

Efficacy of curry leaf formulated feed on the growth, nutritional status and feed utilization efficiencies of Indian major carp, *Catla catla* (Hamilton, 1822)

Rasathi.B

(Reg.No:17PZO012)

The thesis submitted to
Avinashilingam institute for home science and higher education for women
Coimbatore -641043

In partial fulfillment of the requirements for the
Degree of Master of Zoology

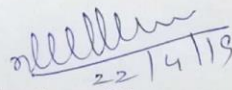
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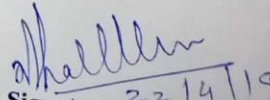
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Signature of the
Head of the department


Signature of the
the Supervisor



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INTRODUCTION

Aquaculture has a great role to play in the welfare of mankind. It is emerging as one of the most viable and promising enterprises for providing notional and food security for humans. Food production from agricultural resources cannot keep pace with the ever increasing human population around the globe (Manivannan *et al.*, 2012). Aquaculture has been one of the fastest growing branches of animal protein production. Aquaculture has become an important economic activity in many countries. (Nagendra Prasad *et al.*, 2016).

Human and marine life stock both depend on wild stock fish as food. Lately, investment of modern fishing fleets and processing factories are used to take advantage of the force behind this division of the food industry, in a natural reaction to cater for the increasing global requirements for fish and fishery products. The doubling in increased fish consumption in the developing countries since the 1970s (Delgado *et al.*, 2003) is contributing to the collapse of natural fish stocks where it is caused by factors such as illegal fishing, unfair fishing and over- fishing. Thus, to combat these problems faced by the fishing industry, many are turning to aquaculture for a more reliable and steady supply of fish as food.

Aquaculture is one of the quickly developing systems on the planet, which has risen as an industry conceivable to supply protein rich food all through the world. Fish is an essential dietary animal protein source in human nutrition. Production of sea-going species through freshwater fisheries and aquaculture for protein supply is being energized all through the world. Global aquaculture has grown dramatically over the past 50 years to around 52.5 million tons (68.3 million including aquatic plants) in 2008 worth US\$ 98.5 billion (US\$106 billion including aquatic plants) and accounting for around 50 per cent of the world's fish food supply. Asia dominates this production, accounting for 89 per cent by volume and 79 percent by value, with China by far the largest producer (32.7 million tons in 2008) John Bostock *et al.*, (2010).

Fish has been recognized as valuable resource of high quality food in human diet. Aquaculture is a low energy expenditure and protein yielding system in comparison to other agriculture sectors. Since aquaculture is affected by multiple factors, many characteristics must be considered and analyzed for product. The physical and chemical characteristics of water

bodies, seed quality, stocking density, seasonal changes, cultural systems, feeding and harvesting patterns are important factors (Sahu *et al.*, 2000).

The development of aquaculture industries has been considered as a means of supplying the future demand for aquatic products; in particular, to provide a major World-wide protein source (Shpigel *et al.*, 1993). Another report pointed out that Millions of people were dependent on aquatic production as their main protein source (Becker and Focken, 1998). It was predicted that aquaculture would have to provide more than 50% of the total demand for seafood products (Tidwell *et al.*, 2001) and reach at least 100 million tons per annum by 2030 to maintain the current per capita consumption (FAO, 2006b). Within the Asian countries, fish protein provides approximately 45 percent of the total protein consumed (Prein and Ahmed, 2000). Although Japan, the European Union and USA have higher per capita consumption of fish and fish products, the share of fish protein in terms of total animal protein consumption is far less than that in many developing countries (Gupta *et al.*, 2005).

Fish tissue contains all the basic amino corrosive and minerals viz., iodine, phosphorus, potassium, iron, copper and vitamin A and D in alluring concentrations. The essential nutrients for fish are amino acids, fatty acids, vitamins, minerals and energy-yielding macronutrients (protein, lipid and carbohydrate) (Stefanie M Hixson 2014).

The intensification of fish culture has led to dependence on artificial feeds. Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost. Reducing the feeding costs could be a key factor for the successful development of aquaculture. Fish have high dietary protein requirement. The significance of qualitative and quantitative feeds is well recognized and the level of dietary protein is of fundamental importance, because it significantly influences growth, survival and yield of fish. Therefore, considerable research effort is needed to determine the quantity and quality of dietary protein necessary to achieve optimum growth performance of fish.

Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals. Guidelines for nutrient adequacy for some farmed fish species suggest the minimum nutrient requirement to promote growth and prevent signs of nutrient deficiency. Protein is required in the diet to obtain amino acids, which are utilized to synthesize new proteins or maintain existing proteins in tissues while excess protein is converted

to energy. Lipids supply essential fatty acids and energy in the diet. Depending on species, protein and lipid are the main source of energy for fish. Feeds in aquaculture are formulated with a balance of nutrients in order to meet specific nutrient requirements for different species, life stages and other purposes (Stefanie 2014).

Lipid is the term used to refer to fats, oil, and waxes, in which fatty acids are the key components (Takeuchi *et al.*, (1978). Watanabe *et al.*, (1975) and Takeuchi *et al.* (1977) reported that common carp need 0.5 to 1.0 percent of linoleic and linolenic acids, in the diet. In short, dietary lipids play an important role in fish nutrition to generate energy and to maintain biological structure and function of all membranes (Sargent *et al.*, 1999).

It is well-known that fish can convert protein to lipid and/or carbohydrate to gain energy. One gram of lipid can liberate 39.3 kJ, which is much more than the same amount of carbohydrate (17.2 kJ/g) or protein (22.6 kJ/g) (Webster and Lim, 2002). Within certain limits, increasing dietary lipid levels improves diet utilization (Watanabe *et al.*, 1979, Johnsen *et al.*, 1993, Peres *et al.*, 1999) because the extra lipid is used to provide biologically useful energy thus sparing protein. (Watanabe, 1982, Beamish *et al.*, 1986), and reducing losses of organic matter and nitrogen (Lee *et al.*, 1973).

Carbohydrates are cheaper and more abundant than protein. Absence of an appreciable quantity of digestible carbohydrate from the diet means that fish have to metabolize more fat for energy (Ufodike *et al.*, 1983). It would therefore be economical and beneficial if cheap carbohydrate foods could be incorporated into fish feed without compromising growth and conversion efficiencies. Together with lipid, availability of digestible carbohydrate could minimize the use of protein as an energy source, which is the most expensive component (Alvarez-Gonzales *et al.*, 2001).

Carbohydrate are non-essential in fish diet as the energy they supply can be replaced by protein or lipids and requirements can be met by gluconeogenesis (Hemre *et al.*, 2002) however, carbohydrates and especially starches can be generally utilized well (Wilson, 1982) and they can also enhance protein sparing effect for growth (Jauncey, 1982). Their digestion and utilization is species dependant with carnivorous fish being less able to utilize them than omnivorous and herbivorous (Krogdhal *et al.*, 2004). Carbohydrate digestion is an extracellular procedure that involves hydrolysis of complex carbohydrates in the stomach, intestine and caecum as well as in

the brush-border section of intestines where enzymes such as maltase and sucrose are present (Harpaz *et al.*, 2001).

The availability of natural food is limited in densely stocked ponds in the case of semi- intensive and intensive culture systems. Thus supplementary feeding of fish is a usual practice in a fish culture Endeavour. A diet is considered a complete one when it contains balanced levels of all essential nutrients such as protein, lipid, carbohydrate, energy, vitamins and minerals, which promote their biological and physiological activities.

Artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support (Jhingran, 1991). The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion and chemical composition of fish (Hassan *et al.*, 1996; Jena *et al.*, 1998; Erfanullah and Jafri, 1998). Worldwide, approximately 80% of carps and 65% tilapia are cultured without modern compound feeds (Naylor *et al.*, 2000).

Feed is considered to be the major constituent of aquaculture inputs. The higher the culture technology applied, more the culture system relies on exogenous feed supply with natural food becoming less significant. Thus, the dependence switches away from natural food to supplementary feeding. Feed formulation is the process of combining feed ingredients to form a mixture that will meet the specific goals of production. These goals may be rapid growth rate, successful reproduction and induction of a vitamin deficiency or establishment of minimum dietary nutrient requirement (Mukhopadhyay, 1999).

Fish feed to obtain sufficient energy to meet the requirements of metabolism, growth, reproduction etc (Nose and Halver, 1981). Energy requirement of fish is met more from protein and lipid because most fishes may have obligate need for amino acids as an energy source. The dependence of carnivorous fish on a high protein diet derives from a highly developed capacity to metabolize protein and also for a limited ability to digest carbohydrates (Cowey and Luguët, 1983) though carbohydrates are as such not essential for cyprinids.

