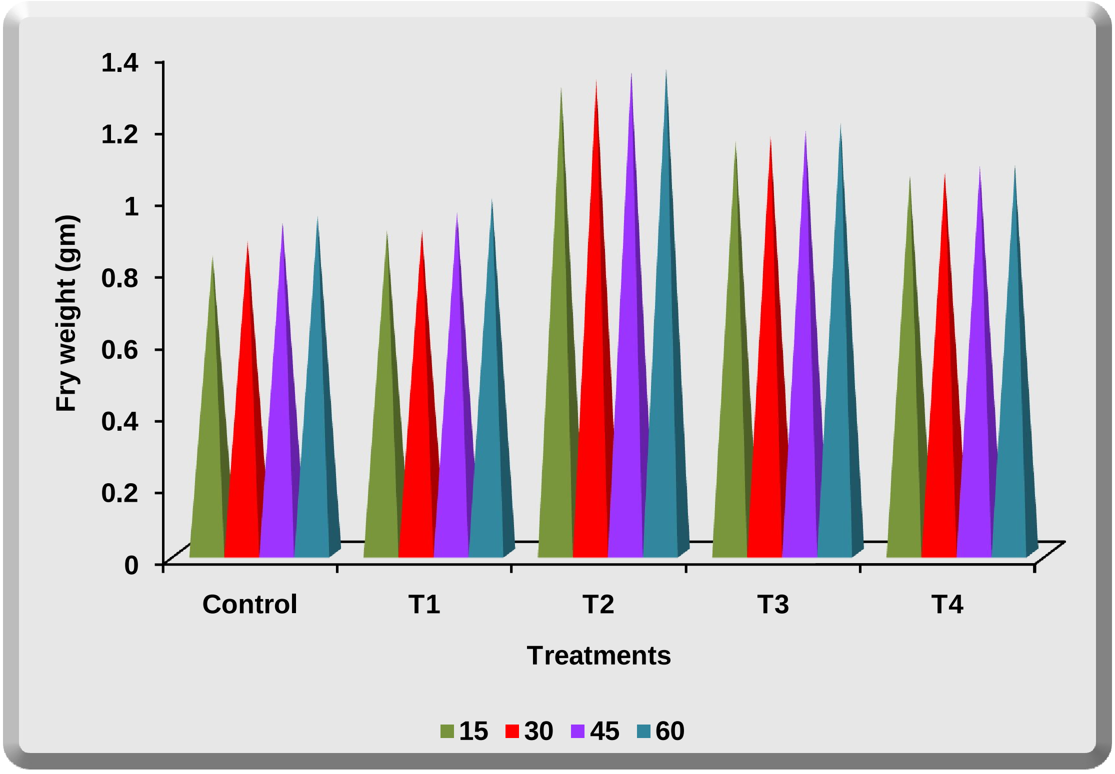

FIGURE 9

FRY WEIGHT (gm) OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

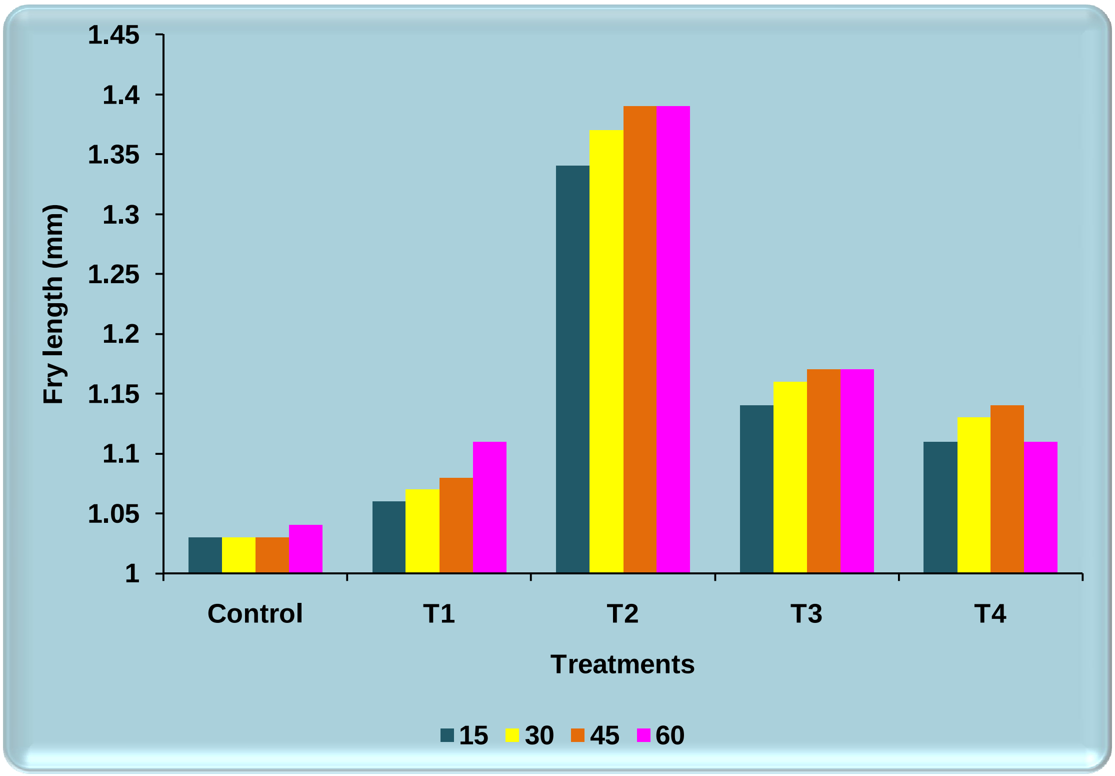

FIGURE 10

