Efficacy of fruit waste formulated feed on growth and reproduction of the ornamental fish, *Poecilia sphenops*

KARTHIKA, K

(12PZO006)

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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Signature of the Supervisor

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INTRODUCTION

1. INTRODUCTION

Aquaculture has been one of the fastest growing food production sectors over the past two decades in many countries of Asia. In the recent five years Asia has contributed 90.8% to the world's total aquaculture production (Pandey, 2004). Fisheries help in raising the standards of national economy by bringing invaluable foreign exchange and improving the balance of payment position of the century (Kumar, 2003).

Ornamental fish culture plays a vital role in the trade and economy of a country. Ornamental fishes usually mean attractive colorful fishes of various characteristics which are kept as pets in confined space of an aquarium and are usually kept in glass aquarium and hence popularly known as "aquarium fishes" and are mainly grouped into two categories, viz. oviparous (egg - layers) and viviparous (live-beares). Generally ornamental fish culture requires more concern and care in terms of maintenance and feeding, however an increasingly important option is the animal protein production.

This activity requires high quality feeds with high protein content, which should contain not only the necessary nutrients but also complementary additives to keep organisms healthy and promote favourable growth. The feed of fish and their nutrient value is one of the most important factors in production cost and health of fish. Diets for aquatic animals can only be effective if they are formulated to contain the full array of necessary nutrients at appropriate concentrations relative to each other along with appropriate factors inducing rapid consumption on a consistent basis Conklin and Piedrahita (2003).

Aquarium fish feed is plant or animal material intended for consumption by pet fish kept in aquariums or ponds. Fish foods normally contain macro nutrients, trace elements and vitamins necessary to keep captive fish in good health Rüdiger and Baensch (1996).In ornamental fish, a correct formulation of the diet improves the nutrient digestibility, supply the metabolic needs and reducing the maintenance cost and at the same time the water pollution Yohana and Wilson (2011). In natural conditions, fish can regulate and maintain their food intake and therefore their nutritional requirements, reducing the possibility of suffering nutritional deficiencies.

In aquaculture the artificial feed sector has made tremendous development in production of sustainable and nutritious feeds. The quality of supplementary feeds forms determining criteria in the yield and profit. The cost of feed is the single largest and most important operating expense. It is feasible to use cheap and easily available ingredients, which are palatable and have high growth efficiency. In addition to cheaply available animal (fish meal, poultry meal and blood meal, etc.) and plant byproducts (cereals, pulses, soybean meal, groundnut oilcake, rice brawn, sunflower oil, coconut oil and linseed oil, etc.), vegetable waste, fruits waste, greens, and herbals are also used as supplementary feed ingredients for better survival and growth (Bhavan, 2013).

The medicinal plants have phytochemicals, the bioactive substances which are responsible for the biological activities they exhibit, for example the protection of health against chronic degenerative diseases (Fukumoto and Mazza, 2000). The herbal biomedicine active principles in the aquaculture have the characteristics of growth promoting ability, tonic to improve the immune system, anti-microbial capability, anti-stress characteristics and stimulating appetite due to presence of alkaloids, flavonoids,

pigments, phenolics, terpenoids, starch, steroids and essential oils. Furthermore, the uses of herbal medicines will reduce the applications of synthetic antibiotics, hormones etc. (Citarasu, 2010).

Despite the economic importance of this sector, the nutritional information for ornamental fish is scarce and often few or even no data of the nutritional requirements is available (Chong *et al*., 2003) Ornamental fish have basically the same nutritional requirements as other farmed fish. Prepared feed of complete artificial diets supply all the ingredients of protein, fats, carbohydrates, vitamins, minerals and trace element necessary for the optimal growth, reproduction and health of the fish. Most fish use complete diets, those containing all the required protein (15-50%), lipid (10-25%), carbohydrate (10-25%), ash (5-10%) and in addition about 5 percent other material such as trace element, vitamins, minerals, supplementary and complement (probiotic, prebiotic and enzymes) (Craig and Helfrich, 2009). Feed acceptability, palatability and digestibility vary with the ingredients and feed quality. The efficiency of nutrient use by ornamental fish can contribute to the formulation of appropriate diets, as well as helping to decrease the elimination of nitrogen and phosphorus in excreta, thereby favouring the maintenance of the water quality and reducing environmental pollution caused by effluence (Zuanon and Salaro, 2011).

Formulated feeds play an important role as major input in aquaculture demand. Even though information regarding the nutritional requirement of commercial fishes is available, the development of economic feed formulation for optimal growth of this species is still needed for cost-effective intense farming. Generally, aquaculture farmers used commercially available feeds prepared from animal and plant sources and their byproducts. However, such feeds are high cost and non-affordable to small farmers.

For sustainable aquaculture, locally available foodstuffs suited for small- scale farmers are needed. The cereal grains, pulses, vegetable wastes are being used in aquaculture feed for both functional and nutritional rules (Bhavan *et al*., 2011). Therefore several studies had been carried out using some fruits wastes such as mango seed kernel, banana peel and papaya peel were incorporated as feed ingredients/ supplements, which are available free of cost. Many by-products including fruits wastes contain polyphenols with potential application as food antioxidants and polyunsaturated fatty acids (Baydar *et al*., 2006). Further fruit wastes could serve as appetizers thereby increasing the secretion of digestive enzymes, which facilitates better digestion and absorption of nutrients, which in turn ultimately promotes the growth. Therefore, these fruits wastes can be taken as ingredients in on-farm feed management for promoting sustainable aquaculture of freshwater prawns.

Orange peel is a good source of dietary fiber, anti-oxidant and phenolic compounds, which could be useful in the formulation of functional foods. The application of using citrus fruits in formulating a fish feed, would be highly cost-effective (Spier *et al*., 2011). Rincon *et al*. (2005) assessed the dried fruit of orange (*Citrus sinensis*) chemical composition some trace elements, such as ascorbic acid, carotenoids dietary fiber, total polyphenols and their antiradical efficiency, using the 2, 2 - diphenyl - l - picrylhidracyl (DPPH).

Among live bearers, the black mollies (*Poecilia sphenops*) are a very popular group of ornamental fish species due to existence of the variety of body colours and 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). Fish selected for the present study is blackmolly, *Poecilia sphenops*.

Taxonomical Hierarchy

Kingdom	:	Animalia
Phylum	:	Chordata
Subphylum	:	Vertebrata
Class	:	Pisces
Order	:	Cyprinodontiformes
Family	:	Poeciliidae
Genus	:	*Poecilia*
Species	:	*sphenops*
Common name	:	Black molly

Live bearing species of the family poeciliidae such as guppies (*Poecilia reticulata*, platies (*Xiphophorus maculatus*), swordtails (*Xiphophorus helleri*) and molly (*Poecilia latipinna, Poecilia sphenops*) are popular ornamental or aquarium fishes produced in many Asian countries (Chong *et al.,* 2004). *Poecilia sphenops* is a common live bearing ornamental fish and breed easily. The live bearing poeciliidae breed easily in captivity (Ling *et al.,* 2006) and in view of this, farmers do not pay much attention to provide the fish with nutritionally balanced feed. Owing to the use of unbalanced feed in fish farming causes problems relating to small brood size, deformed fry and low survival have been reported by farmers. Ultimately poor broodstock nutrition will lead to the production of inferior quality fry with low survival rate. Therefore in the present study was carried out to evaluate the possibility of using cheaper source of supplementary feed, especially fruit waste (orange peel) as a feed additive on growth, biochemical composition, relative

fecundity, fry survival and fry weight of the ornamental fish, black molly, *Poecilia sphenops.*

The objectives of the present study are:

1. To formulate the different types of fish feeds using orange peel in different concentration.
2. To analyse the composition such as protein, carbohydrate and fat content in the control and four different feeds and compared.
3. To compare the growth rate(Length and Weight) of *Poecilia sphenops* 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 black molly, *Poecilia sphenops* grown in the control and four different feeds.
5. To find out the fry survival of black molly, *Poecilia sphenops* during different days of the experiment in the control and four different treatments.
6. To study the weight fry of black molly, *Poecilia sphenops* during different days *of the experiment in the control and four different treatments.*
7. To compare the biochemical composition such as water content, protein, carbohydrate, fat in the muscle tissue of *Poecilia sphenops* before and after the experiment growth rate of *Poecilia sphenops* relative fecundity, fry survival and weight during different days of the experiment in the control and four different treatments and results were subjected to statistical analysis to recommend the best treatment.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The available literature pertaining to the present study entitled “Efficacy of fruit waste formulated feed on growth and reproduction of the ornamental fish, *Poecilia sphenops*” are reviewed and presented below.

Arumugam *et al.* (2013) conducted the experiment to evaluate the growth of *Macrobrachium rosenbergii* fed with mango seed kernel, banana peel and papaya peel incorporated feeds. Basal diet equated to 35% protein was prepared by using soybean meal, groundnut oilcake, horse gram and wheat flour. Each fruit waste powder was separately incorporated with basal diet at a proportion of 10%. Sunflower oil was used as lipid source. Egg albumin and tapioca flour were used as binding agents. Vitamin B-complex with Vitamin-C was also mixed. Feed without any fruit waste was served as control. The results indicated the fact that mango seed kernel incorporated feed was produced the best performance, followed by better performance of banana peel and good performance of papaya peel. These fruits wastes incorporated feeds enhance digestive enzymes activities and act as appetizer, which in turn enhances food utilization and ultimately yielded better survival and growth of *M. rosenbergii* PL. Therefore, these fruits wastes have considerable potentials in sustainable development of Macrobrachium culture.

Ahmad *et al.* (2011) conducted to evaluate the use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization, and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Five iso nitrogenous (30.3% crude protein) and iso-caloric (4.5 kcal/g diet) diets were formulated to contain0.0 (control), 5, 10, 15, or 20 g CSM/kg diet. Fish (3.6±0.3 g) were distributed at a rate of 20 fish per 100-L aquarium and three aquaria have been assigned for each treatment. Fish were fed one of the

tested diets at a rate of 4% of live body weight twice daily; six days a week for 12 weeks. The CSM supplementation enhanced fish growth over the control diet; the highest fish growth and feed utilization were obtained when fish fed on a diet containing 10 g CSM/kg diet. There were no significant changes in fish survival among the different treatments and its range was 98.5–100% suggesting that CSM had no toxic effect. Moreover, CSM has no impact on moisture and protein contents in whole fish. Total lipid increased significantly and total ash contents decreased significantly with increasing CSM levels. A dietary CSM level of 12.5 g/kg provided the best fish performance based on second-order polynomial regression analysis of growth parameters against dietary CSM levels.

Aly *et al*., (2008) carried out an experiment to determine the effects of orange peel on survival, growth, resistance and quality of *Oreochromis niloticus*. It was proved that the doses rates of orange induced a similar effect enhancing immunity and health status, consequently orange peel improved the growth performances.

Arathi *et al*. (2014) investigated the effect of dietary protein on the growth and reproductive performance of the indigenous ornamental fish, *Puntius vittatus(*DAY) and evaluated the impact of varying levels of dietary protein on the growth and reproductive performance and also to develop a protein balanced diet for the ornamental breeding system.

Arulvasu *et al.* (2013) investigated the effect of natural sources of carotenoid pigments from Rosa rubiginosa petals on growth, survival and coloration of ornamental swordtail Xiphophorus helleri fish fry. The results of this experiment concluded that the natural sources of carotenoid pigments extract from R. rubiginosa petals have a positive role in the growth, survival and colour development of fish fry.