In pond culture, Indian major carps are generally fed on a mixture of rice bran and various varieties of oil cakes. In later form formulated feed containing dietary protein is provided for these species. For increasing productivity, emphasis is being laid on developing cost effective complete supplementary feeds. Survival rates normally range from 30 to 40 percent; however, survival often remains low due to improper management. The non-availability of commercial feed, forcing the farmers to resort to the conventional bran oil cake mixture, is another limiting factor for the growth and survival of fry. The survival level of catla in nursery ponds is normally lower than that for rohu and mrigal (FAO, 2014a). The major ingredients are rice bran, de-oiled groundnut cake, cottonseed meal, sunflower meal, soybean meal, mustard oil cake, wheat bran, common salt and mineral mixture (Nandeesh, M.C. *et al.*, 2013). Groundnut oil cake is used traditionally with rice bran in a 1:1 ratio for carp culture in India (shodhganga, 2014). Muhammad A. and Muhammad S. (2004) stated that, it is necessary to use artificial feed of good quality.

Common carp is very much like other teleosts in the way they digest carbohydrates. In the study of Foodie (1983), common carp grew well when fed on diets containing up to 45% of rice. Digestibility of up to 97% was recorded. Very low protein as well as carbohydrate digestibility in the control (neither cassava nor rice added) indicated that carp need carbohydrates. However, a high proportion of carbohydrate in diets could lead to high lipid deposition (Keshavanath *et al.*, 2002). Common carp like most other fish species cannot digest high molecular carbohydrates. Kaushik (1995) found that common carp cannot digest cellulose. Lesel *et al.*, (1986) did not find any cellulose activity in trout, goldfish or grass carp.

Soybean meal has been the most frequently studied dietary ingredient as a fish meal replacer in diets for many fish species because of its high protein content, relatively well balanced amino acid profiles, reasonable price and steady supply (Storebakken *et al.* 2000).

Oilcakes are also widely used for substitution of fish meal in diets of fishes. Groundnut oilcake is mostly used in fish diets due to its easy availability in market. Plant protein ingredients especially soybean and ground nut oil cake have Successfully substituted fish meal with nutritional and economic benefits (Fagbenro, 1999).

Egg albumin was taken. It contains 15% of proteins dissolved in water. It contains about 40 different types of proteins. It has high nutritive value. The proteins in egg white are,

ovalbumin: 64%, ovotransferrin: 12%, ovomucoid: 11%, ovomucin: 1.5%, globulin: 8%, lysosomes: 3.5% (USDA, 2008).

Plant products contain huge amount protein, different amino acid and fatty acids which are not available in animal protein (Mondal and Payra, 2015). Use of plant protein in fish feed industry has been tried for various commercial culture fish species as the formulation of feed is specific to species based on their specific requirements. The advantages lie not only in the availability and economic benefits but also that these plant products also have less amount of phosphate and nitrogen than animal protein, therefore, reducing the chances of eutrophication of pond.

. The plant *murraya koenigii* is an aromatic and more or less deciduous shrub or a small tree found throughout India up to an altitude of 1 500 m commonly in forests often as gregarious under growths. The species is native to India. It commonly occurs in the foothills of Himalaya, Assam, Sikkim, Kerala, Tamil Nadu, Andhra Pradesh and Maharashtra.

M.koenigii leaves have a green color and characteristic odour and taste. Exstipulate, bipinnately compound, 30 cm long, each bearing 24 leaflets having reticulate venation; several compounds have been isolated from different morphological parts of the plant. The alkaloids and essential oils are the most studied phytoconstituents of the plant. Apart from them, terpenoids, phenolics, minerals, protein, fat, carbohydrate, fibre, carotene, nicotinic acid, vitamin C.

The leaves are fragrant, strongly aromatic spicy, bitter, acrid, cooling and weakly acidic in taste. The essential oils impart intense characteristic flavor to the plant parts. Fresh leaves, dried leaf powder and essential oils are extensively used for flavoring soups, curries, chutneys, sausages, fish and meat dishes, pickles, butter milk preparations, curry powder blends, seasonings, ready-to-eat and many modern and other food preparations.

The whole plant is considered to be a tonic and stomachic. Roots and bark are stimulant and are applied externally for skin eruptions and poisonous bites, influenza, fever, itching, dropsy, bronchial asthma, eruptions, diarrhea, body aches, fresh cuts, kidney pains and used in dysentery.

Curry leaves boiled in oil till they are reduced to a blackened residue form an excellent hair tonic in retaining the natural pigmentation and also stimulating hair growth. South Indian women have mixed the leaves with fenugreek for centuries and applied as a paste to keep hair long, black and gleaming. Pesticide activities of curry leaves have also been reported.

Taxonomic position:

Phylum: Chordata

Class: Osteichthyes

Order: Cypriniformes

Family : Cyprinidae

Sub family: Cyprinae

Genus: *Catla*

Species: *Catla catla*

Compressed body is comparatively large with broad head. Mouth is wide, upper lip is thin and covered by skin of snout. Lower lip is moderately thick, broad and continuous post labial groove. Dorsal profile is more convex than that of abdomen. Gill opening is circular and body deepest at origin of dorsal (Talwar and Jhingran, 2001). Pectoral fins are long and extend to pelvic fins. Scales are conspicuously large, lateral line complete with 40-43 scales. In life, its colour is grayish on back and flanks, silvery-white below, fins dusky.

Catla is one of the renowned and the fastest growing of major carps. This fish is found in ponds, lakes, ox-bow lakes, beels, streams, rivers, canals etc. It is non-predatory and its feeding is restricted to the surface and mid waters. It is abundantly found in the Buriganga, Padma, Meghna and other principal rivers of Bangladesh. It resides in fresh or brackish water, being found within the tidal influences (Day, 1989).

Catla is a cultured and highly growing species, if it get proper food it may be weighted at 4 kg at only one year. Very delicious food and supply a huge amount of protein for Bangladeshis people. So, its demand is extensive. According to Basu *et. al.*, (1993), its flesh contains 19.2%

crude protein, 2-5% fat and 70% water. According to Ghosh *et al.* (1933), per gramme of its liver-oil contain 583 IU vitamin-A (Rahman, 2005).

The Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* are the most important, prestigious, commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste. They contribute about 67% of total freshwater fish production (ICLARM, 2001) and their total production is about 3.02 million tonnes (FAO, 2006).

Catla catla is one of the exotic fish species currently used in culture based fishery in inland reservoirs in Sri Lanka. *Catla* stocks are maintained in freshwater fish breeding stations of the National Aquaculture Development Authority (NAQDA) of Sri Lanka for continuous seed production for culture systems.

Nandeesh, *et al.* (2013) stated that in most of the country, the stocking of undersized fish seed continues to be a major hurdle to increasing fish production. Although a huge amount of carp seed is easily produced by employing the Chinese and/or 'jar' hatchery systems, the survival from spawn to fry stage is mostly still less than 20 percent. The fry are then reared to fingerling size if there is enough space, or else they are used for stocking once they reach 3–4 cm.

Saleem Chaudhry and Abid (2002) stated that the commercially important indigenous fish species of Pakistan are extensively cultured under semi-intensive culture system. The need for commercial producer of major carps has created interest to obtain maximum growth of fingerlings during the first year at high stocking densities. The intensive culture system is the first step towards industrial production methods offering several advantages (Meshe, 1983). This system is exclusively feed based and is expected to be main stay for increasing fish production in the years to come (Sinah, 1991). It is essential to work out specific whole feed based nutritional requirements of each species of major carp as each species has different dietary protein requirements (Chaudhary and Sheri, 1999).

The suitability of using formulated feed sources will provide cost effective management practices for efficient fish growth with cheaper feed ingredient under culture system in this herbivore fish, Indian major carp (*Catla catla*) for aquaculture candidate species. Indian major carp, *Catla catla* is a valuable herbivorous food fish in India.

THE OBJECTIVES OF THE PRESENT STUDY ARE

1. To prepare the fish feed with curry leaf in different composition with basal ingredient.
(Control and 3 different feed)
2. To estimate the biochemical compositions such as protein, carbohydrate, lipids and amino acid in the control and experimental feed.
3. To estimate the growth rate (length and weight) of *Catla catla* by feeding the control feed and experimental feed prepared from basal ingredients and prawn waste in different proportions.
4. To estimate and compare growth rate (length and weight) of *Catla catla* in the control and three different treatments during 60 days of the experimental period.
5. To estimate and compare the biochemical composition (protein, carbohydrate, lipids and amino acid) in control and fishes grown in three different feeds.
6. Comparison of feed utilization efficiencies – Feed Efficiency (FE), Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) in the fishes grown in the control and three different treatments.

REVIEW OF LITERATURE

The available literature pertaining to the present study entitled “**Efficacy of curry leaf formulated feed on growth, nutritional indices and feed utilization efficiencies of Indian major carp, *Catla catla* (Hamilton, 1822)**” is given in this chapter.

FISH FEED FORMULATION AND ITS EFFECT ON GROWTH

Johny *et al.*, (2000) studied the effect of dietary protein content on growth, food conversion and body composition of *Oreochromis niloticus* fingerlings fed with fish meal diet. They found that specific growth rates and body protein content increased with higher dietary protein.