FRY LENGTH (mm) OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

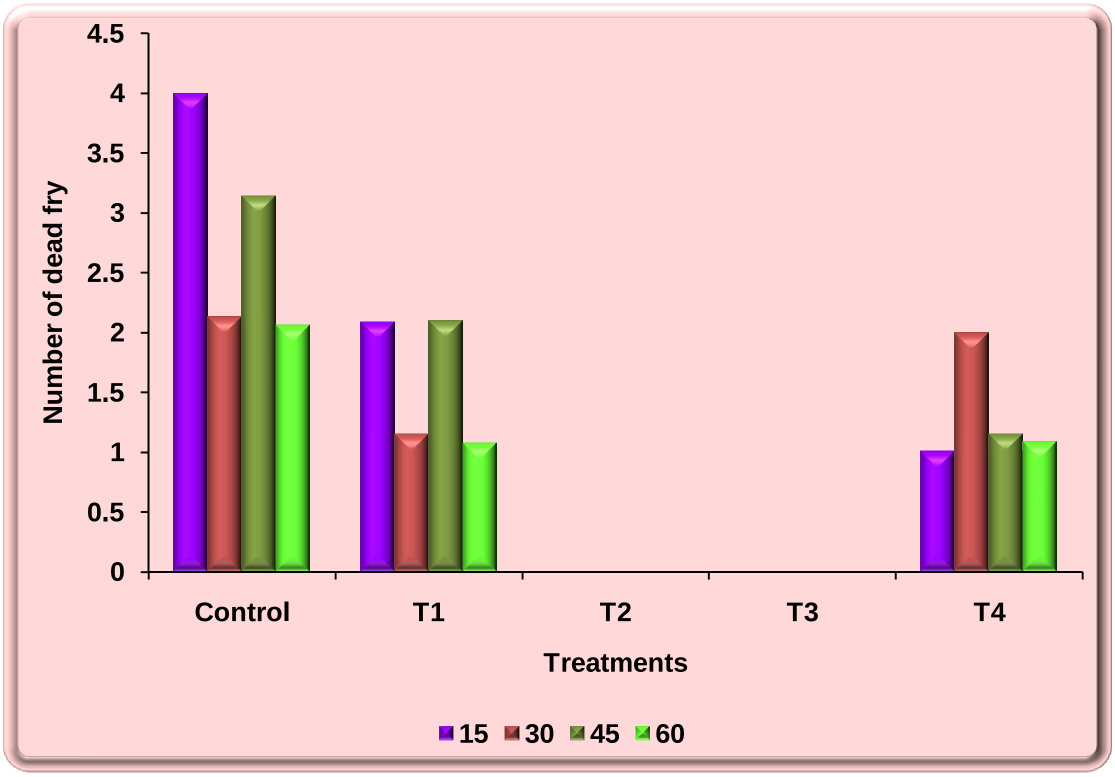

FIGURE 11

DEAD FRY OF WHITE MOLLY, *P. sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