Bhavan, *et al*., (2013) studied the effects of fruits wastes (apple, grape and orange) incorporations on the growth of the freshwater prawn *Macrobrachium rosenbergii*. Basal diet equated to 40% protein was prepared by using soybean meal (36%), groundnut oilcake (30%), cow gram (11%), wheat bran (5%) and maize bran (4%). Each fruit waste powder was separately incorporated with basal diet at three proportions viz., 5%, 10% and 15% by replacing right quantity of basal diet. Sunflower oil (3 ml) was used as lipid source. Egg albumin (5 ml) and tapioca flour (5%) were used as binding agents. Vitamin B-complex with Vitamin-C (1%) was also mixed. Feed without any fruit waste was served as control. Therefore, concentrations of total protein, amino acids, carbohydrate and lipid were recorded to be increased. Therefore, these fruits wastes have considerable potentials for utilizing as supplementary feed ingredients in sustainable aquaculture development of Macrobrachium and brine shrimp nauplii (control). The results indicated that FPFE can be added to a diet for African catfish at 5% of diet formula. However, brine shrimp nauplii fed to larvae had the highest percentage Survival and highest growth rates of any of the treatments.

A conventional and unconventional feed in fish nutrition was carried out the information on supplemental feed and its formulation for effective culture fisheries management. The results reported live feed for fish larvae, fish foods, fish feed ingredients, some common conventional feed stuff, animal and plant sources of unconventional feeds for culture fish, fish feed formulation and feeding methods to provide more information for the effective management of fish farming were Abowei *et al. (*2011).

Diab *et al*., (2002) reported that incorporation of citrus pulp in molly diets improved feed intake and feed conversion ratio. They

added that incorporation of orange peel in black molly diets improved feed conversion ratio and protein efficiency ratio significantly compared to the control unsupplemented group.

Hakim *et al*., (2010) conducted an experiment by using orange peel(citrus pulp)supplementation in the black molly (*Poecilia sphenops*) diets at different levels, on growth performance, nutrients utilization and whole bodies chemical composition. Result obtained are specific growth rate and survival rate were increased with fruit waste (orange peel) also supplementation.

Hon *et al.,* (2009) investigated the effect of dried sweet orange (Citrus pulp) fruit pulp meal on growth performance of ornamental fish black molly, *Poecilia sphenops*. The sweet orange peel meal was analyzed for its proximate nutrients and its crude fibre constituents. Their results suggested a possibility that sweet orange fruit pulp meal can be used as a replacement feedstuff for grown in ornamental fish.

Inaolaj (2006) was conducted to investigate the effect of Fermented Pineapple (*Ananas comosus*) Peel Meal (FPPM) on Growth Performance and Digestibility of Oreochromis niloticus. One hundred and twenty fingerlings of Tilapia (Oreochromis niloticus) with average weight of 13.94 ± 1.96 (Mean ± S.D) per plastic bowl (33L) in the wet laboratory. Four (4) iso-nitrogenous diets containing 35% crude protein in which maize was replaced by FPPM at 0% (TD1), 25% (TD2), 50% (TD3) and 75% (TD4) levels were formulated. The fingerlings were fed at 5% body weight per day for 56 days. The results revealed that the Weight Gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Production Value (PPV) values of 35.60g, 2.25% day, 2.58 and 0.56 respectively were highest in fish fed diet TD4.

Johny *et al*., (2000) studied the effect of dietary protein content of growth food conversion and body composition of *Poecilia sphenops* fed with fish meal diet and that reported specific growth rates and body protein content increased with dietary protein.

Kithsiri, *et al.* (2010) studied growth and reproductive performance of female guppy, *Poecilia reticulate* (peters) fed with different nutrient levels in the used in guppy farming in evaluated for their effect on growth and reproductive performance of females.

Kumar *et al*., (2012) studied the utilization of fruit wastes in single cell protein. The result showed that tested fruit wastes were highly susceptible to hydrolysis. A comparative study of fruit wastes revealed that cucumber peel generates higher amount of protein followed by that of orange with 53.4% crude protein respectively per 100gm of substrate used in the single cell protein.

Lee et al.,(2010) studied the Effects of Dietary Paprika and Lipid Levels on Growth and Skin Pigmentation of Pale Chub (Zacco platypus) Six diets (designated as P0L8, P0L17, P8L8, P8L17, P16L8 and P16L17) were formulated to contain 0%, 8% and 16% paprika with 8% and 17% lipid, respectively. The results suggest that feeding a diet containing 8% paprika and 8% lipid for 6 weeks could improve skin pigmentation of pale chub without any adverse effects on growth performance**.**

Mandal *et al*., (2010) reported growth and pigmentation development efficiencies in fantail guppy, *Poecilia reticulate* fed with commercially available feeds. The result showed that the fantail guppy utilized the live organisms more efficiently than the artificial diet.

Mgbenka and Orji (2014) conducted an experiment to investigate the use of Fresh Palm Fruit Extract as a Feed Ingredient in the Diet of Larval African Catfish, Clarias gariepinus. Three

experiments were conducted. In Experiment 1, the acceptance time of pelleted diets sprayed with fresh palm fruit extract (FPFE), commercial palm oil (COM). In Experiment 2, the effects of five diets on growth and survival of African catfish larvae were determined: (1) bambara nut waste based (BW) diet; (2) bambara nut waste-based diet with 5% of diet formula of FPFE (BWP); (3) bambara nut waste-based diet with 5% of diet formula of FPFE plus 1.5% of diet formula of Spirulina powder (BWPS); (4) fish waste-based diet (FWP); and (5) brinc shrimp, Artemia sp., nauplii (control). In the BWP, BWPS, and FWP diets, fresh palm fruit extract was sprayed on the diets as a feed attractant. In Experiment 3, the effects of seven diets on growth and survival of the African catfish larvae were investigated: (I) BW; (2) BWP; (3) FWP; (4) a bambara waste-based diet with 5% COM (BWC); (5) a fish waste-based diet with 5% of formula as COM (FWC); (6) a fish waste-based diet with neither FPFE.

Milad *et al*., (2012) studied the growth and reproduction performance by different feed types in fresh water angel fish and showed that breeders benefit from inclusion of prepared granulated feed and living earthworm during their growth and reproductive stages by simultaneous using them for achieving better results.

Nithya *et al*., (2012) reported the effect of photoperiod on the growth rate of black molly,*Poecilia sphenops* from larvae to adult in mass culture. It was reported that more light periods can enhance the larval developmental problems and increase the aquaculture production.

Oyebamiji *et al. (*2013) carried out an experiment to assess the water parameter and bio chemical composition of fish black molly, (*Poecilia sphenops*).The results were compared with the standard levels recommended for the various nutrients and statistically analyzed of varience (ANOVA) at 5% probability level.

Prasad and Suneesh (2013) investigated the digestive enzyme characterization of threatened yellow catfish Horabagrus brachysoma (gunther) (teleostes : siluriformes: horabagridae) at two life stages. The results shows that in both juveniles and adults all major digestive enzymes viz. carbohydrases, proteases, and lipases were present. Among the carbohydrases, amylase, maltase and lactase were present whereas cellulase was lacking in the two life stages studied. The enzyme sucrase was present in the adults but lacking in the fingerlings. Among proteases, pepsin showed the utmost activity followed by trypsin and peptidase. The enzyme lipase was present in the intestine of adults while it was weakly present in fingerlings. Based on the findings it can be concluded that the fish has the ability to digest all major types of nutrients and possibly which will help to accept wide spectrum of feed components. The present findings will help to formulate a more economically viable and nutritionally balanced diet for the artificial rearing of this cultivable catfish.

Sahu *et al*., (2007) reported that orange peel has many beneficial health properties which are attributed to inorganic compound in present or formed in crushed fruit wastes.

Shalaby *et al*., (2006) investigated the effect of orange peel in black molly, *Poecilia sphenops* and reported that the incorporation of orange peel in black molly diets increased significantly final weights and specific growth rate and increase was more pronounced at higher incorporation levels.

Shim *et al*., (2000) suggested that 30-40% dietary protein should be the optimal level for feeding guppy and the female fish fed 30-40% dietary protein levels and 9-10.5% lipid levels gained the highest mean body weight and length.

Soltan and Laithy (2008) conducted an experiment to determine the effects of fruit wastes and some spices as feed

additions on the performance and behavior of the Black molly, *Poecilia sphenops*. Results of the experiment indicated that,supplementation of the fruit wastes and spices(orange peel) significantly improved survival rate of molly.Feed intake,feed intake,feed utilization and growth performance of Black molly incuding final body weight,final body length and protein efficiency ratio,were significantly high.

The effect of replacing soybean meal protein by other plant protein sources on growth performances and economical efficiency of Nile tilapia(*Oreochromics niloticus*) cultured in tanks. The results of their experiment showed that replacement of 30% of soybean meal protein by sesame seed cake protein improved significantly final body weight of Nile tilapia were studied Hakim *et al*. , (2008).

The studies of Gabor *et al*., (2010) suggested the effect of some phyto additives on growth, health and meal quality on different species of fish and also reported that the effect of phyto additives (fruit waste) modulators and anti oxidant stimulants did not cause any threat to fish studied.

Yousefian *et al*., (2012) investigated the food requirements and dietary in aquarium fish. These include water, proteins, lipids, carbohydrate, vitamins, minerals and other supplementary additive such as probiotic and prebiotic. In their environment fish have developed a wide variety of feeding specializations to acquire essential nutrients and utilize varied food sources. In past decade the nutritional requirements of various fish species have understood and technological advances in feed manufacturing have been obtained. This resulted in development and use of formulated and manufactured feeds to replace natural feeds in the aquaculture industry. The production and trade of ornamental fish is a profitable alternative in the aquaculture.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Experimental fish selection

Fresh water ornamental live bearer fish, *Poecilia sphenops* (Figure 1) were collected from a fish aquarium in Papanayakkan palayam, Coimbatore District, Tamil Nadu. Fishes were kept in large aquarium tank in the laboratory for 15 days to get acclimatized (Figure 2). During this period they were fed with control feed (rice bran and ground nut oil cake). Water was changed alternative days to ensure sufficient oxygen supply to fish.

Preparation of feed and feed formulation

The orange peel were collected from the fruit stall, dried and ground into fine powder. The feed ingredients used in the feed were orange peel powder, groundnut oil cake and rice bran powdered and tapioca powder (as binding agent) (Figure 3).

The ingredients of each diet were mixed thoroughly. The dry powder constituents were blended at first to make a homogenous mixture and cooked by adding tapioca powder with required quantity of hot water to form soft dough. The pellets were prepared by feeding the dough through an extruder having a perforated disc with 0.8 cm diameter holes. The moist noodles were dried of approximately 0.5 cm in length. Four different feed with orange peel powder for 4 different treatments and one without orange peel powder as control was prepared for the present study.

Four different treatments selected for the present investigation includes.

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)

- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran, oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

Feeding Trials

The experiments were carried out for 60 days. The feed trials were carried out in five treatments in three replications. Each trough contains ten individuals in the control and in the four different treatments (Figure 4). Prior to stocking biochemical composition were analysed in the live bearer, black molly, *Poecilia sphenops*.

During the experimental period *Poecilia sphenops* in the control and four different treatments were fed with five per cent of their body weight. Every fortnight the fishes were weighed and the quantity of the feed were also adjusted according to the growth of the fish. The rational feed was given once a day. Biochemical composition such as protein, carbohydrate and fat were calculated in prepared fish feeds. The length (cm) and weight (in gm) of *Poecilia sphenops* biochemical composition such as water content, protein, carbohydrate and fat were calculated in the muscle tissue of the fishes grown in control and four different treatments before and after the experiment and the results were subjected to statistical analysis and were compared.