Lee *et al.*, (2001) investigated the effects of dietary herbs on growth and body composition of juvenile *Abalones, Halitosis disus hannai*. Sivam (2001) reported that the garlic has several beneficial effects including antioxidant, antihypertensive and antimicrobial properties. Diab *et al.*, (2002) reported that incorporation of *Allium sativum* in tilapia diets improved feed intake and feed conversion ratio. They added that incorporation of garlic or fennel in Nile tilapia diets improved feed conversion ratio and protein efficiency ratio significantly compared to the control unsupplemented group.

Nadeesha *et al.*, (2001) noticed the growth performance of two Indian major carps, *Catla catla* and *Labio rohita* fed diets containing different level of *Spirulina plantesis* replaced fish meal protein from the standard diet at 25%, 50%, 75% and 100% levels. The result showed that no significant difference in the final weight attained by catla at all level of *Spirulina* feed compared to the fish meal based control diet. However the replacement of fish meal by more than 25% *Spirulina* resulted in significantly superior growth of rohu. SGR, protein efficiency ratio was improved rohu with higher levels of *spirulina* inclusion while in catla did not differ significantly from the control diet. In both species the digestibility of dry matter protein and fat was found to improve marginally with increasing levels of *spirulina* incorporation. Fat content was significantly higher in *spirulina* diets. The result of the present study clearly demonstrates that *S.platesis* could be exploited as a protein source for incorporation in Indian major carp diets. Cost effective technologies are available for the cultivation of *S.platesis* using various organic

wastes (Venkataraman and Becker, 1986) and these techniques could be effectively used for large scale production of *spirulina*, they concluded that usefulness of spirulina for partial or complete replacement of fish meal in the diets of Catla and Rohu.

Ji *et al.*, (2007) investigated the effect of dietary medicinal herb on growth and some specific immunity in juvenile red sea bream *pagrus major*. They concluded that survival, specific growth rate, feed efficiency, condition factor and hemoglobin levels were higher in fish given herbal diets.

Abdul kadhar *et al.*, (2014) studied the effect of live feed on the survival and growth of fry of *Catla catla* using three different live feeds cyclopoid, cladoceran, mixed diet (cyclopoid and cladoceran) carried out in 100ml tank for 40 days. Commercial feed (C) used as control. Result showed that cyclopoid and cladoceran mixed diet significantly better survival rate (54.80 ±2.43%) cyclopoid had showed significantly ($p < 0.001$) better growth- length (26.03 ±1.88mm) and weight (61.07±3.53mg) than those fed with other food types protein, carbohydrates and lipids showed higher level in cyclopoid diet. while comparing the three diets cyclopoid, cladoceran and mixed diet the cyclopoid diet can be used as live feed for effective production of catla fry.

Sanchez camargo *et al.*, (2011) investigated the proximate composition and extraction of carotenoids and lipids from Brazilian red spotted shrimp waste (*Farfantepenaeus paulensis*). The shrimp waste was found to have high protein and ash contents, but low lipid content. Guipu Li *et al.* ,(2011)analyzed the lipid content and fatty acid composition in the edible eat of twenty nine species of wild and cultured freshwater and marine fish and shrimps. Both the lipid content and fatty acid composition of the species where specified their unique food habit and trophic levels. Most of the marine fishes demonstrated higher lipid content than fresh water fish ,where as shrimps had the lowest lipid content all the marine fish and shrimps had 16much higher total n-3 PUFA than n-6 PUFA,while most of the fresh water fish and shrimps demonstrated much lower total n-3 PUFA than n-6 PUFA. This might be the biggest difference in fatty acid composition between marine and freshwater species. The cultured fresh water fish demonstrated higher perencentages of total PUFA, total n-3 PUFA than n-6 PUFA, and EPA+DHA than the wild freshwater fish.

Moron kola *et al.*, (2011) studied the proximate and mineral contents of crab (*Callinectes amnicola*) from the shore of Ojo river Lagos and Nigeria. The crude protein ranged between 19.2-28.3g /100g and the studies showed that the crab tissue rich in protein (28.00±0.071%). crude fiber and crude fat were found the range from 0.02 to 11.70% and 0.6-1.020%. The crude fiber is higher in the walking legs with the value of 11.070±0.037% when compare to tissue and the crunchy part with the value of 0.023±0.07 and 6.680±0.074% respectively. The moisture ranged from 67.37±0.026 to 70.046±0.049 in all analyzes. Ash content recorded an average value of 1.040±0.017%, 1.300±0.001% and 1.041±0.02% in crunchy, walking legs and tissue respectively therefore, crabs *Callinectes amnicola* could be a balanced human diet and could be employed as an alternative dietary supplement used as protein.

Tank culture experiment was conducted by Bello *et al.*, (2012) to assess the growth responses and nutrient utilization of walnut leaf and onion bulb residues *Clarias gariepinus*. Result showed that the fish on Bulp and walnut residue-based diets increase significantly the growth rate and nutrient utilization respectively.

Fall *et al.*, (2012) studied the effect of replacement of soybean meal by shrimp shell on the growth of hybrid tilapia (*Oreochromis niloticus x O.aureus*) reared under brackish water. The result showed that fish fed diet containing 100% shrimp shell meal (SSM) exhibited the lowest specific growth rate (SGR). There were no significant differences of SGR in the fish fed 0, 33, 50 and 67% SSM. The fish fed 0, 33 and 50% SSM had better feed conversion rate (FCR) value than those fish fed with 67 and 100%SSM supplement. The survival of juveniles ranged from 97 to 100% begin 97% for 0, 50, and 67% SSM and 100% for 33 and 100%SSM. The Weight gain was highest in fish fed diet without SSM, intermediate in fish fed diets with 33, 50 and 67% SSM, and lowest in fish fed diet with 100% SSM. The highest body protein content was observed in fish fed diets with the 50 and 67% SSM compared to the initial fish. The lowest body protein content was obtained with the fish fed diet with 100% SSM. The tilapia fed diet containing 33 and 67% SSM had the highest body lipid content compared to the initial fish. There were no significant differences in body lipid content among the fish fed diet with 0, 50 and 100% SSM. No difference in body ash content was observed among treatments.

Khan *et al.*, (2012) studied the replacement of fish meal by plant protein source in Nile tilapia (*Oreochromis niloticus*) diet and reported growth performance and feed utilization. The

result indicating the growth performance tended to decrease with increase in inclusion level of rice polish and mustard oil cake. The control diet (Fish meal 35) recorded the highest body weight gain (BWG) ($363.79 \pm 59.32\%$) and the least ($330.24 \pm 32.32\%$) was in diet FM25. specific growth rate (SGR) was followed the same trend and no significant differences of SGR was observed among the diets ($p > 0.05$). feed intake (FI) of different diets was ranged between 30.33g and 35.08g per fish at the end of this experiment. Feed intake was also declined with the increase in inclusion level of rice polish, through the feed conversion ratio (FCR) and protein efficiency ratio (PER) were not significantly different ($p > 0.05$) among the diets. The result of this study revealed that partial replacement of fish meal by rice polish and mustard oil cake would be cost effective without any significant change in growth performance.

Antolovic *et al.*, (2012) observed the effect of soybean meal (SBM) inclusion and treatment on growth of juvenile saddled bream. The result showed that, no significantly difference in body weight of fish fed with the other three diets. When compared to the control (diet 1), fish fed with the diets 2 and 3 did not show any significant difference in body protein content. Body fat content was significantly higher in fish fed with the control diet. Whole body ash content was significant higher in fish fed with diet 4.

Citarasu (2010) reported that hormones antibiotics, vitamins and several other chemicals have been tested in the aquaculture operations for various remedies and the alternatives herbal bio-medicinal products in the aqua cultural operation, that have the characteristics of growth promoting ability and tonic to improve the immune system acts as appetite stimulants. They increase consumption, include maturation and have antimicrobial capability and also anti stress characteristics that will be of immense use in the culture of shrimps and other fin fishes without any environment and hazardous problems.

Kasthuri *et al.*, (2012) studied the immune modulatory effect of *Adhatoda vasica* in *Oreochromis mossambicus*. This study attempted to identify the possible immune stimulatory activity of the medicinal plant *Adhatoda vasica*, so that it can be applied to aquaculture industry for the maintenance of health in the cultivable fishes. The active principle of *A. Vasica* responsible for the immune modulatory effect of the extract is yet to be identified. After confirming the immune modulatory properties of *Avarice* in studies involving other immunological parameters particularly those of nonspecific immunity and sufficient field trails,

the plant extract can be used in finfish aquaculture farms. Results of this study showed that use of immunotimulants, adjuvant and vaccine carries in fish culture offers a wide range of attractive methods for inducing and building up protection against diseases.

Oyebamiji *et al.*,(2013) carried out an experiment to assess the water parameter and biochemical composition of two fish species (*Tilapia niloticus* and *Sardinella aurita*)obtained from Azikwe River, Nigeria. The results compared with the standard levels recommended for the various nutrients and metals and thereafter statistically analyzed of variance (ANOVA) at 5% probability level.