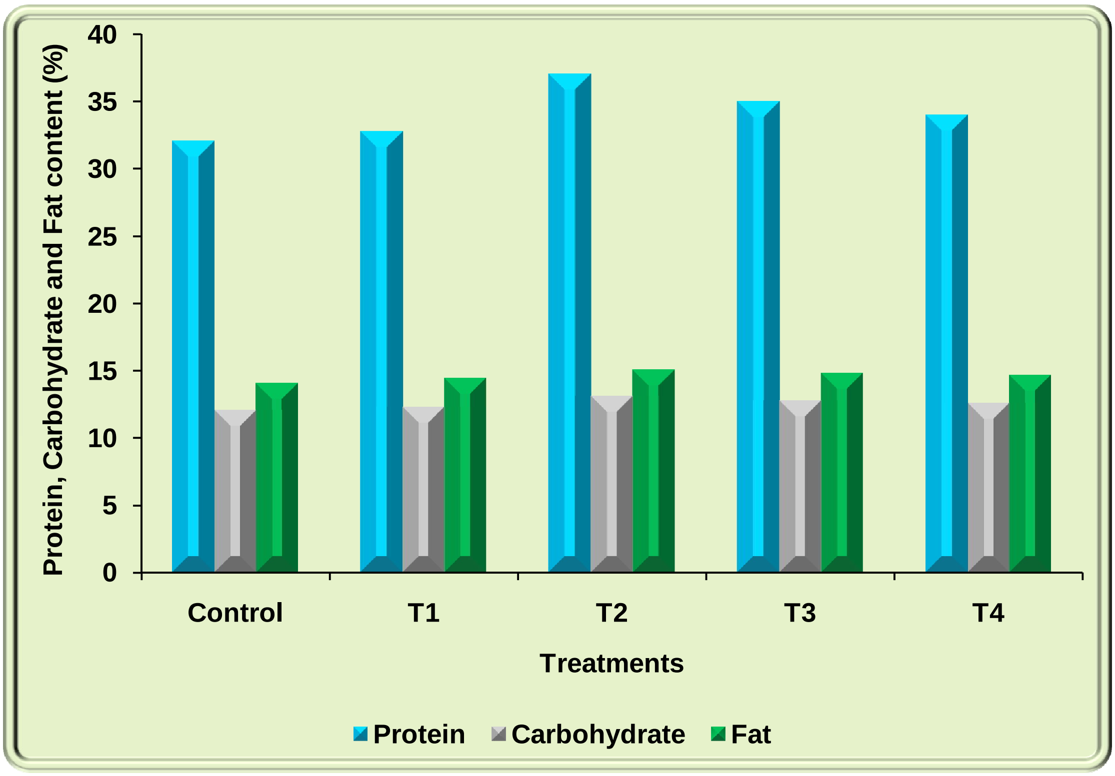

FIGURE 12

PROTEIN, CARBOHYDRATE AND FAT CONTENT (%) IN THE CONTROL AND FOUR DIFFERENT FEEDS

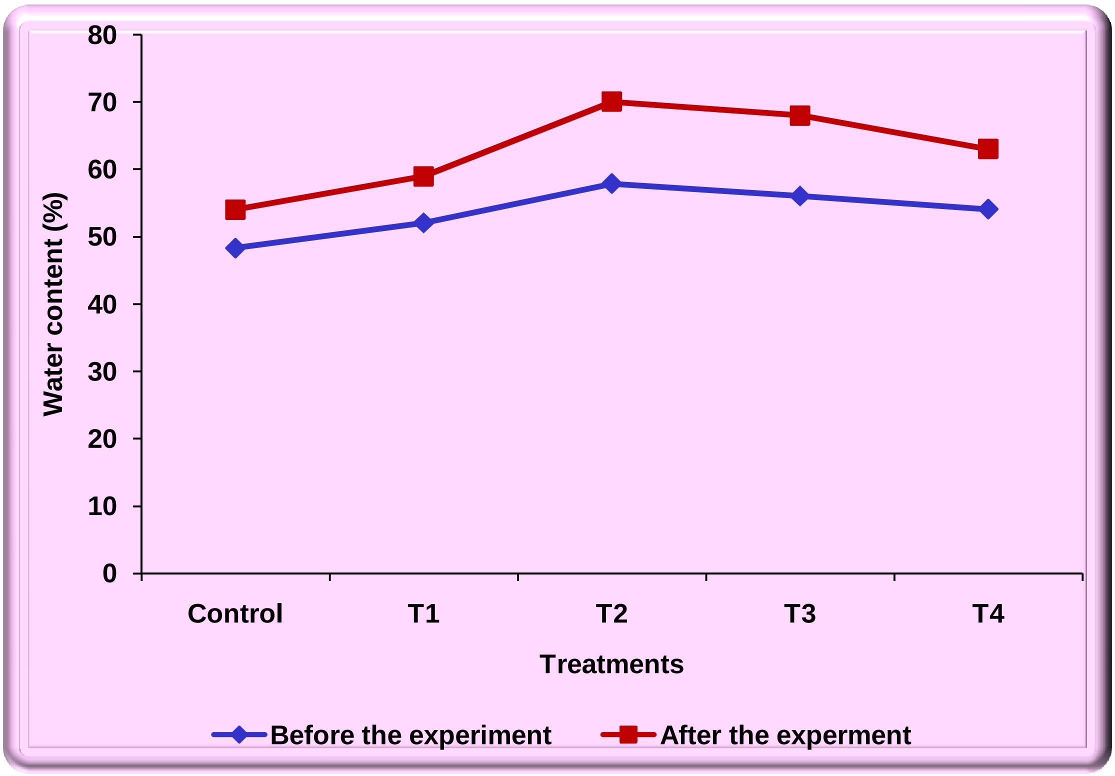

FIGURE 13

WATER CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

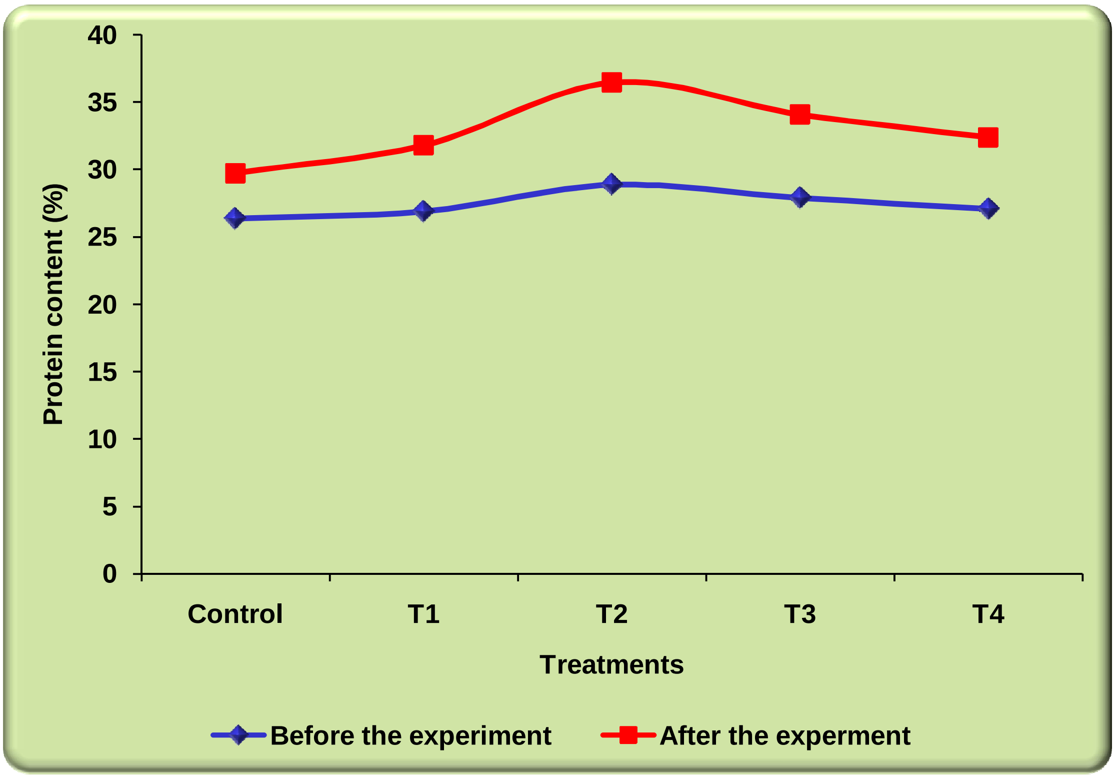

FIGURE 14

PROTEIN CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

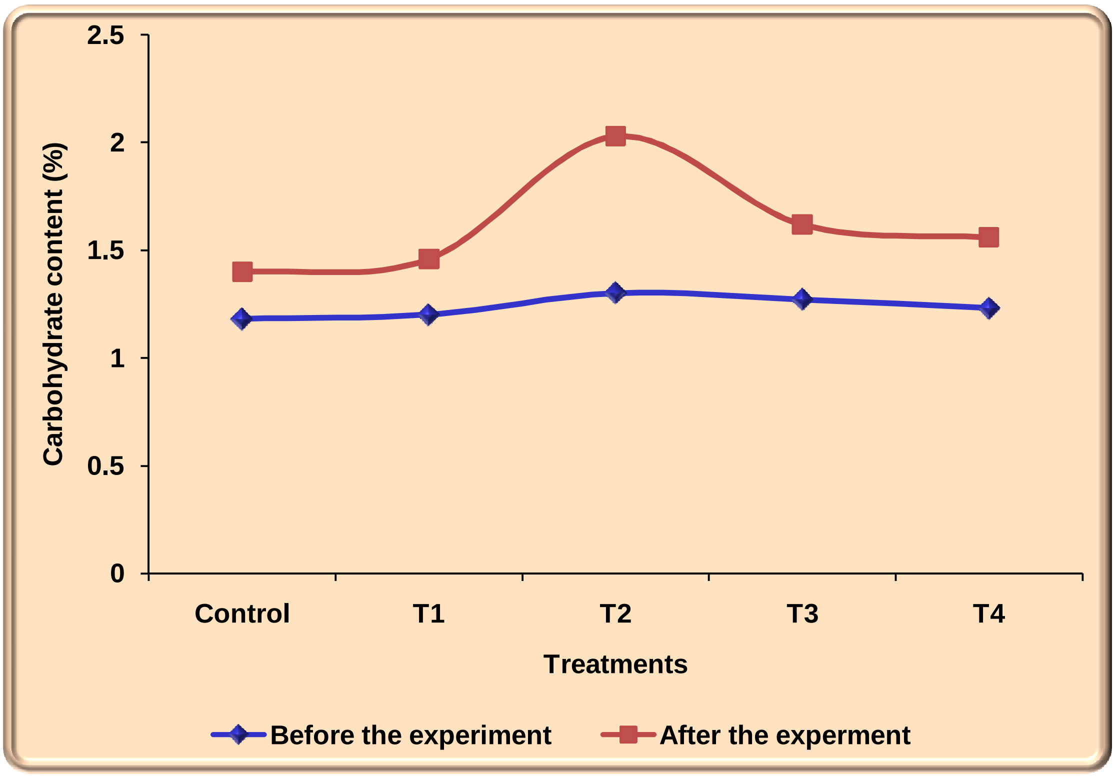

FIGURE 15

CARBOHYDRATE CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

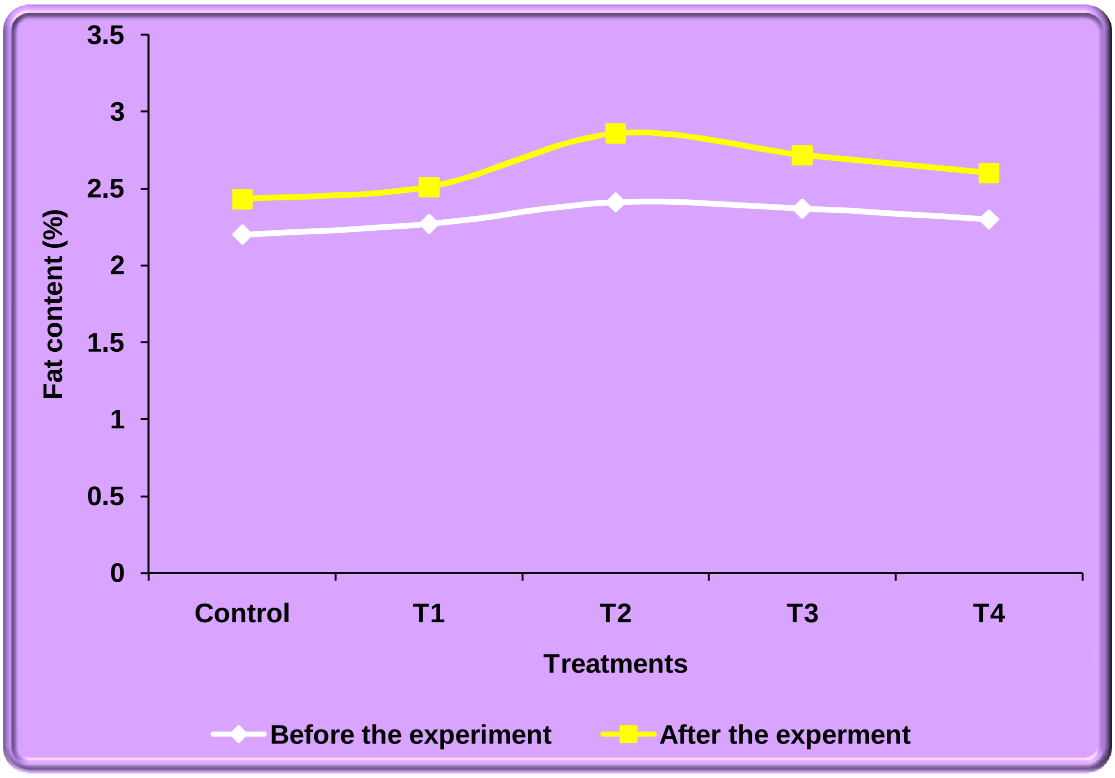

FIGURE 16

FAT CONTENT (%) OF *P. sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

5. DISCUSSION

Live bearing ornamental fish, white molly *P. sphenops* grown in four different treatments showed more weight and length gain than the fishes grown in the control. During 60 days of the experiment the maximum weight (5.20 gm) and length (5.50 cm) were observed in T_2 fishes fed with rice bran and oil cake 99 gm and probiotic (*Bifilac*) 1 gm. The weight and length were minimum in control fishes where the feed contains only rice bran and oil cake in the ratio of 1 : 1.