Growth assessment and reproductive performance

The growth parameters such as length (cm), and weight (gm) of spawning black molly, relative fecundity (total fry production throughout experimental period / mean weight of female in gram), fry survival (total live fry after time / total fry production X 100) and fry weight (g) were calculated.

Figure 1 - Ornamental fish, black molly, *Poecilia sphenops*

fish

Figure 2 - Acclimatization of *Poecilia sphenops* in the laboratory condition

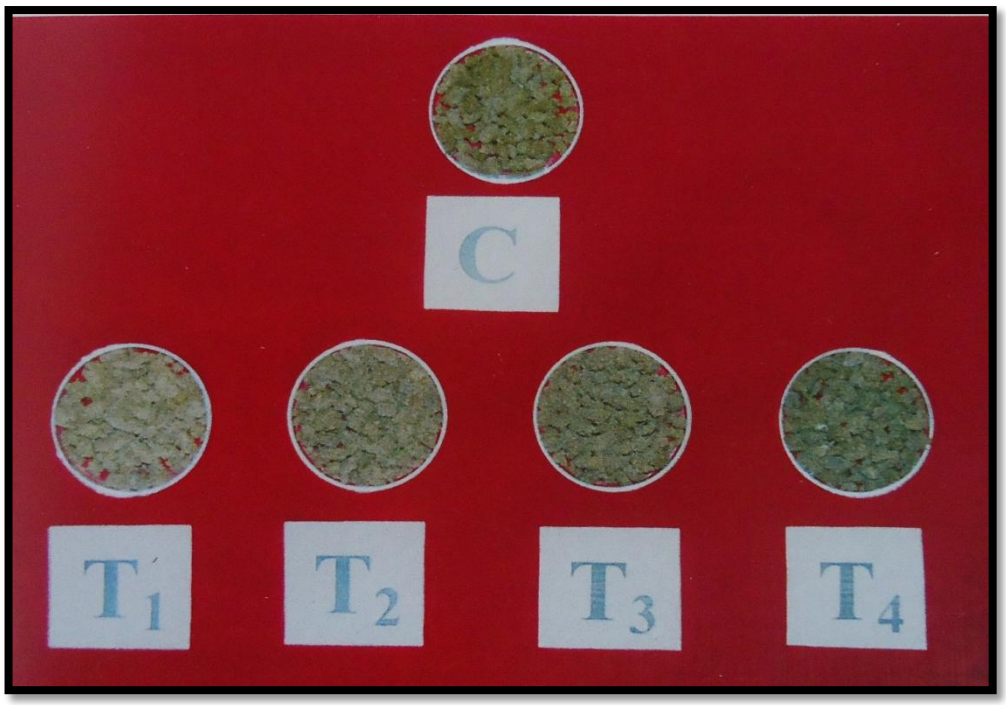

Figure 3 - Fish feed prepared from rice bran, groundnut oil cake and orange peel in different concentrations

Figure 4 - Experimental setup shows *Poecilia sphenops* in control and four different treatments

Biochemical Estimation of Feed and Experimental Animal

Black molly, *Poecilia sphenops* were brought to the laboratory to estimate the biochemical composition. In the laboratory they were washed and the surface moisture was removed by using blotting paper. Biochemical composition such as water content, protein, carbohydrate and fat were analysed in the muscle tissues of *Poecilia sphenops*.

The proximate composition of the formulated feed (one time) in control and four different treatment feeds and experimental animal, before and after 60 days was estimated during the experimental days by following the standard methods.

1. Water Content

Water content was calculated in muscle tissues of *Poecilia sphenops* in the control and in the replicates. The dissected tissue were placed in separate vials (weighed to 100 mgs) and dried in hot air oven for 24 hours at 80°C until attaining constant weight.

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

2. Protein Estimation

The protein was estimated by adopting the method of Lowry *et al*. (1957).

Reagents

Reagent A

Two per cent sodium carbonate in 0.1 N sodium hydroxide w/v (2 gm / 10 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.

1 N 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 Trichloroacetic Acid

5 gm of trichloroacetic acid in 100 ml distilled water.

Procedure

Standard Preparation

For plotting the standard curve, a set of standard were run 0.1, 0.2, 0.3, . . . , 1 ml 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-ciocateau 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 was homogenized with 1 ml of 0.9 per cent sodium chloride solution. 1 ml of five per cent trichloroacetic acid was added, and then centrifuged at 8000 rpm for 20 minutes. The precipitate was dissolve 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 muscle tissue of *Poecilia sphenops* in the control fish and also in the T_1, T_2, T_3, T_4 fed fishes were taken and the same procedure described for the feed sample 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 Estimation

Carbohydrate was estimated in the feeds and fishes in control and in replicates fed with treatments by Hedge and Hofreiter (1962).

Reagent

5 per cent trichloroacetic 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 standard were run 0.1, 0.2, 0.3, ..., 1 ml in a series of test tubes. The volume in each test tube was made up to 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.

Sample Procedure

100 mg of feed and also of muscle tissue of *Poecilia sphenops* in control and treatments (T_1, T_2, T_3 and T_4) were homogenized in 1 ml of 0.9 per cent sodium chloride solution. 1 ml of 5 per cent trichloroacetic 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 – 15 minutes and then cooled in dark. After 30 minutes the OD value was read at 620 nm in calorimeter.

4. Fat Estimation

Total fat was resoluted by gravimetric method using chloroform methanol mixture (3 : 1) (Folch *et al*., 1957) in tissues of *Poecilia sphenops* in the control and four treatments.

Reagents

Chloroform methanol mixture.

Procedure

100 mg of muscle tissue were weighed and ground well with 5 ml of chloroform methanol mixture. In the same way control and treatment feeds were also ground well with 5 ml of chloroform methanol mixture. The homogenate 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.

In methanol layer was removed without disturbing the chloroform layer. The chloroform was evaporated in an oven at about 60°C. The break was weighed. The difference between the final weight and initial weight of the beaker will give the lipid content of the tissues.

The amount o lipid present in the sample is expressed in percentage.

Statistical Analysis

Results were subjected to ANOVA – two - way analysis ($P < 0.05$) of variance to evaluate the mean differences among individual diet treatments and fish carcass composition at 0.05 level of significance.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The results of the present investigation on the “Efficacy of fruit waste formulated feed on growth and reproduction of the ornamental fish, *Poecilia sphenops*” are presented below.

(A) Biogrowth Parameters

The biogrowth parameters such as weight and length in *Poecilia sphenops* during 15, 30, 45 and 60 days of the experiment in the control and four different treatments are presented.

1. Weight of spawning female

Weight (gm) of spawning female in *Poecilia sphenops* during different days of the experiment in the control and four different treatments are shown in Table 1 and Figure 5.

During 15 days of the experiment the maximum weight was noted in the fish grown in T_4 (4.12gm) followed by T_3 (4.10gm), T_2 (3.71gm) and T_1 (3.60gm). The minimum weight (3.50gm) was bbserved in the control fish. Similarly results were observed in 30^{th}, 45^{th} and 60^{th} day of the experiment. During 30 days experiment, highest weight (4.50gm) was recorded in the fishes grown in T_4 feed followed by T_3(4.30gm), T_2 (3.92gm), T_1(3.91gm) whereas the lowest weight (3.70gm) was observed in control. During 45 days of the experiment highest weight was noted in T_4 (4.90gm) followed by T_3 (4.79gm) T_2 (4.50gm) and T_1 (4.42gm) and the lowest weight (3.90gm) was noticed in the control fishes.

During 60 days of the experiment the weight was increased in T_4 (5.30gm) followed by T_3 (5.07gm), T_2 (4.91gm) and T_1 (4.90gm). The weight was decreased (4.00gm) in the control fishes.

The growth rate (weight) of black molly during 15, 30, 45 and 60 days in the four different treatment and control were found to be statistically significant.

2. Length of spawning female

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

During 15 days of the experiment the fishes grown in T_4 (4.90cm) followed by T_3 (4.21cm), T_2 (4.10cm) and T_1 (4.02cm). The minimum length (3.90cm) was recorded in control fishes. During 30 days of the experiment the maximum length was observed in T_4 (5.32cm) followed by T_3 (4.51cm), T_2 (4.50cm) and T_1 (4.30cm). The minimum length was recorded in control fishes (4.22cm).

During 45 days of the experiment the length was noted in T_4 was (5.82cm) followed by T_3 (4.90cm), T_2 (4.82cm), T_1 (4.80cm) and control (4.50cm). During 60 days of the experiment the highest length (6.00cm) was observed in T_4 showed by T_3 (5.32cm), T_2 (5.20cm) and T_1 (5.01cm). The lowest length was noticed in control (4.90cm).

The growth rate (length) of black molly during 15, 30, 45 and 60 days in four different treatments and control were found to be statistically significant.

Among the different treatments analysed T_4 shows maximum growth rate (length) during 15 days, 30 days, 45 days and 60 days of the experiment.

The growth rate of the T_4 fed fishes unanimously increased during the experimental period and this is perhaps due to the high composition of energy, proteins and dietary fibre present in the fruit waste.

A similar study was carried out by Deka *et al*., (2003), where an integrated fruit waste composition of orange, pineapple and sweet lime was fed to the *H. rohita* and a distinctive increased growth rate was noted in the fish fed with pineapple, due to the presence of

‘bromelain’ (proteocytic enzyme), which might have induced the better utilization of protein orange fruit contains several bioactive compounds like ferulic acid, hydrocynnamic acid, cyadin, glucosides, carotenoikd, anaringin, flavonoids, chesperidin, narirutin, naringin and eriocitrinl, ascorbic acids, volatile oils, pectins and coumarins (Abeysingh *et al*., 2007; Kelebek *et al*., 2008 and Xu *et al*., 2008).

3. Relative fecundity

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

Relative fecundity was maximum in T_4 (73.14) followed by T_3(17.06),T_2(13.35) and T_1 (12.08).The minimum relative fecundity was observed in control (6.05) fishes.

Relative fecundity

According to Yehuda, (1996), the quality and quantity of the feed are the important factors affecting the reproduction of fishes. In coherence to the above, the results of the current investigation revealed high fecundity rates in the feed formulated with high composition of orange peel wastes. Similarly, James and Sampath (2002), reported that the high percentage of fecundity of the ornamental fish Betla splendens was found when live feed was fed *Artemia* sp. and the spawning activity was three times higher than the other group of fishes fed with pelleted food. The rate of fecundity of the fishes also depends upon the protein content and the presence of essential amino acids which would stimulate the gonad development.

4. Fry survival

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

During 15 days of experiment the fry survival was highest in T_4 (19.00) followed by T_3 (18.01), T_2 (16.02) and T_1 (15.00). The lowest fry survival of (7.01) was observed in the control fish. During 30 days of experiment the fry survival was maximum in T_4 (21.03) followed by T_3(20.02), T_2 (18.03) and T_1 (17.01). The lowest fry survival (8.03) was noticed in the control fed fishes

During 45 days of the experiment highest fry survival was recorded in T_4 (23`01) followed by T_3 (21.00) and T_2 (20.00) and T_1 (19.02). The lowest fry survival (9.00) was noticed in the control fishes.

During 60 days of the experiment fry survival was highest in T_4 (25.02) showed by T_3 (23.03), T_2 (22.03) and T_1 (20.01) respectively. The minimum fry survival was observed in control fishes (10.02).

The growth rate (fry survival) of black molly during 15, 30, 45 and 60 days in four different treatments and control were found to be statistically significant.