Metwaly (2009) studied the effect of garlic (*Allium sativum*) in some antioxidant activities in *Tilapia nilotica* (*Oreochromis niloticus*) .The results showed that, weight gain and growth performances of *Oreochromis niloticus* significantly increased in all group fed on garlic with addition of garlic in any form to fish diet can promote growth rate, decrease mortality rate and increase the antioxidant activity in fish was suggested.

Gaber *et al.*,(2012) studied the effect of partially replacing corn meal by date stone on growth performance in Nile tilapia (*Oreochromis niloticus*) fingerlings, diets supplemented with digestarom. The result revealed that, mean final weight (g/fish), SGR (%/day), feed conversion ratio, PPV and PER, were significantly ($p \leq 0.05$) affected by the levels of DS (Data stone) and level of D (Digestarom).

Elegbede *et al.*, (2013) studied the proximate and mineral compositions of common crab species [*Callinectes pallidus* and *Cardisoma armatum*] of Badegry Creek, Nigeria. The proximate composition of meat and shell of both crabs were significantly different ($p > 0.05$), both crabs were high in calcium, potassium, magnesium and manganese. Zinc in meat of both crabs were not significantly different ($p < 0.05$), but sodium, iron and copper of meat (both crabs) were significantly different ($p > 0.05$).

A hold and van (2004) studied the dietary fatty acids and the stress response of the fish, arachidonic acid in sea bream and tilapia. The result indicates that short feeding period with a relatively minor dietary change in a single fatty acid , arachidonic acid, is sufficient to considerable alter several physiological function in Mozambique tilapia and gilthead sea bream. The two spices showed completely opposite responses to a stressor after their diets were

supplemented with arachidonic acid, while it had a positive effect on the osmoregulatory capacity of both species.

The effects of vitamin C supplementation on the growth of *Heterobranchus longifilis* fingerlings and reported that fish receiving the vitamin C supplemented diet had significantly improved weight gain, protein efficiency ratio, specific growth rates, feed efficiency ratio and survival rate. And they concluded that vitamin C is essential in the nutrition of these fishes by Ibiyo *et al.*, (2006).

Onion bulb can be used as a growth promoters and health management in African cat fish, *Clarias gariepinus*. The result of the study showed that onion bulb could increase body weight gain, feed intake and feed efficiency and it does not cause any damage to the physiological system and organelles of the organism. (Kumar and Anantharaja 2007).

Hagbayan and Mehrgan, (2015) studied the effect of replacing fish meal in the diet with enzyme-treated soybean meal (hp310) on growth and body composition of rainbow trout fry. The result showed that diets containing 75% and 100 % HP310 had significantly higher feed conversion ratio and lower feed intake, weight gain and specific growth rate compared to fish feed diets containing higher levels of fish protein ingredients ($p < 0.05$).

Bahrevar and Faghani- Langroudi., (2015) observed the effect of fish meal replacement by blood meal in fingerlings rainbow trout (*Oncorhynchus mykiss*) on growth and /fillet quality traits. These result showed that blood meal is not a suitable protein source as fish meal replacement for fingerlings rainbow trout. Growth, nutrient utilization and body composition were either not improved or were significantly influenced by gradually replacing fish meal by blood meal.

Soares *et al.*, (2015) observed the replacement of fish meal by protein soybean concentrate in practical diets for pacific white. Result showed that after 42 days, the weight gain of shrimp fed diets with 0 and 25% protein replacement was higher than that observed in shrimp fed 100% replacement, and there were no differences among those fed the other diets. Feed efficiency and survival did not differ among shrimp fed different protein replacements. There was a negative linear trend for growth parameters and feed intake as protein replacement with

soybean protein concentrate increased. Fish meal by – product can be replaced by up to 75% soybean protein concentrate, with no harm to the growth of pacific white shrimp.

Kathirvel *et al.* , (2014) studied the proximate ,amino acids and fatty acid and minerals analysis of box crab, *Calappa lophus* (herbst, 1782) from parangipettai, southeast coast of India. The *Calappa lophus* appendage and body region was average quantity of protein, carbohydrates, fat, ash and moisture in the level of (22.19 gm, 21.45 gm), (8.34 gm, 5.45 gm), (55.18 gm 61.19 gm), (2.56 gm, 2.98 gm), (73.11 gm, 82.98 gm) respectively.

Reddy *et al.*, (2014) investigated the study on the natural status of the fresh water prawn *macrobrachium rosenbergii* from Nellore coast, India. Fifteen different samples were selected randomly for the estimation of the proximate composition. The average values of the proteins, carbohydrates, lipids, ash and moisture ash in cultured and frozen prawns were recorded ash 74.24 ± 0.49 , 5.50 ± 0.34 , 9.09 ± 0.09 , 9.71 ± 0.19 , 77.14 ± 0.19 and 60.55 ± 0.35 , 8.23 ± 0.18 , 7.98 ± 0.13 , 21.61 ± 0.42 and 74.93 ± 0.23 , respectively. The higher amount of proteins, lipids was identified in the cultured prawn.

Gunalan *et al.*, (2013) studied the biochemical composition in *Litopenaeus vannamei* the results rewarded that *L. vannamei* species can be considered as a good source of fatty acids as well as protein rich space. Keramah, (2013) investigated the effect of replacement of fish-meal with crab-meal on growth and feed utilization of African giant cattle fish heterobranchus longifilis fingerlings. The result showed that fish feed with 40% crud protein (CP) fish-meal diet had the best growth as indicated in mean weight gain, 6.99 ± 1.70 g, specific growth rate, $4.05 \pm 0.19\%$ day⁻¹, feed conservation ratio, 1.12 ± 0.06 and protein efficiency ratio of 2.28 ± 0.12 . Diets with fish-meal (FM) performed better than crab meal (CM) diet. Percentage survival rate for FM and CM containing diets varied between 91.0 ± 1.63 and 100% and the condition of fish was not significantly different ($P > 0.05$). A part from whole body levels of CP in FM feed fish, moisture and fat contents were unaffected by dietary treatments.

Soltan and laithy (2008) conducted the experiment to determine the effect of probiotics and some spices as feed additives on the performance and behavior of the Nile Tilapia (*Oreochromis niloticus*). Result showed that supplementation of the basal diets with probiotics *B.subtilis* or Biogen and spices (garlic and fennel) significantly improved survival rate of Nile

Tilapia. A combination of *B.subtilis* and garlic and fennel showed the best fish survival rate but did not significantly differ from those recorded for the diets supplemented with probiotics or spices alone. Feed intake, feed utilization and growth performance of Nile Tilapia including final body weight, final body length, weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio were significantly high.

Mandeville *et al.*, (2009) observed the proximate analysis of the commercial shrimp waste indicated the presence of 94.6% protein and 4.2% fat on dry basis. Abdullash *et al.*, (2009) reported the proximate fatty acids (FA) and element compositions of two shrimp species, deep seawater rose shrimp (*Parapenaeus longirostris*) and red shrimp (*Plesionikamartia*). Amount of lipids in *P.martia* was found as 1.1 and 2.61% proportion of lipids in both shrimp was lower than that of marine fish.

Yang *et al.*, (2004) investigated the effect of replacement of dietary fish meal by meat and poultry by-product meal on growth and feed utilization of gibel carp, *Carassius auratus*. The results showed that feeding rate for that meat and bone meal (MBM₅₀) group was significantly higher than for other groups except the poultry by-product meal (PBM₅₀) group ($p < 0.05$). Growth rate in the MBM₁₅ group was significantly higher than that in the control ($p < 0.05$), while there was no significant difference in the growth between the control and other groups ($p > 0.05$). Feed efficiency and protein efficiency ratio in MBM₁₅ was significantly lower while that in MBM₁₅ was significantly higher ($p < 0.05$). Replacement of fish meal by MBM at 500 g kg⁻¹ protein significantly decreased apparent dry matter digestibility (ADCD) and gross energy (ADCE) while apparent protein digestibility (ADCP) was significantly decreased by the replacement of MBM or PBM ($p < 0.05$). The result suggests that MBM and PBM could be replaced up to 500 g kg⁻¹ of fish meal protein in diets for gibel carp without negative effects on growth while 150 g kg⁻¹ replacement by MBM protein improved feed utilization.

Ali Aberoumand (2011) conducted the study to compare the proximate composition and energetic value of three selected marine fishes and prawn. Prawn contained the highest protein compared to other fishes. The highest fat content among the three studied fish species was in 10 yellow fin Tuna fish at 6.89±2.76% while the fat content of prawn was 1.06%. Yellow fin Tuna fish contained the highest energetic value of 33.6 kcal/g of the entire sample.

Siva reddy et al (2013) observed the proximate composition of the prawn, *macrobrachium rosenbergii* from Andhrapradesh coast, India. Ten different samples were selected from both the sexes for the experimentation. Protein, moisture, fats and ash contents in female and male *macrobrachium rosenbergii* were measured as 25.92±0.40, 75.96±0.15, 5.01±0.42, 1.84±0.05 and 23.14±0.47, 74.16±0.75, 3.35±0.61 and 1.52±0.09 respectively. There was significant different between male and females in case of proteins, moisture, fat and ash.