Taoka' *et al*. (2006) observed that probiotics stimulated the food digestibility and non specific immune response by improving its growth and reproductive performance of ornamental fish. Tukmechi and Bandboni (2013) conducted growth studies to determine the suitability of probiotic sources in the diet of mollies *P. sphenops*. At the conclusion of the experiment highest weight was attained in *P. sphenops* fed with diets supplemented with probiotics and lowest weight was seen in the control fishes fed with only basal diet, which supports the present findings where the maximum weight and growth rate was seen in T_3 fishes fed with higher level of supplemented feed containing probotics.

In the present study *P. sphenops* fed with one gram of probiotics used in T_2 treatment showed maximum growth rate (weight and length) than the other treatments which contains 0.5 gm, 1.5 gm and 2.0 gm of probiotics. The fishes in all the treatments showed more growth rate (weight and length) than the control fishes where control feed contains only rice bran and oil cake. The fish fed on the probiotic feed supplement showed higher growth rate which is in positive correlation with dietary protein intake (Kumar, 2006).

Abasali and Mohamed (2011) reported the highest value of fry survival in platy, *Xiphophorous maculalus*. Tamaru *et al*. (2001) observed the gradual increase in fry production with each spawning may be attributed to the positive correlation between fish body weight and fry production. In the present study *P sphenops* also showed highest fry survival in the probiotic supplemented fishes and maximum values were recorded in T_2 (36.67) of probiotic incorporation during the experimental period.

Fernandez *et al*. (2006) reported reproduction is gated by the state of body energy reserve and is sensitive to different metabolic cues, the neuroendocrine mechanisms responsible for the tight coupling between energy homeostasis and fertility are represented by metabolic hormones and neuropeptides that integrate the hypothalamic center governing reproduction, controlling the expression and release of growth hormone releasing hormone (GHRH).

Goldin and Gorbach (1992) observed the probiotic bacteria produce B group vitamins and the production and supply of vitamin B complex and certain unknown stimulants could have played a key role in the elevated reproductive performance of the probiotic feed fed fish. In the present study, total fry production was higher in probiotic supplemented groups than the control.

Dietary probiotic supplementation had beneficial effects on relative fecundity of white molly, *P. sphenops.* Efficiency of probiotic incorporation on reproductive performance of *P. sphenops* resulted better than control.

The relative fecundity among the four different treatments and the control showed highest level (75.11) in the T_2 where the feed was incorporated with maximum level of probiotics. The decreased fecundity (7.32) was noted in the control where the feed contains only rice bran and oil cake without protective inclusion

The average number of dead fry were found to vary significantly in the *P. sphenops* with the higher values being recorded in control feed fed fish and the lower values in probiotics feed fed fishes. The average weight and length of released fry were recorded in the fry hatched in the control and four different treatments. Maximum weight and length was observed in T_2 (1.39 mm and 1.35 gm) fish feed. The synthesis of B complex, vitamins, particularly thiamine (vitamin B_1) and vitamin B_{12} by the probiont *Bacillus subtilis* could have accounted for the reduced numbers of dead and deformed fry in the probiotic diet fed fish was studied by Ghosh *et al*. (2007). This may be the reason for the reduction of dead fries in the probiotic experimental group (0.5 gm to 2.0 gm) than the control fish.