4. Fry growth rate (weight)

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

During 15 days of the experiment, the fry weight (gm) was increased in T_4 (1.32gm) showed by T_3 (1.09 gm) T_2 (1.06 gm) and T_1 (0.82gm). The decresed fry weight was noticed in control (0.65gm).

During 30 days of the experiment, the maximum fry weight was observed in T_4 (1.34gm) followed by T_3 (1.12gm), T_2 (1.08gm) and T_1 (0.91gm). The minimum fry weight was observed in the control (0.86gm). In 45^{th} day of the experiment, the highest fry weight was noticed in T_4 (1.36 gm) followed by T_3 (1.15gm) T_2 (1.09gm) and T_1 (0.94gm). The lowest fry weight was noticed in control (0.90gm)

On 60th day of the experiment, the maximum fry weight was noticed in T_4 (1.39 gm), followed by T_3 (1.17gm) T_2 (1.12 gm) T_1 (0.97gm). The minimum fry weight was noticed in control (0.92gm)

The growth rate (fry weight) of black molly during 15, 30, 45 and 60 days in four different treatments and control were found to be statistically significant

The survival rate of the fry was reported to be highest in the T_4 feed. In contrary, Bhavan *et al*., (2013), reported the minimum survival rate of 10% in the fresh water prawns *M. rosen,* higher rate of survival in the case of apple and grape wastes. It is significant to not that the requirements of protein and lipid content will be high during the young stages of fishes compared to the adult fishes. In addition, Deka *et al*., (2003), reported that the fruit wastes do not possess any detrimental effect on the health and the body tissue content of the finger lings was least affected due to feeding of fruit wastes.

(B) Biochemical Composition (Protein, Carbohydrate and Fat) in Control and Four Different Feeds are Presented are Table 6 and Figure 10.

(1) Protein

The protein content was maximum in T_4 feed (25.04 per cent) followed by T_3 (24.01 per cent), T_2 (23.00per cent) and T_1 (22.02 per cent). Minimum protein content was noticed in control feed (20.00 per cent).

(2) Carbohydrate

The carbohydrate content was increased in T_4 feed (20.02 per cent) followed by T_3 feed (18.02 per cent), T_2 (17.03 per cent), T_1 (15.00 per cent) and minimum carbohydrate content was noticed in the control (14.01 per cent).

(3) Fat

The fat content was maximum in T_4 feed (18.01 per cent) followed by T_3 (16.02 per cent), T_2 (14.00 per cent), T_1 (12.01 per cent) and minimum value was observed in control feed (11.02 per cent).

Tacon (1994), stated that the digestibility of protein, energy and individual amino acids are the prime consideration as the basis of feed formulation in fish, with information gained for different raw materials such as plant by products commonly used in the feed manufacturing industries. In the present investigation, the selected fruit (*Citrus sinensis)* formulated in different concentration along the basal diet was studied and the results revealed that high protein content (25.04%), carbohydrate (20.02%) and fat (18.01%) was found

in T_4 feed, which had the highest composition of orange peel powder (10gms).

Al-Shahib and Marshall, (2003), reported the nutrition and biochemical composition of date palm (Phoenix dactylifera) to contain high % of carbohydrate (44.88%), fat (0.2-0.5%), protein (2.3-5.6%) and a high percentage of dietary fibre (6.4-11.5%) which are could be used effective was in food formulated Bhonsau *et al.,* (2010) reported the formulation of fish feed using several ingredients from plant sources such as turmeric powder (*Curcuma longa*) for the antibacterial, anti flammatory and hepato protective properties. Moreover it also functions as antioxidant and adjunction in feed preparation. Whereas, the addition of garlic paste promotes the antibiotic and antibacterial properties.

Similarly, pepper powder was also used as a major constituent which perhaps be used as a stimulant for appetite (Porman and Deans, 2000) and also possess 0.6 – 2.6% of essential oil and

vitamins A, B. and C. However, based on the reports of (USDA, 2008) cumin (Cuminium cyminium) powder possesses 44.2 gm of carbohydrate, 17.8 gm protein, 105 gm fat and dietary fit 10.5 gm. Several researches in animals indicated that it could stimulate the pancreas enzymes and also an significant factor of nutrient digestion and assimilation.

(C) Biochemical Composition (Water Content, Protein, Carbohydrate, Fat) Before and After the Experiment in *Poecilia sphenops* are presented

(1) Water content

Water content (%) in the muscle tissue of *Poecilia sphenops* in the control and four different treatments before and after the experiment is shown in Table 7 and Figure 11.

The water content in the muscle tissue of *Poecilia sphenops* before the experiment was increased in T_4 (59.02 per cent) followed by T_3 (56.03 per cent), T_2 (55.10 per cent) and T_1 (54.06 per cent). Minimum value was noted in control (46.01 per cent).

After the experimental period water content was highest in T_4 (70.69 per cent) followed by T_3 (69.02 per cent), T_2 (67.02 per cent) and T_1 (58.02 per cent). The lowest water content was analysed in the control fishes (53.04 per cent).

Kumar *et al*., (1995), studied water content, protein, lipid and carbohydrates contents in relation to body weight in air breathing fish *Anabas testudineus*, and reported that the nutrient level increases in relation to the body weight, which agree with the increased levels of nutrients in *Poecilia sphenops* grown in T_4 diets.

(2) Protein

The protein content (%) in the muscle tissue of *Poecilia sphenops*in the control and four different treatments before and after the experiment is shown in Table 8 and Figure 12.

The highest value of protein content was found in T_4 fishes (28.88 per cent) followed by T_3 (27.60 per cent),T_2(26.90 per cent) and T_1 (25.89 per cent) Minimum protein content was recorded in control (25.36 per cent).

After the experimental period protein content in the muscle of *Poecilia sphenops* was highest in T_4 (36.47 per cent) followed by T_3 (33.10 per cent), T_2 (32.40 per cent) and T_1 (31.80per cent). Minimum protein content was found in control fishes (27.70per cent).

The protein content in the muscle tissue of *Poecilia sphenops* in the four treatments were found to be statistically significant.

Protein content in the muscle tissue of *Poecilia sphenops* among the four different treatments the maximum value was observed in T_4 during 15,30,45 and 60 days. The minimum value was observed in the control treatment.

Satia (1974) observed a general increase in the protein content in the carcass of rainbow trout (*Oncorhynus mykiss*)in relation to the amount present in the diet.Ogino and Satio (1979) also reported that there was a linear relation between protein content of the diet and body protein content of the young carp *Cyprinus carpio*.There was also significant difference ($P<0.05$) was observed in the fish diet although the protein levels were significantly different in the diet.

Dahlgren (1980) conducted an experiment with three types of feed with different protein levels and recorded higher growth and reproductive performance in the fishes fed 35% to 47% protein levels.

(3) Carbohydrate Content

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

The carbohydrate content in the muscle tissue of *Poecilia sphenops* before the experiment was maximum in T_4 fishes (1.29 per cent) followed by, T_3 (1.27 per cent), T_2 fishes (1.19 per cent) and T_1 fishes (1.17 per cent).Minimum carbohydrate content was found in control(1.16 per cent).

After the experimental period carbohydrate content in the muscle of *Poecilia sphenops* was highest T_4 fishes (1.68per cent) followed by T_3 (1.64 per cent), T_2 (1.56 per cent) and T_1 (1.42 per cent). Minimum carbohydrate content was recorded in control (1.35per cent) fishes.

The carbohydrate content in the muscle tissue of *Poecilia sphenops* in the four treatments was found to be statistically significant.

Carbohydrates represent a broad group of substances which includes sugars, starch, gums and cellulose. These make up three fourth biomass of the plant material, but are present in meagre quantities in animal body as glycogen, sugars and their derivates. In, the present investigation, the carbohydrate content of the muscle tissue of *P. sphenops* fed with T_4 feed was (1.29%) highest. This conveys that the maximum utilization of the carbohydrate content was made by *P. sphenops* from the T_4 on comparison with the $T_{2,}$ T_3 and control. A report by NRC (1993), conveyed that no dietary requirement of carbohydrates has been demonstrated in fish and warm water fishes tends to utilize greater amount of dietary carbohydrate than the cold water and marine species. Olaleye-loaheed Inaolaji (2011), reported that, *Tilapia* efficiency utilized the

partially hydrolyzed starch than sugars such as glucose and sucrose (Edwing and Meng, 1996).

(4) Fat Content

Fat content (%) in the muscle tissue of *Poecilia sphenops* in the control and four different treatments before and after the experiment are shown in Table 10 and Figure 14.

The fat content in the muscle tissue of *Poecilia sphenops* before the experiment was maximum in T_4 fishes (2.35 per cent) which was followed by T_3(2.39 per cent), T_2 (2.30 per cent) and T_1 fishes (2.24 per cent). Minimum fat content was found in control fishes (2.14per cent).

After the experiment fat content was maximum in the muscle tissues of *Poecilia sphenops* before grown in T_4 (2.76 per cent) followed by T_3 (2.70 per cent), T_2 (2.58 per cent), T_1 (2.48 per cent). Minimum fat content was recorded in control fishes (2.40 per cent).

The fat content in the muscle tissue of *Poecilia sphenops* in the four different treatments were found to be statistically significant when compared to control.

These present result was supported by Jeyachandran and Raj (1976) where they observed a positive correlation between the fat content of the feed and the fat in the fish.

Andrews (1971), reported the energy and lipid requirements of cat fish and inferred that the environmental temperature influences the level of fat in cat fish. It was evident that other factors such as dietary protein and energy will also influence body composition. The significant influence of supplevite on fish muscle lipid levels clearly indicates the effect on metabolic process of *Poecilia sphenops.* The growth promoting effect might be due to better utilization of carbohydrate as energy sources and subsequent retention of

excessive lipid in muscle tissue. Similar increase in muscle protein level was observed by Keshavanath *et al*. (1991) in rohu and common carp fingerlings fed with “staface 20” which contains 2 percent viginiamycin as a non-hormonal feed additive. There may also be interactions between the various vitamins and minerals in the veterinary feed as did studied by Lee *et al*. (2003) and James *et al*. (2004).