Gunalan et al (2013) studied the biochemical composition in *Litopenaeus vannamei* the results rewarded that *L. vannamei* species can be considered as a good source of fatty acids as well as protein rich space. Keramah, (2013) investigated the effect of replacement of fish-meal with crab-meal on growth and feed utilization of African giant cattle fish *heterobranchus longifilis* fingerlings. The result showed that fish feed with 40% crud protein (CP) fish-meal diet had the best growth as indicated in mean weight gain, 6.99±1.70g, specific growth rate, 4.05±0.19% day⁻¹, feed conservation ratio, 1.12±0.06 and protein efficiency ratio of 2.28±0.12. Diets with fish-meal (FM) performed better than crab meal (CM) diet. Percentage survival rate for FM and CM containing diets varied between 91.0±1.63 and 100% and the condition of fish was not significantly different (P>0.05). A part from whole body levels of CP in FM feed fish, moisture and fat contents were unaffected by dietary treatments.

Hakim *et al.*, (2008) studied the effect of replacing soybean meal protein by other plant protein sources on growth performances and economical efficiency of mono sex Nile Tilapia (*Oreochromis niloticus*) cultured tanks. The result showed that replacement of 30% soy bean meal protein by sesame cake protein improved significantly final body weight of Nile Tilapia.

Amar *et al.*, (2008) investigated the efficacy of fermented prawn shell waste as a feed ingredient in Indian white prawn, *Fenneropenaeus indices*. The result showed the enhanced growth could be observed in prawn fed with Feed134 and Feed 124, incorporated with the fermentation products generated using *Bacillus* spp. The percentage survival rate of prawn after 7 days of challenges was found to be highest for groups feed diet F111 incorporated with fermentation products generated using *Bacillus* spp.

3. MATERIALS AND METHODS

Experimental animal

Freshwater fish, *Catla catla* were collected from the Pollachi district, Aliyar. They were safely brought to the laboratory in oxygenated plastic bags. They were stocked in fish tank and acclimatized for one week in ground water. During this period they were fed with control (.Rice bran, Soya bean meal, Coconut oilcake, Egg albumin, Sunflower oil, Tapioca flour, Vitamin E). The tank was cleaned periodically and water was infused at regular intervals to ensure sufficient oxygen supply to fish.

Experimental feed and feed formulation

The feed ingredients fish meal, soy meal, groundnut oilcake and rice bran was purchased from local market, sun dried and separately powdered using mixture grinder. The diets were prepared using such as FM, soybean meal, groundnut oilcake, rice bran, sunflower oil, egg albumin, tapioca flour and vitamin E and served as control feed. The FM in the basal diet was replaced with dried curry leaves at the following concentrations, 50%, 75%, and 100% and served as experimental feeds. The FM served as control feed. Egg albumin and tapioca flour were used as binding agents. Vitamin E and a pinch of salt were also added (Table 1). These substances were mixed with hot water and made into dough and boiled for 30 minutes. Then the boiled feed was taken out side, cooled and finally made into noodles. The moist noodles were dried for 3-5 days in the sun to prevent fungal attack and were broken into pieces of 0.5cm in length. Three different diets and control were prepared for the present investigation.

TABLE-1**Experimental treatments control and three different treatments**

Ingredient	Control (BI+FM)	Diet-1 (BI+FM+R50)	Diet-2 (BI+FM+R75)	Diet-3 (BI+FM+ R100)
Groundnut oil cake	25	25	25	25
Rice bran	10	10	10	10
Soybean meal	25	25	25	25
Egg albumin	6	6	6	6
Sunflower oil	2	2	2	2
Tapioca flour	6	6	6	6
Vitamin E	1	1	1	1
Fish meal	25	12.5	6.25	0
Curry leaves	-	12.5	18.75	25
Total	100	100	100	100

BI- Basal Ingredient, FM- Fishmeal, R- Replacements

Experimental design

The experiment was carried out in three different treatments in three replication and control. Each trough contained about eight individuals in the control and in three treatments. The study focused on the bio chemical composition both in the fish *Catla catla* and fish feed and also determination of nutritional indices in experimental fishes. The water level in each trough was maintained throughout the experimental period.

During this time, experimentally prepared curry leaves feed was given to the fishes. Before feeding biochemical composition such as protein, carbohydrate, fat, amino acid, moisture and ash were calculated in prepared fish feeds. Every fifteen days, growth performance were analyzed in terms of length and weight and biochemical composition such as protein, carbohydrate, fat, amino acid moisture and ash were calculated in the muscle tissues of the *Catla catla* fishes in control and three different treatment before and after the experiments. The medium was changed two days once in order to remove the faecal and unconsumed wastes

Evaluation of growth performance and feed utilization efficiency

The evaluation of growth performance and feed utilization efficiencies in *Catla catla* was performed by using a number of criteria. The growth and feed utilization efficiencies such as feed efficiency (FE), food conversion Ratio (FCR) and Protein Efficient Ratio (PER) was determined in the present investigation.

1. Growth measurement

Growth performance of fish was determined in terms of initial and final individual fish weight (mg/g) and length (cm/m)

Calculation

$$\text{Percentage of weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Percentage of length gain} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \times 100$$

$$\text{Specific growth rate (Ln)} = \frac{\text{Final body length} - \text{Initial body length}}{\text{Number of experimental days}} \times 100$$

$$\text{Specific growth rate (wt)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of experimental days}} \times 100$$

2. Feed utilization efficiencies

$$\text{Conservation Ratio (FCR)} = \frac{\text{Food fed (g)}}{\text{Weight gain (g)}}$$

*- as fed basis i.e. Dry weight

** - Wet or fresh weight gain

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Live weight gain (g)}}{\text{Protein consumed (g)}} \times 100$$

$$\text{Productive Protein Value (PPV)} = \frac{\text{Body protein gain (g)}}{\text{Protein consumed (g)}} \times 100$$

$$\text{Feed Efficiency (FE)} = \frac{\text{Weight gain (g)}}{\text{Food fed (g)}}$$

Biochemical composition

The moisture content (%) was estimated by subtracting the dry weight of the sample from the wet weight (APHA, 2005). Total protein (mg/g) content of the samples was estimated using bovine serum albumin as a standard (Lowry *et al.*, 1951). Carbohydrate (mg/g) content was estimated using the methodology of Dubois *et al.*, 1956 with glucose as a standard. Lipid (%) content was accessed by adopting Folch *et al.*, 1957 method. Amino acid (mg/g) content was estimated using methodology of Moore and Stein, 1948 method. To determine the ash content (APHA, 2005), samples were incinerated in muffle furnace at 600°C for 4 hours and. All the parameters were determined (in triplicates) on dry weight basis.

1. Estimation of total protein (Lowry et al., 1951)

Principle

Protein reacts with folin ciocalteus reagent to give a colored complex. The color formed is due to the reaction of alkaline copper with the protein at the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the color depends upon the amount of these aromatic acids present and thus varies for different proteins.

Reagents

- 1) **80% ethanol:** 80 ml of ethyl alcohol was dissolved in 20 ml distilled water.
- 2) **NaOH (0.1N):** 400 mg of NaOH was dissolved in 10 ml of distilled water.
- 3) **NaOH (1N):** 4 g of NaOH was dissolved in 100 ml of distilled water.
- 4) **Solution A :** 2 g of sodium carbonate was dissolved in 100 ml of 0.1N NaOH.
- 5) **Solution B:** Solution B was prepared by dissolving 500 mg of copper sulphate in 1% sodium potassium tartarate (1 g of sodium potassium tartarate in 100 ml of distilled water).
- 6) **Solution C:** Solution C was prepared by mixing 50 ml of solution A with 1 ml of solution B.
- 7) **Folin ciocalteus reagent:** Folin ciocalteus reagent was prepared by mixing 1 ml of folin ciocalteus reagent with 1 ml of distilled water.
- 8) **Blank:** 5 ml of solution C, 0.5 ml of 1N NaOH and 0.5 ml of folin ciocalteus reagent served as the blank solution served as the standard.
- 9) **Standard:** Bovine serum albumin (BSA) at the concentration of mg/ml and different dilutions form this stock solution

Procedure

Standard preparation

For plotting the standard curve, a set of standard were run 0.1, 0.2, 0.3, 0.4.....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 alkanin copper reagent was added, mixed and allowed to stand for 10 minutes at room temperature.

0.5 ml of 1N folin- ciocalteus reagent was then added to each tube and shaken well, the blue color 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 value on Y-axis and standard concentration on X-axis.

Sample preparation

100 mg of feed was homogenized with 2 ml of 80% ethanol. Then it was centrifuged at 5000 rpm at 4°C for 15 minutes. The precipitate was dissolved in 1N NaOH and made up to 5 ml. from this, 0.5 ml was taken and then 5 ml of folin ciocalteus reagent was added and the intensity of the color developed was read at 660 nm in a spectrophotometer.