Supplementation of feed with probiotics significantly increased the fecundity, fry production of spawning females and length and weight of fry in all the treated groups. The number of dead fry were significantly lower in fish fed with the probiotic feeds. The use of higher concentration of the probiont in diet did not always lead to significantly improved reproductive performance of the spawners.

The beneficial influence of *Bifilac* probiotic on reproductive performance was possibly due to alternation of the fish intestinal microflora and improving the beneficial mixed bacteria culture by probiotic ingredients, particularly *Streptococcus facalis, Clostridium butyricum, Bacillus mesentericcy* and lactic acid bacillus. Higher survival rate and lower dead fry could be linked to the intestine probiotic bacterial population which produces B complex vitamins. It is important to define the probiotic levels administrated to fish to avoid over dosing and under dosing with resultant study considered different probiont levels and concluded that a probiotic concentration of 1 gm was sufficient for enhanced reproductive performance and that the use of higher concentration of probiotic cells did not always yield significantly better results.

These results coincide with the present investigation which proves the *P. sphenops* fed with feed prepared from probiotics showed highest weight, length, relative fecundity, fry survival, fry weight, fry length and lowest dead fry.

The water content was maximum in *P. sphenops* grown in T_2 (70.05 per cent) followed by T_3 (68.01 per cent), T_4 (63.04 per cent) and T_1 (59.02 per cent) respectively. The minimum water content was recorded in control fishes (54.08 per cent). Kumar *et al*. (1995) studied water content, protein, lipid and carbohydrate in relation to body weight in air breathing fish.

The nutrient level increases in relation to body weight which agree with the increased level of nutrients in *P. sphenops* grown in T_2.

Protein content was found to be maximum in T_2 (37.06 per cent) feed prepared from 99 gm rice bran, oil cake and 1 gm of probiotic (*Bifilac*) followed by T_3 feed (prepared from 98.5 gm rice bran, oil cake and 1.5 gm probiotic (*Bifilac*) were the protein content was estimated as (35.02 per cent).. After 60 days of the experiment the protein content in the *P. sphenops* was found to be maximum in T_2 (36.47 per cent) and minimum in control (29.73 per cent). The level of protein in the *P. sphenops* proportionately increased with the increase of protein in the feed. Supplementation of fish diets with probiotics or species optimized protein use for the growth which can decrease the amount of feed necessary for fish growth, which could result in reducing production. Ringo and Gatesoupe (1998) studied a similar improvement in the biological value of the diets supplemented with probiotics. The results of the chemical analysis for protein, carbohydrate and fat of the fish fed with different feeds are compared with protein, carbohydrate and fat present in different feeds. It is observed that probiotic bacteria (*L. sporgeny*) along with basal diet showed higher protein content than the control where the basal diet alone was used for the feed preparation.

The highest level of carbohydrate content was analysed in T_2 feed (13.06 per cent) and the lowest level was observed in the control feed (12.03 per cent). After the experiment the maximum carbohydrate content was estimated in the *P. sphenops* grown in T_2 (2.03 per cent) and minimum carbohydrate content was observed in control (1.40 per cent) fishes. Skalli *et al*. (2004) observed a positive correlation between the carbohydrate content of the feed and the carbohydrate content in the fish.

The maximum value of fat content was observed in T_2 feed (15.02 per cent) and the minimum value was analysed in the control feed (14.03 per cent). After the experiment the highest level of fat content was estimated in the *P. sphenops* grown in T_2 (2.86 per cent) and lowest level of fat content was observed in control (2.43 per cent) fishes.

The biochemical constituents such as water, carbohydrate, protein and fat value of *P. sphenops* fed with 99 gm rice bran and oil cake and 1 gm of probiotic (*Bifilac*) showed increased value after the experimental period that the fishes fed with basal diets. Among the different treatments the biochemical constituents was high in the treated fishes than the control fishes. The findings of Limin *et al*. (2006) supports the present findings. They inferred that probiotics could enhance the growth and biochemical composition of fishes.

In the present investigation, the fishes fed with 99 gm rice bran, oil cake and 1 gm probiotic (*Bifilac*) showed higher weight, length, fry survival, fry weight, length and biochemical composition such as water content, protein, carbohydrate, fat value than the fishes grown in control. Collectively, this study showed that female live bearers benefit from inclusion of probiotics in diet during their reproductive stages. In aquaculture practices probiotic can be effectively used in the preparation of fish meal which will enhance the growth and reproduction of ornamental fishes.