TABLE – 1

WEIGHT (gm) OF SPAWNING FEMALE IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	15 days	30 days	45 days	60 days
C	3.50±0.20	3.70±0.18	3.90±0.11	4.00±0.40
T_1	3.60±0.14	3.91±0.09	4.42±0.12	4.90±0.14
T_2	3.71±0.09	3.92±0.10	4.50±0.17	4.91±0.18
T_3	4.10±0.10	4.30±0.11	4.79±0.08	5.07±0.20
T_4	4.12±0.12	4.50±0.18	4.90±0.20	5.30±0.11
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 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 2

LENGTH (cm) OF SPAWNING FEMALE IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	15 days	30 days	45 days	60 days
C	3.90±0.11	4.22±0.18	4.50±0.15	4.90±0.16
T_1	4.02±0.18	4.30±0.12	4.80±0.10	5.01±0.21
T_2	4.10±0.19	4.50±0.10	4.82±0.16	5.20±0.11
T_3	4.21±0.10	4.51±0.15	4.90±0.12	5.32±0.20
T_4	4.90±0.08	5.32±0.20	5.82±0.13	6.00±0.18
SEd	**0.12381**			
CD (P<0.05)	**0.25024**			

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 3

RELATIVE FECUNDITY OF BLACK MOLLY *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	Relative fecundity
C	6.05
T_1	12.08
T_2	13.35
T_3	17.06
T_4	73.14

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 4

FRY SURVIAL OF BLACK MOLLY IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	15 days	30 days	45 days	60 days
C	7.01±0.50	8.03±0.60	9.00±0.50	10.02±0.30
T_1	15.00±070	17.01±0.60	19.02±0.70	20.01±0.70
T_2	16.02±0.40	18.03±0.70	20.00±0.60	22.03±0.40
T_3	18.01±0.20	20.02±0.80	21.00±0.20	23.03±0.90
T_4	19.00±0.90	21.03±0.80	23.01±0.50	25.02±0.60
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 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 5

FRY WEIGHT (gm) OF BLACK MOLLY IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

Treatments	15 days	30 days	45 days	60 days
C	0.65±0.48	0.86±0.06	0.90±0.05	0.92±0.02
T_1	0.82±0.02	0.91±0.03	0.94±0.04	0.97±0.06
T_2	1.06±0.04	1.08±0.05	1.09±0.08	1.12±0.04
T_3	1.09±0.07	1.12±0.03	1.15±0.05	1.17±0.07
T_4	1.32±0.03	1.34±0.06	1.36±0.05	1.39±0.08
SEd	**0.09657**			
CD (P<0.05)	**0.19519**			

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 6

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

Treatments	Protein	Carbohydrate	Fat
C	20.00±0.70	14.01±0.10	11.02±0.60
T_1	22.02±0.60	15.00±0.40	12.01±0.20
T_2	23.00±0.40	17.03±0.20	14.00±0.50
T_3	24.01±0.80	18.02±0.30	16.02±0.80
T_4	25.04±0.50	20.02±0.60	18.01±0.10
SEd	0.0741	0.0863	0.0884
CD(P<0.05)	0.1651	0.1922	0.1970

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 7

WATER CONTENT (%) IN THE MUSCLE TISSUE OF *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Water content (%)	
	Before experiment	After experiment
C	46.01±0.11	53.04±0.22
T_1	54.06±1.96	58.02±0.06
T_2	55.10±0.34	67.02±0.28
T_3	56.03±0.31	69.02±0.22
T_4	59.02±0.65	70.69±0.05
SEd	**0.57402**	
CD (P<0.05)	**1.19739**	

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 8

PROTEIN CONTENT (%) IN THE MUSCLE TISSUE OF *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Protein content (%)	
	Before experiment	After experiment
C	25.36±0.06	27.70±0.09
T_1	25.89±0.09	31.80±0.07
T_2	26.90±0.06	32.40±0.11
T_3	27.60±0.08	33.10±0.10
T_4	28.88±0.08	36.47±0.07
SEd	**0.06700**	
CD (P<0.05)	**0.13976**	

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 9

CARBOHYDRATE CONTENT (%) IN THE MUSCLE TISSUE *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Carbohydrate content (%)	
	Before experiment	After experiment
C	1.16±0.06	1.35±0.05
T_1	1.17±0.03	1.42±0.02
T_2	1.19±0.08	1.56±0.08
T_3	1.27±0.08	1.64±0.04
T_4	1.29±0.09	1.68±0.08
SEd	**0.05287**	
CD (P<0.05)	**0.11029**	

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran,oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

TABLE – 10

FAT CONTENT (%) IN THE MUSCLE TISSUE *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

Treatments	Fat content (%)	
	Before experiment	After experiment
C	2.14±0.14	2.40±0.01
T_1	2.24±0.07	2.48±0.08
T_2	2.30±0.05	2.58±0.06
T_3	2.35±0.06	2.70±0.09
T_4	2.39±0.09	2.76±0.13
SEd	**0.07470**	
CD (P<0.05)	**0.15582**	

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

- Rice bran and oil cake 100 percent (control)
- Rice bran, oil cake 97.5 percent and orange peel powder 2.5 percent (T_1)
- Rice bran, oil cake 95.0 percent and orange peel powder 5.0 percent (T_2)
- Rice bran, oil cake 92.5 percent and orange peel powder 7.5 percent (T_3)
- Rice bran, oil cake 90.0 percent and orange peel powder 10.0 percent (T_4)

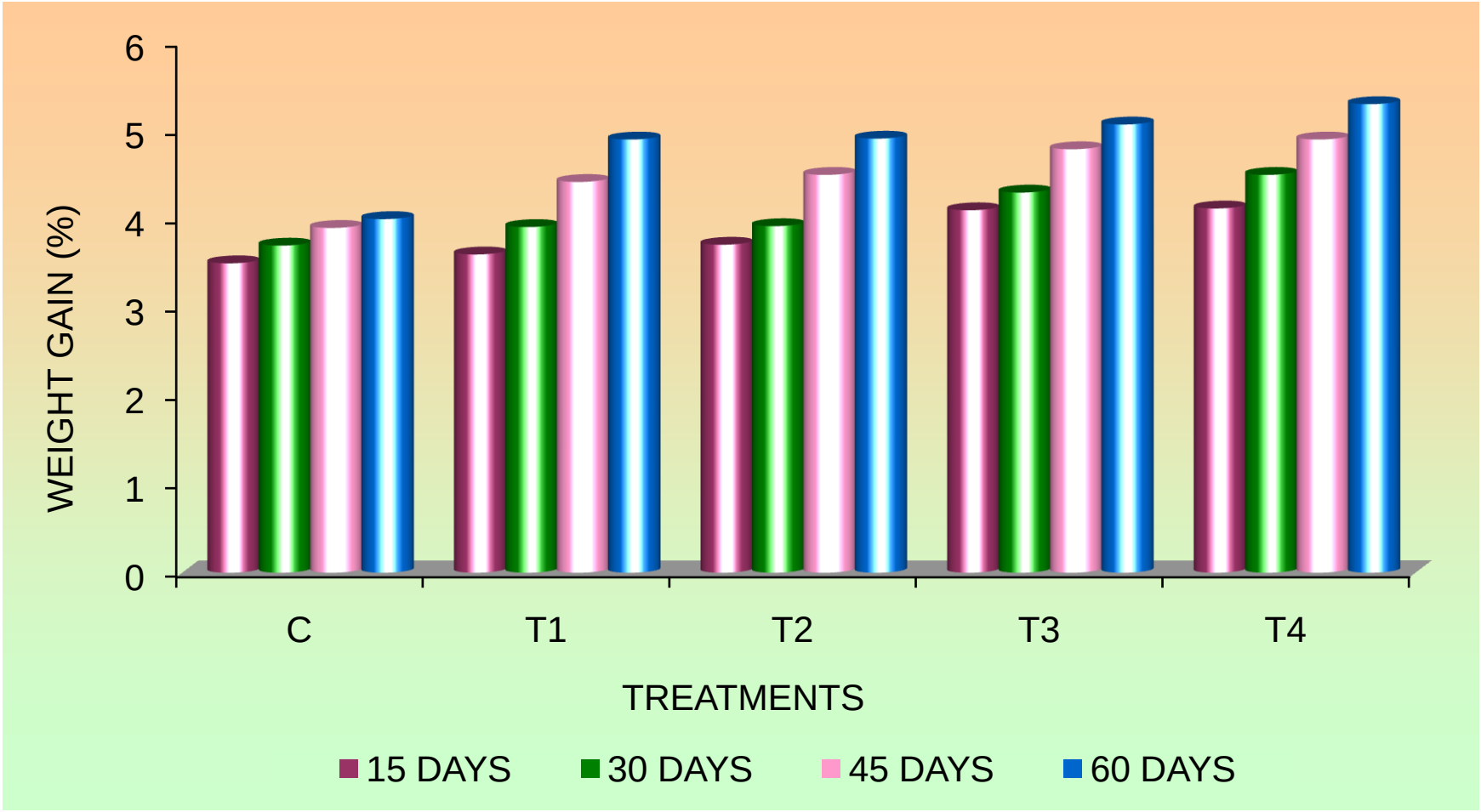

FIGURE 5 – WEIGHT (gm) OF SPAWNING FEMALE IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

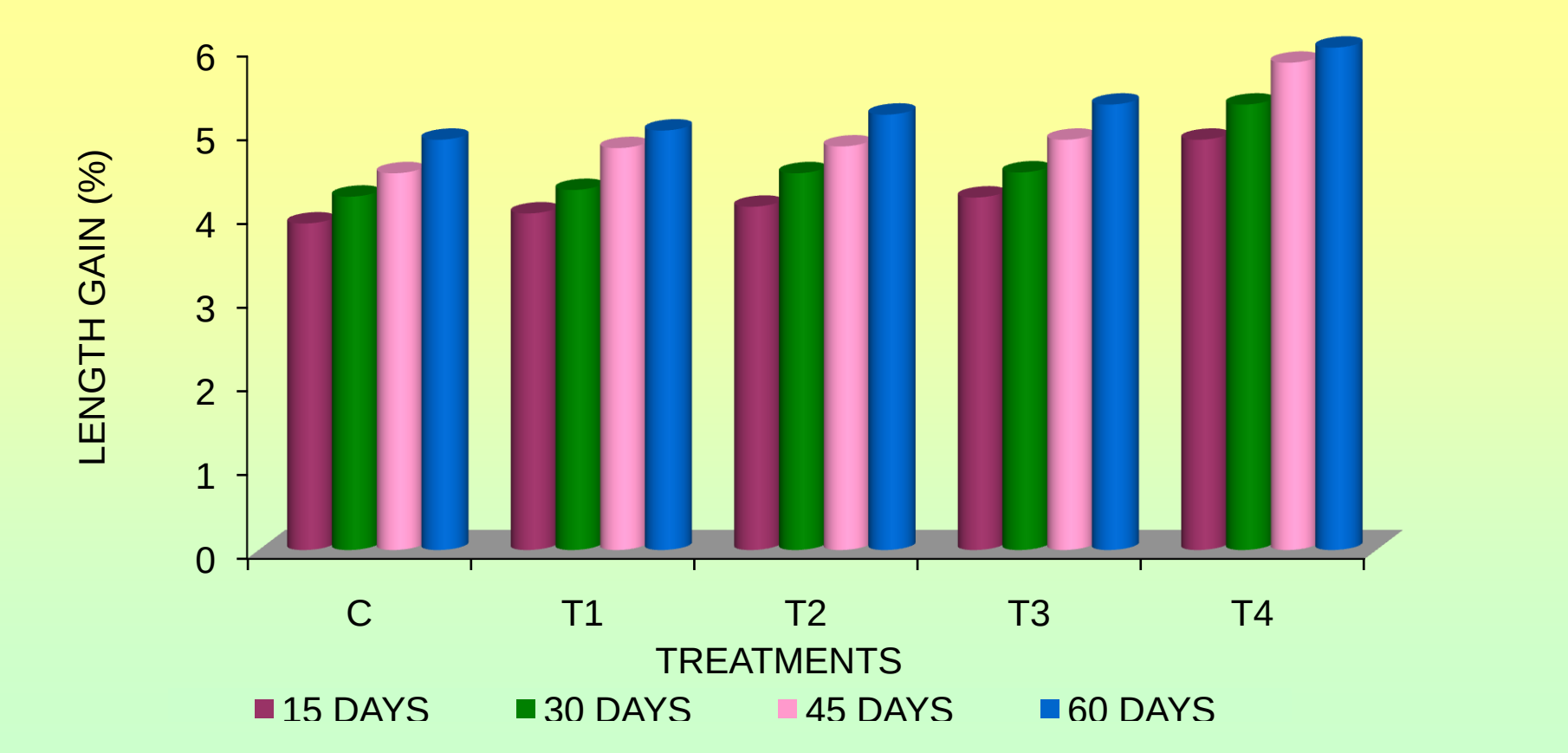

FIGURE 6 - LENGTH (cm) OF SPAWNING FEMALE IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