Calculation

$$\text{Protein present in the sample (\%)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times \frac{\text{Conc. of the standard (mg)}}{\text{Weight of the sample (mg)}} \times 100$$

2. Estimation of carbohydrate (Roe et al., 1995)

Principle

Sulphuric acid hydrolyzes the di- and oligosaccharides into monosaccharides and converts the monosaccharides into furfural or furfural derivatives, which react with anthrone and produce a complex colored product.

Reagents

- 1) **80% ethanol:** 80 ml of ethanol was dissolved in 20 ml of distilled water.
- 2) **Anthrone reagent:** 200mg of anthrone powder was dissolved in 50 ml cold concentrated sulphuric acid. To this, 0.5 ml of thiourea was added to stabilize the color.
- 3) **Standard:** 100 mg of D-glucose was dissolved in 100 ml of saturated benzoic acid and different dilutions from this stock solution served as a standard.

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 volumes in each test tube were 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 / tissue was taken and it was homogenized well using 2 ml of 80% ethanol. Then it was centrifuged at 5000 rpm for 15 minutes at 4°C. To the clear supernatant (0.5ml), 4 ml of anthrone reagent was added and the test tubes were kept in a boiling water bath for 15 minutes. The test tubes were taken out and kept in a dark room for 10 minutes and finally the color developed was measured at 620 nm in a spectrophotometer.

Calculation

$$\text{Carbohydrates present in the sample (\%)} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

3. Estimation of lipid (Folch et al., 1957)

Principle

The quantitative determination of lipid by gravimetric method using chloroform methanol mixture (3:1).

Reagent

Chloroform methanol mixture (3:1)

Procedure

100mg of fish sample was weighed separately and ground well 5 ml of chloroform methanol mixture. The homogenate was centrifuged, taken in a small weighted beaker and this beaker was placed inside a large beaker filled with water along the side 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 was evaporated in oven at above 60°C. The beaker was weighted and the different between the final and initial weight of the beaker gives the lipid content of the tissue.

Calculation

Lipid present in the Sample (%) = Final weight – Initial weight

4. Estimation of ash and moisture (APHA, 2005)

Principle

The sample was allowed to dry by kept in desiccators. The different between the wet weight of the tissue and its dry weight give the amount of water present in the fresh tissue. On heating the dry material to higher temperature all the organic constituents were burnt leaving only the inorganic constituents in the form as ash.

Procedure

Known amount of wet sample was taken individually on previously weight concave glass and they were kept in desiccators, maintaining 0.5% relative humidity. Dry the tissues in the desiccators till they reached a constant weight. Then the dried materials were transferred individually in silica crucible and kept in a muffle furnace and heated at 550-600°C for 4 h.

Calculation

$$\text{Moisture} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100$$

$$\text{Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

5. Estimation of Amino acid (Moore and Stein, 1948)

Principle

When amino acid is heated with ninhydrin, they undergo deamination and a beautiful blue or purple colour developed, which is of value in both qualitative and quantitative determination of amino acid. The keto acid formed in the oxidative deamination is decomposed by heat into an aldehyde and carbon dioxide.



Hydrantin reacts with some of the ninhydrin to form Rothmans

purple. **Reagents**

- 1) **10% Sodium tungstate:** Prepared by dissolving 10 g Sodium tungstate in 100 ml-distilled water.
- 2) **2/3N.H₂SO₄:** 6.6 ml of concentrated sulphuric acid was diluted to 350 ml with distilled water.
- 3) **Ninhydrin reagent:** It was prepared by dissolving 0.5 g of ninhydrin in 12.5 ml ethanol and stored in refrigerator.
- 4) **1% Leucine solution (standard solution):** 100 mg Leucine was dissolved in 10 ml of 80% ethanol.

Procedure

From each group 1 g of tissue/feed sample was accurately weighed and homogenized individually with 2ml distilled water, to this 1 ml of sodium tungstate and 1 ml 2/3 N H₂SO₄ were added. This mixture was then centrifuged at 3000 rpm for 10 minutes and the supernatant were collected. Three test tubes were taken and labeled as blank, test and standard. 0.5 ml supernatant was added to the test tube 'test', 0.5 to 'standard' and 4.5 ml distilled water was added to both test tubes. 5ml distilled water was added to the blank. 0.5 ml ninhydrin was pipette to all test tubes and were cotton plugged. The tubes were kept in boiling water bath until blue color developed. The tubes were cooled and the intensity of the colour developed was measured with colorimeter at 540 nm.

Calculation

$$\text{Amino acids present in the sample (\%)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times \frac{\text{Conc. of the standard (mg)}}{\text{Weight of the sample (mg)}} \times 100$$

Statistical analysis

Results were expressed as Mean \pm SD. The data obtained were analyzed for significant differences ($p < 0.05$) by two way analysis of Variance (ANOVA) and mean separation were accomplished by Duncan's Multiple Range Test (DMRT) using STATISTICAL software (Stat soft Inc. 1999).



Figure -1: Fresh water fish *Catla catla*



Figure-2: Acclimatization of experimental fish, *Catla catla* in laboratory condition



Figure-3: Fish feed prepared from basal ingredients with fish meal and curry leaves in different concentration

Basal ingredient – soybean meal, groundnut oilcake, wheat bran, sunflower oil, egg albumin, tapioca flour and vitamin E.

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)



Figure-4: Experimental set up shows fresh water fish *Catla catla* in control and three different treatments

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(B

4. Results and discussion

The results of the present investigation on the “Efficacy of curry leaf formulated feed on growth, nutritional indices and feed utilization efficiencies of Indian major carp, *Catla catla* (Hamilton, 1822)” are presented below.

4.1. Growth performances

4.4.1. Weight gain (g)

Weight gain in *Catla catla* during different days of the experiment in the control and three different treatments are presented in table 2 and figure 5.

Among the three different treatments (T1, T2 and T3) and control, the maximum weight gain was observed in the fishes grown in T2 (8.02%) followed by T1 (3.80%), T3 (4.90%) on the 15 day of the experiment and the minimum value (1.30%) was recorded in the control fishes.

On the 30th day, maximum weight gain (16.28%) was noticed in the fishes grown in T2 followed by T3 (15.87%) and T1 (13.83%) and minimum value (0.81%) was observed in the *Catla catla* grown in the control.

On the 45 day, maximum weight gain (25.73%) was observed in the fishes grown in T2 followed by T3 (16.30 %) and T1 showed 23.70% weight gain and the minimum value (20.01%) was recorded in control.

On the 60th day of the experiment maximum value (35.68%) was observed in T2 followed by T1 (31.87%) and T3 (32.04%), minimum value (31.40%) was noticed in the *Catla catla* grown in the control.

During the experimental period (15, 30, 45 and 60 days), among the three different treatments and control the maximum weight gain was noticed in T2 fishes when compare to the other treatments and minimum weight gain was observed in the control. The growth indices showed significantly differences from those produced by the diet. Guo *et al.*(2007) noticed that the highest protein(36.3%) and fat content in the poultry by-product meal increased the weight

gain, specific growth rate and muscle proximate composition (moisture, protein and lipid) in cineaste drum, *Nibea miichthioides*.

4.1.2. Length gain (cm)

Length gain in *Catla catla* during different days of the experiment in the control and three different treatments are presented in Table 3 and Figure 6.

On the 15 day of the experiment, the maximum length gain (10.11cm) was observed in the grown fishes in T1 followed by T2 (7.93cm), T3 (7.27cm) and the minimum value (2.53cm) was estimation in the control fishes. On the 30th day, maximum value (17.58cm) was observed in the T2 followed by T1 (17.10cm) and T3 (7.27cm). Minimum value (9.00 cm) was noticed in the *Catla catla* grown in the control fishes.

On the 45 day of the experiment, the maximum value (29.14cm) was estimated in T1 followed by T2 (25.68cm) and T3 (14.40cm) and the minimum value (11.47cm) was recorded in control fishes. On the 60 day, maximum length gain was recorded in T2 (42.22cm) followed by T1 (37.13cm) and T3 (16.63cm) and the minimum value (15.25cm) was recorded in control fishes.

Among the three different treatments the maximum length gain was observed in T1` fishes during 15, 30, 45 and 60 days and minimum length gain was observed in the *Catla catla* grown in control fishes.

Feed utilization efficiencies

Feed conversion ratio (FCR)

Feed conversion ratio (g) in *Catla catla* during days of the experiment in the control and three different treatments are presented in Table 4 and Figure 7.

On the 15 day of the experiment, the maximum value (1.05g) observed in T2 fishes followed by T1 (0.96g), T3 (0.93cm) and the minimum values (0.90g) was recorded in the control.

On the 30 day, maximum value was noticed in the fishes grown in T2 (0.93g) followed by T1 (0.86g) and T3 (0.78g) and the minimum value (0.71g) was observed in the control.

On the 45 day, maximum value was observed in the fishes grown in T2 (0.79g) followed by T3 (0.72g) and T1 (0.50g). Minimum value (0.71g) was recorded in the control.