SUMMARY AND CONCLUSION

The present investigation was carried out on **"Dietary Probiotic (*Bifilac*) Supplementation in the Reproductive Performance of White Molly (*P. sphenops*)"** and the results are summarized :

Growth rate of female live bearer *P. sphenops*, survival rate, growth rate of fry, dead fry were analysed during 15, 30, 45 and 60 days of the experiment. The proximate composition such as protein carbohydrate and fat were analysed in the control and four different feeds. The biochemical components like water content, protein, carbohydrate and fat were analysed in the *P. sphenops* grown in the control and four different treatments before and after the experiment. The salient findings of the present study includes :

1. The weight (gm) was maximum in T_2 (5.20 gm) fishes fed with 99 gm rice bran, oil cake and 1 gm Probiotic (*Bifilac*) during 15, 30, 45 and 60 days of the experiment.

2. The length (cm) was highest (5.50 cm) in T_2 fishes followed by T_3 (5.10 cm) fishes fed with 98.5 gm rice bran, oil cake and 1.5 gm Probiotic (*Bifilac*). The minimum length (4.90 cm) was recorded in the control.

3. Relative fecundity was maximum in T_2 fishes (75.14) followed by T_3 (18.01), T_4 (14.50) and T_1 (12.10). The minimum relative fecundity was observed in the control fishes (7.32).

4. The maximum fry survival was observed in T_2 fishes (36.67) followed by T_3 (31.00), T_4 (29.00) and T_1 (26.00). The maximum fry survival was seen in the control fishes.

5. The fry weight was highest in T_2 fishes (1.35 gm) and lowest in control fishes (1.35 gm) fed with 50 gm of rice bran and 50 gm of oil cake.

6. The maximum fry length of 1.39 mm was recorded in T_2 fishes fed with 99 gm of rice bran, oil cake and 1 gm of Probiotic (*Bifilac*) followed by T_3 (1.17 mm), T_4 (1.14 mm) and T_1 (1.11 mm). Control fishes showed minimum fry length of 1.04 mm.

7. The highest dead fry was observed in the control (2.06) followed by T_4 (1.09) and T_1 (1.08).

8. Among the different treatments analysed maximum weight, length, fry survival, fry weight and length was reported in fishes grown in T_2 treatment.

9. The protein content in T_2 feed (37.06 per cent) was higher than other feeds. Control feed has minimum protein content (32.03 per cent). Carbohydrate content was maximum in T_2 feed (13.06 per cent) followed by T_3 (12.75 per cent), T_4 (12.57 per cent) and T_1 (12.27 per cent) and lowest level of carbohydrate (12.03 per cent) was recorded in control feed prepared from 50 gm rice bran and 50 gm oil cake. Fat content was highest in T_2 feed (15.02 per cent) and lowest level (14.03 per cent) was observed in control feed.

10. The water content, protein, carbohydrate and fat were increased after 60 days in the fishes grown in the control and four different treatments.

11. The water content was maximum (70.05 per cent) in fishes grown in T_2 followed by T_3 (68.01 per cent), T_4 (63.04 per cent) and T_1 (59.02 per cent). The minimum value (54.08 per cent) was recorded in control fishes fed with 50 gm rice bran and 50 gm oil cake.

12. Fishes grown in T_2 (99 gm rice bran, oil cake and 1 gm Probiotic (*Bifilac*) showed maximum protein content (37.06 per cent) followed by T_3, T_4 and T_1 fishes. Protein content was minimum in fishes fed with 50 gm rice bran and 50 gm oil cake in 1 : 1 ratio (control).

13. The carbohydrate content was highest in T_2 (2.03 per cent) fed fishes followed by fishes grown in T_3 (1.62 per cent), T_4 (1.56 per cent), T_1(1.46 per cent) and control (1.40per cent).

14. Maximum fat content was recorded in T_2 (15.02 per cent) fishes followed by T_3, T_4 and T_1. Fat content was minimum in the fishes grown in control when compared to fishes in other treatments.

15. The results were subjected to statistical analysis to recommend the best treatment. The significant results were found in T_2 treatments.

16. Among the control and four different feeds the T_2 feed showed significant value of protein, carbohydrate and fat.

17. Biochemical composition showed significant values in T_2 fishes fed with 99 gm rice bran, oil cake and 1 gm Probiotic (*Bifilac*).

The present investigation revealed that the commercial Probiotic (*Bifilac*) used in this study can improve the reproductive performance of female White molly fish brood stocks during reproductive stages. There is an ever increasing commercial interest in the ornamental fish trade in India and all over the world. The application for Probiotics in aquaculture will be beneficial especially for improving reproductive efficacy of the ornamental fish.

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