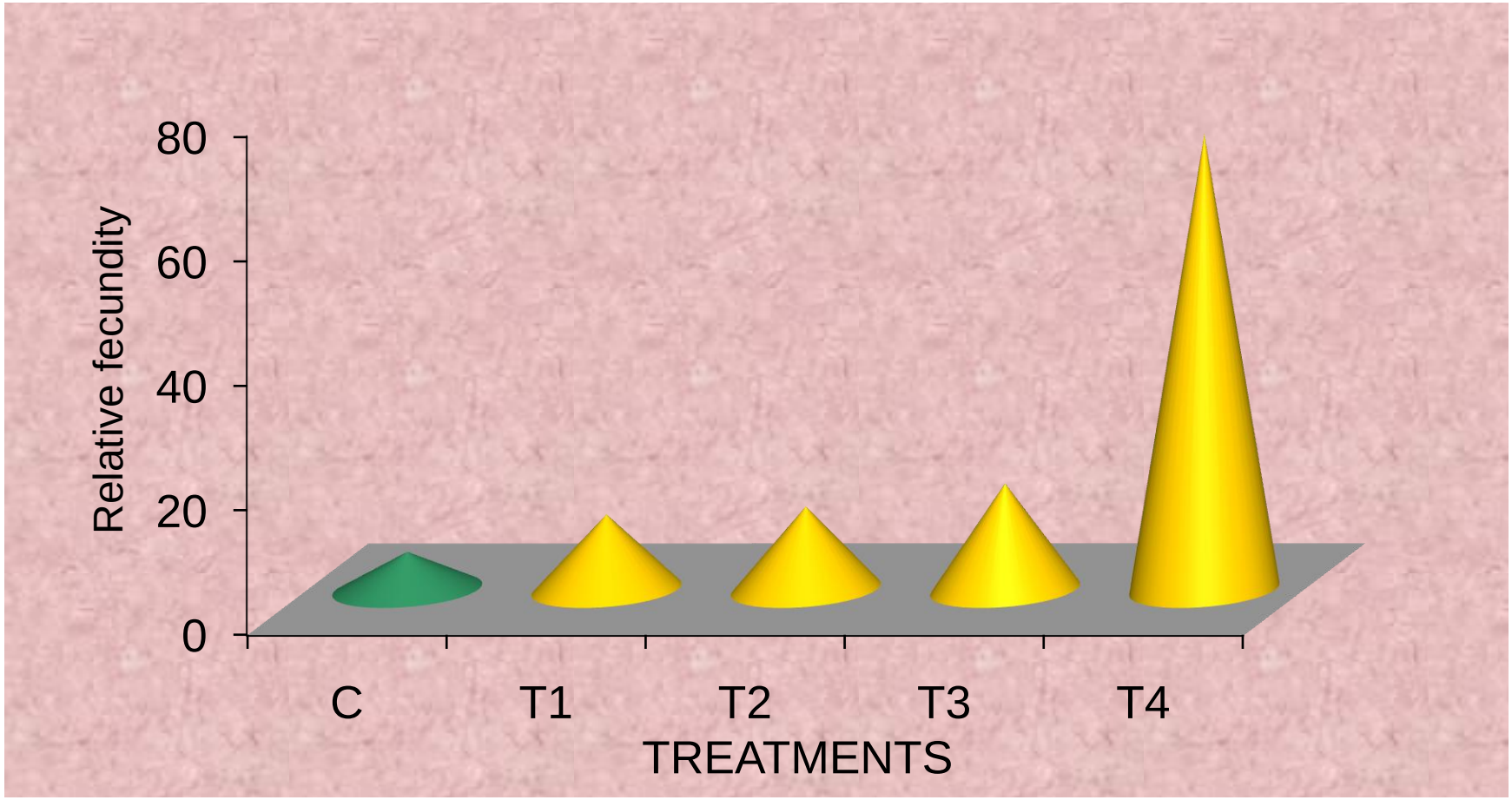

FIGURE 7 - RELATIVE FECUNDITY OF BLACK MOLLY *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

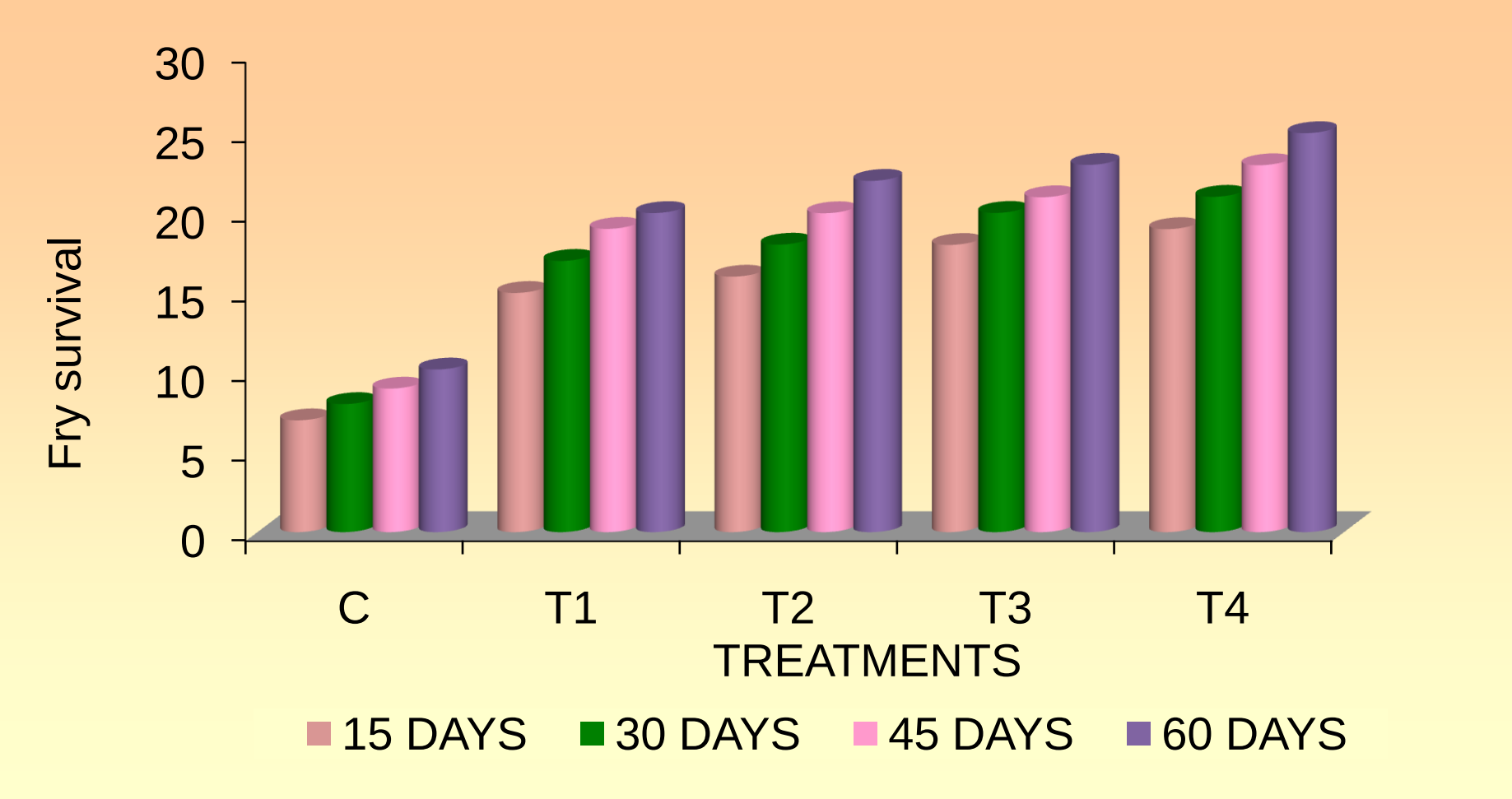

FIGURE 8 - FRY SURVIAL OF BLACK MOLLY IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

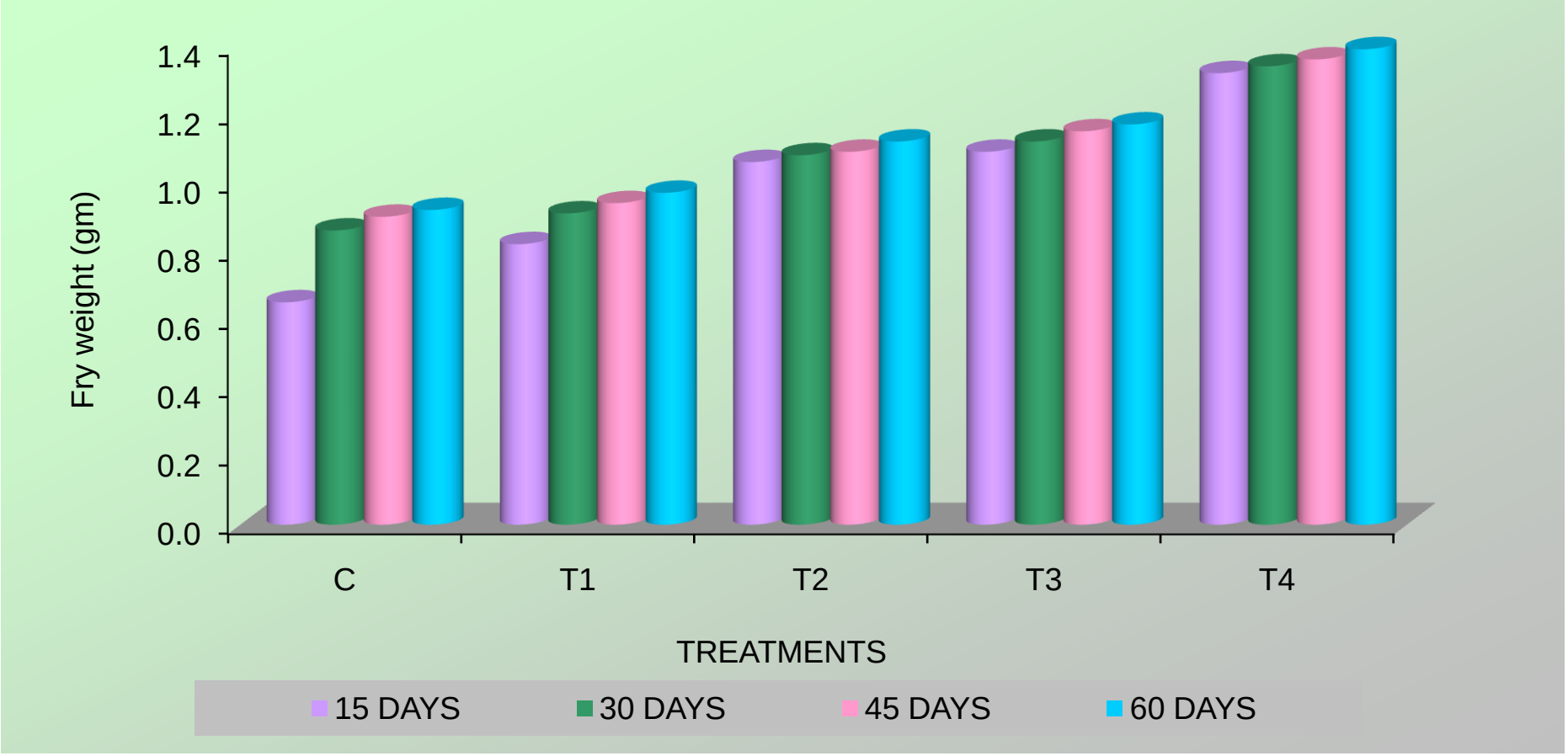

FIGURE 9 – FRY WEIGHT (gm) OF BLACK MOLLY IN *Poecilia sphenops* DURING DIFFERENT DAYS OF THE EXPERIMENT IN THE CONTROL AND FOUR DIFFERENT TREATMENTS