On the 60 day of the experiment maximum value was observed in the T2 (0.68g) followed T3 (0.66g) and T1 (0.60g). Minimum value (0.53g) was noticed in *Catla catla* grown in the control.

During the experimental period (15, 30, 45 and 60 days), among the three different treatments and control the maximum value was noticed in T2 feed and a minimum value was observed in the control. Feed with poor water stability disintegrate rapidly and cause feed waste, water pollution and give poor feed conversion ratio(FCR) (sanhotra,1994). Feeding rate is important for the growth, feed conversion, nutrient retention efficiency and chemical composition of body tissue (Storebakken and Austreng, 1987). The high-energy diet produced the lowest FCR and the highest nutrient retention (Coyle *et al.*, 2004; Zamal *et al.*, 2009)

Feed conversion ratio is the preeminent factor to measure the rate of feed accepted by fish and ultimate fish growth performance (Inayat and Salim, 2005).

Feed efficiency (FE)

Feed efficiency (g) in *Catla catla* during the experimental period in the control and three different treatments are presented in table 5 and figure 8.

On the 15 day of the experiment, the maximum value (1.19g) was observed in T2 fishes followed by T3 (1.05g) T1 (1.03g) and the minimum values (0.91g) was recorded in the control.

On the 30 day, maximum value was noticed in the fishes grown in T2 (1.38g) followed by T1 (1.17g) and T3 (1.27g) and the minimum value (1.07g) was observed in the control.

On the 45 day, maximum value (1.69g) was observed in the fishes grown in T2 followed by T3 (1.39g) and T1 (1.07g). Minimum value (1.01g) was recorded in the control.

On the 60 day of the experiment maximum value (1.89g) was observed in the T2 followed by T3 (1.50g) and T1 (1.62g). Minimum value (1.46g) was noticed in *catla catla* grown in the control.

During the experimental period (15, 30, 45 and 60 days), among the three different treatments and control the maximum value was noticed in T2 feed and a minimum value was observed in the control feed. Per and fcr are also generally related to digestibility of nutrients (Jabir *et al.*, 2012).

Biochemical composition (protein, carbohydrate, lipids, moisture and amino acids) in control and three different feeds

The protein, carbohydrate, lipids and moisture in control and three different feeds are shown in Table 7 and Figure 10.

Protein (mg/g)

The maximum protein content was observed in T2 feed (38 %) followed by T3 (34 %) and T1 (37%). Minimum protein content was noticed (30%) in control feed. The protein content was high in T2 feed when compared to other two feeds and control.

Protein plays a very important role in the maintenance of blood glucose level (Jrueger *et al.*, 1968). Proteins serve as building blocks for cellular and organic structure. Proteins form components of catalysts called enzymes which are essential for the metabolic reactions.

Carbohydrate (mg/g)

The maximum carbohydrates content was recorded in T2 feed (12%) followed by T1 (10%), T3 (18%) and the minimum carbohydrates content was noticed by control feed (4%). Carbohydrates are utilized by the cells mainly in the form of glucose.

Lipids (mg/g)

Lipid content was high in T2 feed (17%) when compared to T3 (14%) and T1 (15%). Lipid content was low in control feed (0.07mg/g). The greater protein and lipid content represents higher energy density (Dempson *et al.*, 2004).

Amino acids (mg/g)

Amino acids was maximum in T3 (mg/g) followed by T2 (5.23mg/g) and T1 (4.90mg/g). The minimum amino acid content was noticed in control feed (4.60mg/g).

Biochemical composition (protein, carbohydrate, lipids, moisture and amino acids) in *Catla catla*

Protein (mg/g)

Protein content in the muscle tissue of *Catla catla* in the control and three different treatments are presented in Table 8 Figures 11.

Protein content in the muscle tissue of *Catla catla* during 15 day, was maximum in T2 (18.10%) fishes followed by T1 (17.87%), T3 (17.10%) and control (16.27%).

During 30 day, maximum protein content was observed in T2 (18.40%) followed by T1 (18.30%) and T3 observed equal value (17.33%) of protein content. The minimum value (16.90%) was observed in *Catla catla* grown in the control.

During 45 day, maximum value was observed in T2 (18.87%) followed by T1 (18.57%) and T3 (17.50%) and minimum value (17.02%) was observed in control fishes.

During 60 day, maximum value (19.57%) was observed in T2 followed by T1 (18.77%) and T3 (17.80%). The minimum value (17.30%) was observed in control fishes.

Among the three different treatments and control the maximum value was observed in the T2 fishes during 15, 30, 45 and 60 days and minimum protein content was observed in the control fishes.

Protein requirement for optimal growth and feed efficiency of fingerling fish usually ranged from 40 to 45 % depending upon species (Harpaz *et al.*, 2001). Protein is one of the most prominent biochemical compounds of cancans and its quantity in this class of organism is largely influenced by the extent of fat. Soybean meal incorporation improved the palatability of feeds and also enhanced the protein digestibility (Jose *et al.*, 2006)

4.4.2 Carbohydrate (mg/g)

Carbohydrate content in the muscle tissue *Catla catla* in the control and three different treatments are presented Table 9 Figure 12.

Carbohydrate content in the muscle tissue of *Catla catla* during 15 day of the experiment was maximum in (12.03%) in T2 followed by T1 (11.30%) and T3 recorded the (10.17%) of carbohydrate content. The minimum value (9.30%) was observed in the *Catla catla* grown in the control.

During 30 day, maximum value was observed in T2 (12.07%) followed by T1 (11.40%) and T3 (10.37%). The minimum value (9.30%) was observed in control fishes.

During 45 day, maximum value was observed in T2 (12.84%) followed by T1 (12.07%) and T3 (10.67%). The minimum value (9.80%) was recorded in control fishes.

During 60 day, maximum value was observed in T2 (13.20%) followed by T3 (10.87%), T1 (12.40%). The minimum value (10.10%) was noticed in control fishes.

Among the three different treatments and control the maximum carbohydrate content was observed in T2 fishes during 15, 30, 45 and 60 days and minimum carbohydrate content was observed in T2 fishes during 15,30,45 and 60 days and minimum carbohydrate content was observed in the control fishes.

Carbohydrates are utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted into glycogen for stora.

Lipids (mg/g)

Lipid content in the muscle tissue of *Catla catla* in the control and three different treatments are presented in Table 10 Figure13.

Lipid content in the muscle tissue of *Catla catla* during 15 day of the experiment, the maximum value was observed in T2 (1.30%) followed by T1 (1.23%) and in T3 (1.10%) of lipid content was noticed. The minimum value (1.10%) was observed in the *Catla catla* grown in the control.

During 30 day, maximum value was observed in T2 (1.87%) followed by T3 (1.27%) and T1 (1.50%) and in control minimum value (1.28%) was analyzed.

During 45 day, maximum value was observed in T2 (2.10%) followed by T1 (1.90%) and T3 (1.50%) and in control minimum value (1.50%) was estimated.

During 60 day, maximum value was observed in T3 (2.50%) followed by T2 and T1 which of lipid (2.10%) and in T3 (1.70%) of lipid and in control minimum value (01.70%) of lipid was observed.

Among the three treatments and control the maximum lipid content was observed in the T2 fishes during 15, 30, 45 and 60 days and minimum lipid content was observed in the *Catla catla* grown in the control.

Lipids are highly efficient as sources of energy and they contain more than twice the energy of carbohydrates and proteins (Okuzumi and Fujii 2000). Deficiency of lipid in the diets has been reported to induce mortality of larvae and pl of shrimp (Chandge and Ral, 1990).

The level of phospholipids in refined fish oil, on the other hand is low. The triglycerides/ phospholipids ratio in the residual lipid content in fish meal is about 2:1 of total lipids (Tocher *et al.*,2008). Phospholipids acts as surfactants in the intestine during lipid digestion and may thus improve lipid emulsification and increase lipid digestibility in fish (Hung *et al.*, 1997; Tocher *et al.*, 2008). Carcass lipid content positively correlated with dietary lipid level irrespective of protein level and carcass protein, fat and ash content increased with increasing dietary lipid (Subhadra *et al.*, 2006).

Moisture (%)

Moisture content in the muscle tissue of *Catla catla* in the control and three different treatments are present in Table 11 Figure 14.

Moisture content in the muscle tissue of *Catla catla* during 15 day, maximum value (49.33%) was recorded in T2 (44.00%), T3 (42.67%) and the in control the maximum value (30%) was noticed.

During 30 day, maximum content (57%) was observed in T3 followed by T2 (49.67%) and T1 (41.67%). The minimum value (36%) was observed in control fishes.

During 45 day, maximum value was observed in T2 (55.67%) followed by T3 and T1 where equal values of moisture content (47.67%) was noticed, in T1 (46.33%) and control was minimum value (45.67%) was estimated.

During 60 day, maximum value (55%) was observed in T3 followed by T2 (53.67%) and T1 (45.33%). The minimum value (34.67%) was observed in control fishes.