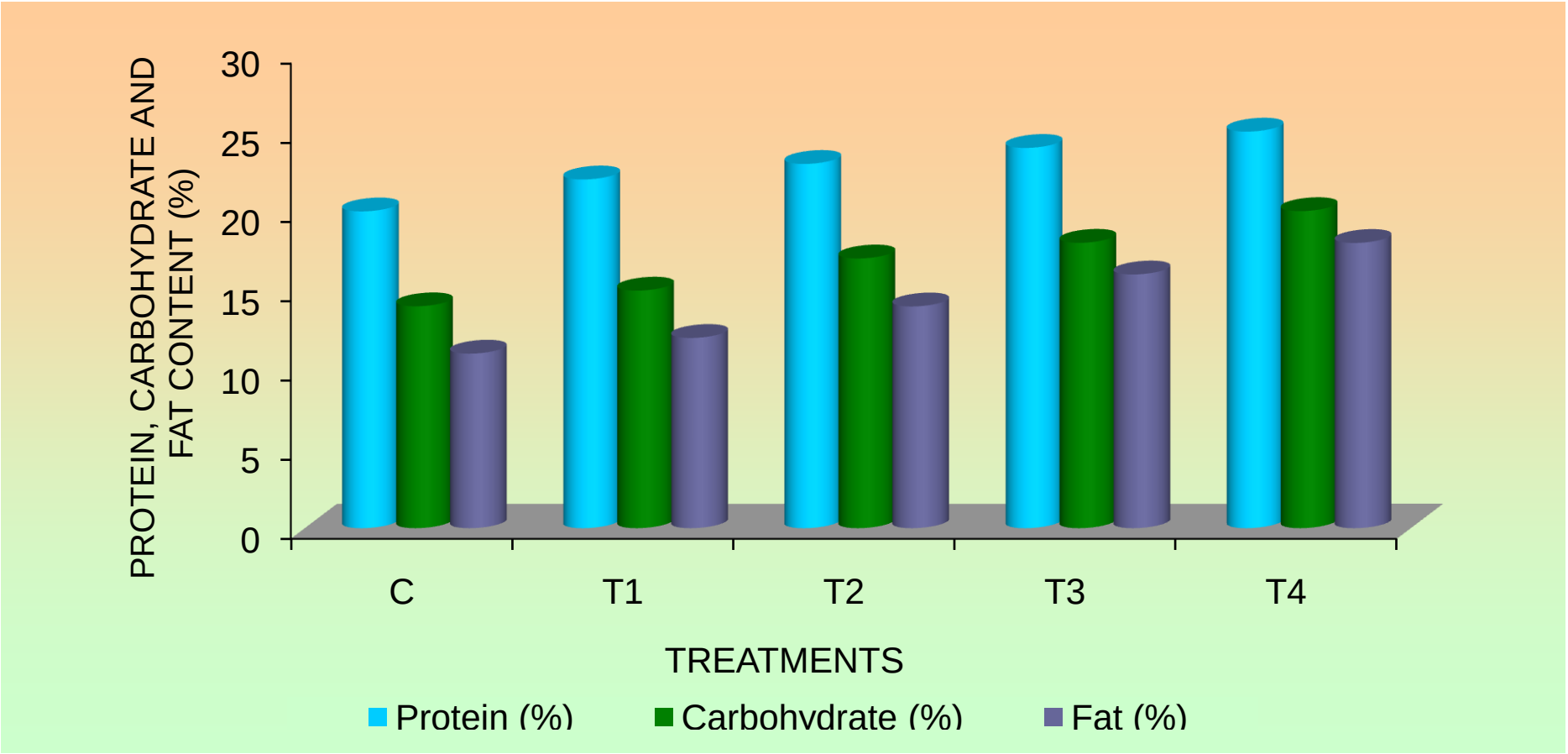

FIGURE 10 - PROTEIN, CARBOHYDRATE AND FAT CONTENT (%) IN THE CONTROL AND FOUR DIFFERENT FEEDS

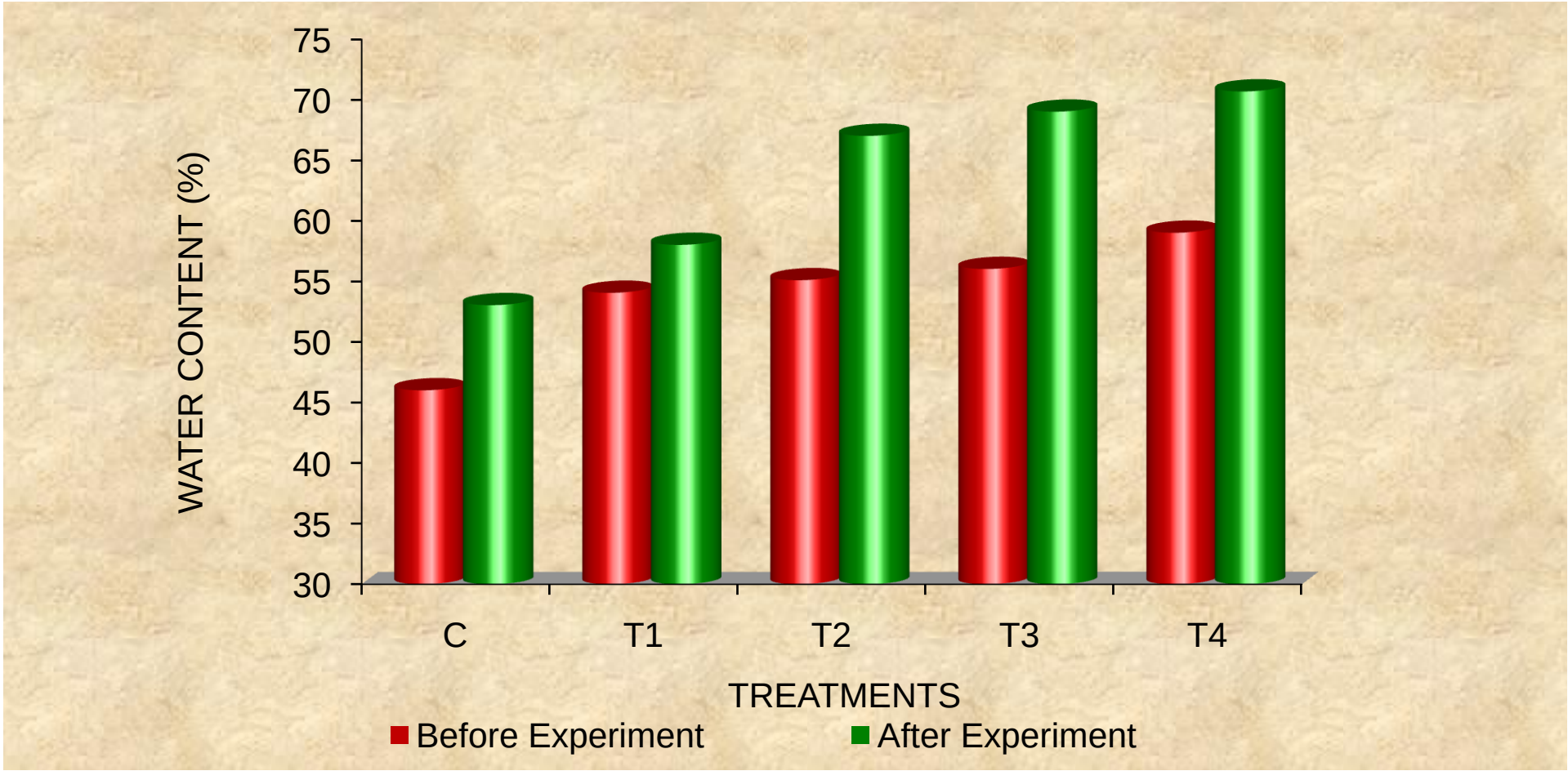

FIGURE 11 - WATER CONTENT (%) IN THE MUSCLE TISSUE OF *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

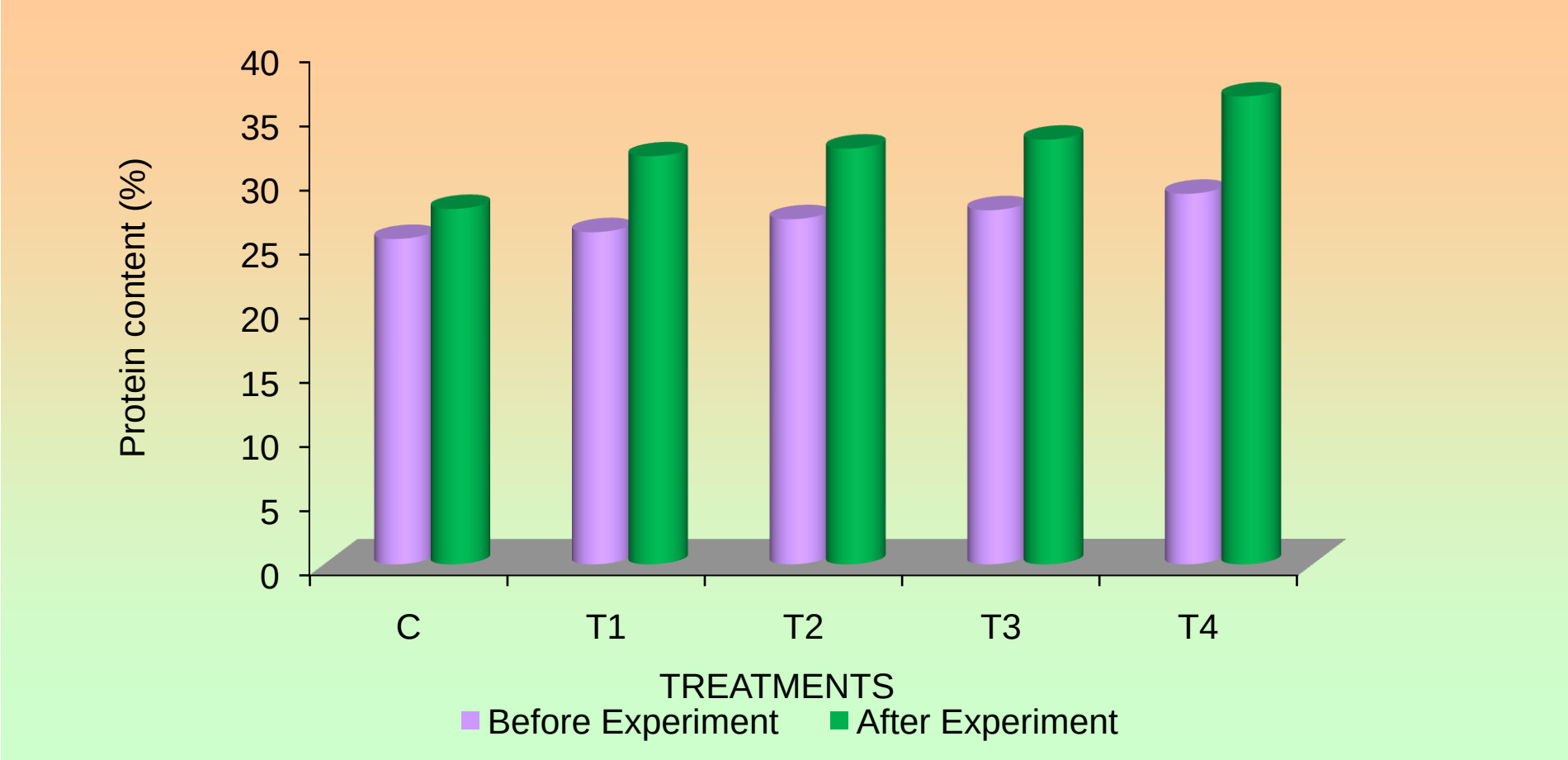

FIGURE 12 - PROTEIN CONTENT (%) IN THE MUSCLE TISSUE OF *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

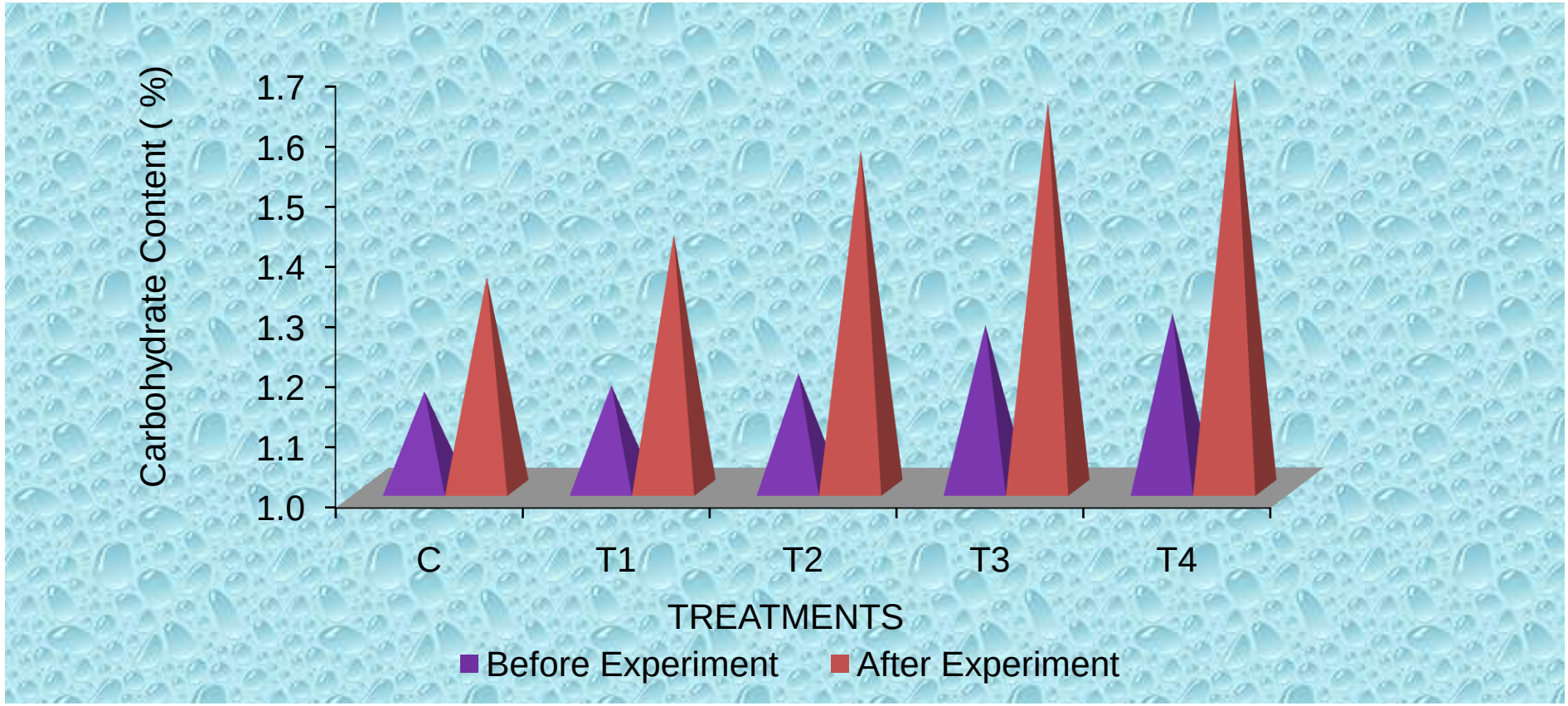

FIGURE 13 – CARBOHYDRATE CONTENT (%) IN THE MUSCLE TISSUE *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENT

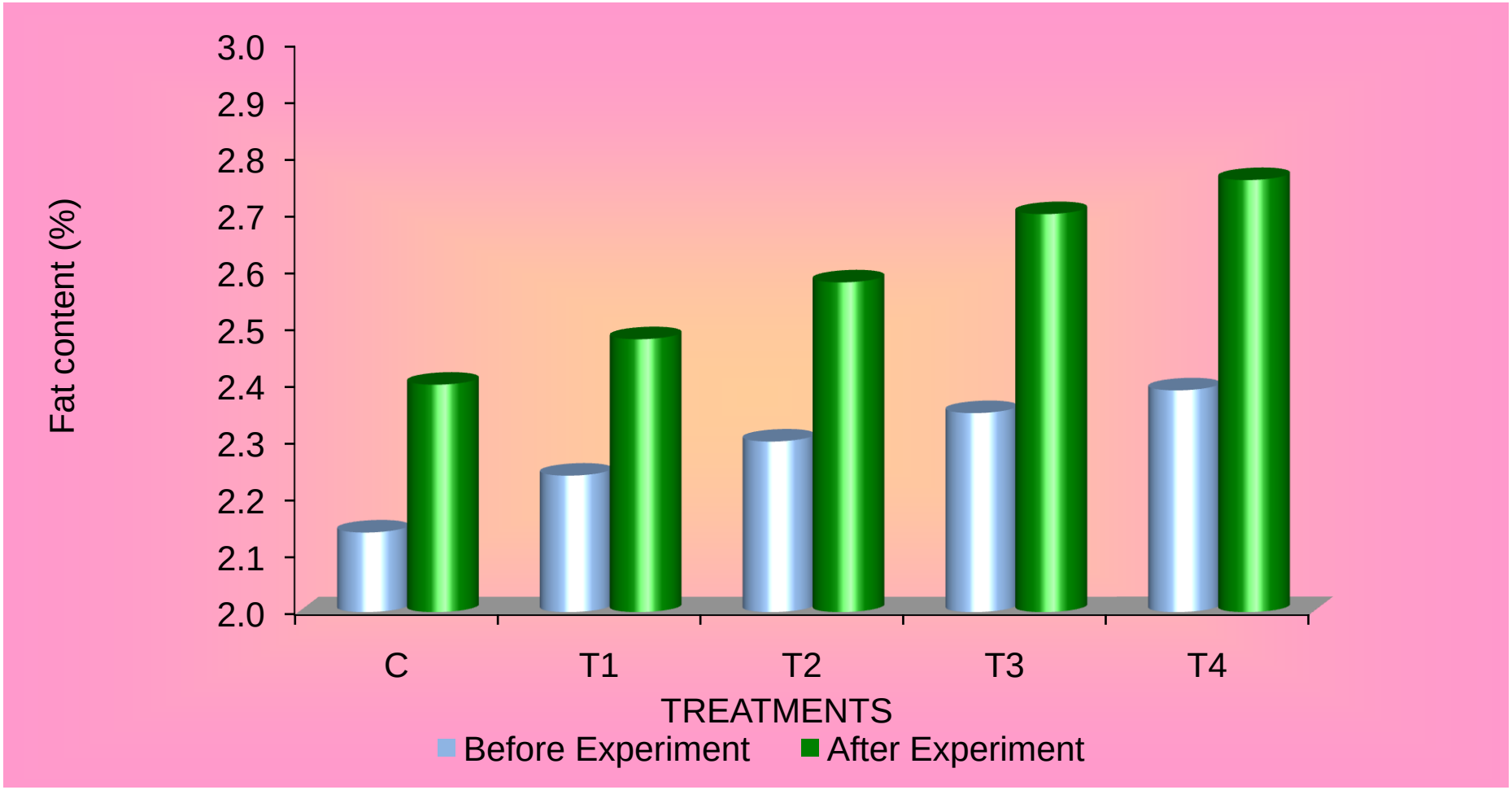

FIGURE 14 - FAT CONTENT (%) IN THE MUSCLE TISSUE *Poecilia sphenops* IN THE CONTROL AND FOUR DIFFERENT TREATMENTS BEFORE AND AFTER THE EXPERIMENTAL

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The present investigation was undertaken on **"Efficacy of fruit waste formulated feed on growth and reproduction of the ornamental fish, *Poecilia sphenops".***

The bio growth parameters such as weight of spawning female and length of spawning female, relative fecundity, fry survival, fry weight, in *Poecilia sphenops"* were analysed during 15, 30, 45 and 60 days of the experiment in the control and four different treatments.

The biochemical composition (protein, carbohydrate and fat) were also analysed in the control and four different feeds. Biochemical compositions (water content, protein, carbohydrate and fat) before and after the experiment in the control fishes and the four different treatments were also analysed.

The salient findings of the present study are as follows :

1. During 15,30,45 and 60 days of the experiment the weight (gm) of spawning female was maximum in T_4 fishes (4.12 gm, 4.50 gm, 4.90 gm and 5.30 gm respectively) fed with rice bran and oil cake 90 percent orange peel 10 percent. The lowest weight was recorded in the control (3.50 gm) fishes.

2. The length of spawning female in *Poecilia sphenops*, was highest in T_4 fishes during 15 days (4.90 cm), 30 days (5.32 cm), 45 days (5.82 cm) and 60 days (6.0 cm) of the experiment. The lowest length was recorded in the control (3.90 cm).

3. Among all the treatments maximum weight and length of spawning female, relative fecundity, fry survival, fry weight, was reported in fishes grown in T_4 treatment.

4. T_4 feed (25.04 per cent) has maximum protein content and minimum protein content was found in control feed (20.00 per cent). Carbohydrate content was also maximum in T_4 feed (20.02 per cent) and minimum was in control feed (14.01 per cent). Fat content was also maximum in T_4 feed (18.01 per cent) and minimum in control feed (11.02 per cent).

5. *Poecilia sphenops* grown in T_4 showed highest water content (70.69 per cent) at the end of the experiment and the lowest water content (53.04 per cent) was observed in control fishes.

6. Highest value of protein content before the experiment in the muscle tissue of *Poecilia sphenops* was in T_4 fishes (28.88 per cent) followed by T_3 (27.60 per cent), T_2 (26.90 per cent), T_1 (25.89 per cent)and control (25.36 per cent)after the experiment maximum was in T_4 fed fish (36.47per cent) followed by T_3 (33.10per cent), T_2 (32.40 per cent) and T_1 (31.80 per cent). Before the experiment minimum protein content was before the experiment was noticed in T_4 fishes and after the experiment in control fishes (27.70 per cent).

7. Maximum carbohydrate (1.68 per cent) was recorded in T_4 fishes, fed with rice bran and oil cake 90 gm and orange peel 10 gm. The minimum carbohydrate content (1.35 per cent) was recorded in control fishes where the orange peel powder was not included in the feed.

8. After the experiment highest fat content (2.76 per cent) was recorded in the fishes grown in T_4 and the lowest level (2.40 per cent) was recorded in control fishes.

9. The results were subjected to statistical ANOVA - two way analysis to recommend the best treatment which had showed highly significant value

of biogrowth parameter, biochemical composition in *Poecilia sphenops* grown in T_4 fed diet.

10. In conclusion of the present study, it was observed that 10% supplementation of orange peel powder (Citrus pulp) had significantly improved the growth rate in terms of weight and length of spawning female. Finally it was proved that the orange peel powder as feed additives represent alternatives solution in aquaculture, feed as growth promoters. More clearly, it showed the significant increase in growth and length performance, feed utilization and bio chemical composition.

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