Among the three different treatments and control the maximum value was observed in T3 fishes during 15, 30, 45 and 60 days minimum moisture content was observed in the control fishes.

Moisture and fat usually vary inversely in fish flesh, while the protein is more constant (Bela and Assem, 1995). Moisture is required for normal functioning of many biological molecules. It is present in two forms, bound to the protein and in the free form (Stanchena, 2003). There exists an inverse relationship between the moisture and lipid content. The percentage of water is a good indicator of its relative content of energy, proteins and lipids (Olagunju *et al.*, 2012).

Amino acids (mg/g)

Amino acid content in the muscle tissue of *Catla catla* in the control and three different treatments are presented in Table 12 Figure 15,

Maximum Amino acid content in the muscle tissue of *Catla catla* during 15 day of the experiment (4.43mg/g) was obtained in T2 followed by T3 (43.3mg/g) and T1 (3.67mg/g) and the minimum value (3.53mg/g) was recorded in the *Catla catla* grown in the control.

During 30 day, maximum amino acid content (5.23mg/g) was observed in t2 followed by T3 (5.20mg/g) and T1 (4.50mg/g) and minimum value (4.33mg/g) was observed.

During 45 day, maximum value (5.96mg/g) was observed in T2 followed by T3 (5.80mg/g) and T1 (4.83mg/g) and control in the minimum value (4.33mg/g) was observed.

During 60 day, maximum value (6.77mg/g) was observed in T2 followed by T3 (6.25mg/g) and T1 (5.50mg/g). The minimum value (5.10mg/g) was observed in control fishes.

Among the three different treatments and control the maximum value was observed in t2 fishes during 15, 30, 45 and 60 days and minimum amino acid content was observed in the control. Amino acid is the buildings blocks of proteins and also plays a central role as intermediates in metabolism (Baldwin, 2003)

Ash (%)

Ash content in the muscle tissue of *Catla catla* in the control and three different treatments are presented in Table 13 Figure 16.

Ash content in the muscle tissue of *Catla catla* during 15 day of the experiment maximum value (50.33%) was obtained in T2 followed by T3 (48.67%) and T1 (37%) and control in minimum value (36.33%) was observed.

During 30 day, maximum amino acid content (50.33%) was observed in T2 followed by T1 (49.67%), T3 (38.33%) and minimum value (37%) was observed in control fishes.

During 45 day, maximum value (59.67%) of ash content was observed fishes grown in T2 followed by T3 (49.33%) and T1 (40.33%) was observed in the *Catla catla* grown in the

control. During 60 day, maximum value (56.33%) was observed in t2 followed by T1 (48%) and T3 (44.67%). The minimum value (44.33%) was observed in control fishes.

Among the three different treatments and control the maximum value was observed in T3 fishes during 15, 30, 45 and 60 days and minimum value was observed in the control fishes. Ash is the measure of the mineral content of a food item, this results indicates that the prawn waste are good sources of miner

Table-2

Weight gain (g) in *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	1.65±0.25	9.06±1.10	20.01±1.48	31.40±0.69
T1	3.29±1.12	13.83±1.88	23.70±3.23	31.89±2.89
T2	8.01±0.58	16.28±0.39	25.72±2.57	35.65±0.94
T3	4.90±1.39	15.87±2.06	16.30±2.52	32.02±2.10
SEd	1.5602			
Cd(p<0.05)	2.9321			

Values are mean ± SD of the three samples in each

group C-Control (BI+FM)

T₁-Treatment 1(BI+FM+R50)

T₂-Treatment 2(BI+FM+R75)

T₃-Treatment 3(BI+R100)

Table-3

Length gain(cm) in *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	2.53±0.21	9.00±0.95	11.47±1.20	15.25±1.36
T1	10.11±2.67	17.10±2.50	29.14±2.16	37.13±0.02
T2	7.93±0.76	17.58±0.75	25.68±1.97	42.22±1.36
T3	7.27±1.32	13.73±1.25	14.40±3.45	16.63±1.42
SEd	2.09878			
Cd(p<0.05)	4.24187			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table4

Feed efficiency (FE (gg⁻¹) in *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	0.91±0.04	1.07±0.03	1.01±0.75	1.46±0.06
T1	1.03±0.01	1.17±1.10	1.69±0.06	1.62±0.04
T2	1.19±0.02	1.38±0.04	1.27±0.03	1.89±0.04
T3	1.05 ±0.07	1.27±0.06	1.39±0.10	1.50±0.08
SEd	0.14767			
Cd(p<0.05)	0.29850			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table-5

Feed conversion ratio (FCR gg^{-1}) in *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	0.93±0.12	0.71±0.04	0.71±0.08	0.53±0.01
T1	0.96±0.01	0.86±0.09	0.50±0.02	0.60±0.02
T2	1.05±0.05	0.93±0.02	0.79±0.02	0.65±0.03
T3	0.93 ±0.06	0.78±0.04	0.72±0.02	0.66±0.01
SEd	0.0335			
Cd(p<0.05)	0.09512			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table -6

Protein efficiency ratio (PER (g gain/ g protein intake) in *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	27.07±1.21	47.97±3.76	53.87±1.69	54.64±7.45
T1	47.03±2.82	62.67±2.56	66.43±4.03	90.33±11.33
T2	64.67±1.88	69.87±5.06	74.97±5.71	69.47±5.64
T3	54.33±4.18	58.47±5.11	57.77±1.99	58.60±1.51
SEd	3.95018			
Cd(p<0.05)	7.98377			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table -7

Protein, carbohydrates, lipids and amino acids (mg/g) in the control and three different feeds

Treatments	Feed			
	Protein	Carbohydrates	lipids	amino acids
Control	20.00±0.21	5.06±0.15	0.07±0.02	4.60±0.36
T1	27.00±0.11	5.53±0.06	0.08±0.03	4.90±0.49
T2	30.00±0.15	7.73±0.59	0.33±0.41	5.23±0.47
T3	34.63±0.31	6.43±0.50	0.11±0.01	6.10±0.35

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table -8

Protein (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	16.27±0.15	16.90±0.20	17.20±1.65	17.30±1.80
T1	17.87±0.25	18.30±0.20	18.57±0.15	18.77±0.15
T2	18.10±0.10	18.40±0.20	18.87±0.15	19.57±0.25
T3	17.10±2.10	17.35±1.46	17.50±0.20	17.80±0.20
SEd	0.66020			
Cd(p<0.05)	1.33434			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table-9

Carbohydrate (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	9.00±0.50	9.30±0.30	9.80±0.10	10.10±0.20
T1	11.20±0.20	11.40±0.20	12.07±0.21	12.40±0.20
T2	12.03±0.15	12.47±0.25	12.84±0.21	13.20±0.30
T3	10.17±0.25	10.37±0.25	10.67±0.25	10.87±0.15
SEd	0.20180			
Cd(p<0.05)	1.68143			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table-10

Lipid (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	1.10±0.65	1.28±0.82	1.50±0.59	1.70±0.64
T1	1.23±0.15	1.50±0.20	1.90±0.20	2.10±0.20
T2	1.30±0.20	1.87±0.31	2.10±0.20	2.50±0.20
T3	1.10±0.20	1.27±0.25	1.50±0.20	1.70±0.20
SEd	0.29391			
Cd(p<0.05)	0.59403			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table -11

Amino acids (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
control	3.53±1.53	4.03±1.53	4.33±1.53	5.10±2.64
T1	3.67±1.15	4.50±2.00	4.83±2.08	5.50±1.00
T2	4.43±1.53	5.27±4.04	5.96±3.21	6.67±2.52
T3	4.20±2.00	5.20±3.00	5.80±1.73	6.26±0.59
SEd	1.68000			
Cd(p<0.05)	3.37526			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

Table -12

Moisture (%) (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	30.00±2.00	36.00±1.00	45.67±9.02	34.67±2.52
T1	40.00±12.53	41.67±10.69	46.33±1.15	45.33±10.50
T2	44.00±3.61	49.67±6.66	55.67±9.24	53.67±9.07
T3	42.67±6.81	57.00±10.39	47.67±7.51	55.00±4.36
SEd	6.91295			
Cd(p<0.05)	13.97189			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM)

T3-Treatment 3(BI+R100)

Table -13

Ash (%) (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

Treatments	No of days during experiment			
	15 days	30 days	45 days	60 days
Control	36.33±1.53	37.00±5.57	40.30±7.37	44.33±2.09
T1	50.33±6.66	49.67±6.66	40.33±4.16	48.00±7.81
T2	37.00±3.00	53.33±1.53	59.67±5.13	56.33±3.21
T3	48.67±6.66	38.33±8.02	49.33±5.13	44.67±5.86
SEd	5.72228			
Cd(p<0.05)	11.56540			

Values are mean ± SD of the three samples in each group

C-Control (BI+FM)

T1-Treatment 1(BI+FM+R50)

T2-Treatment 2(BI+FM+R75)

T3-Treatment 3(BI+R100)

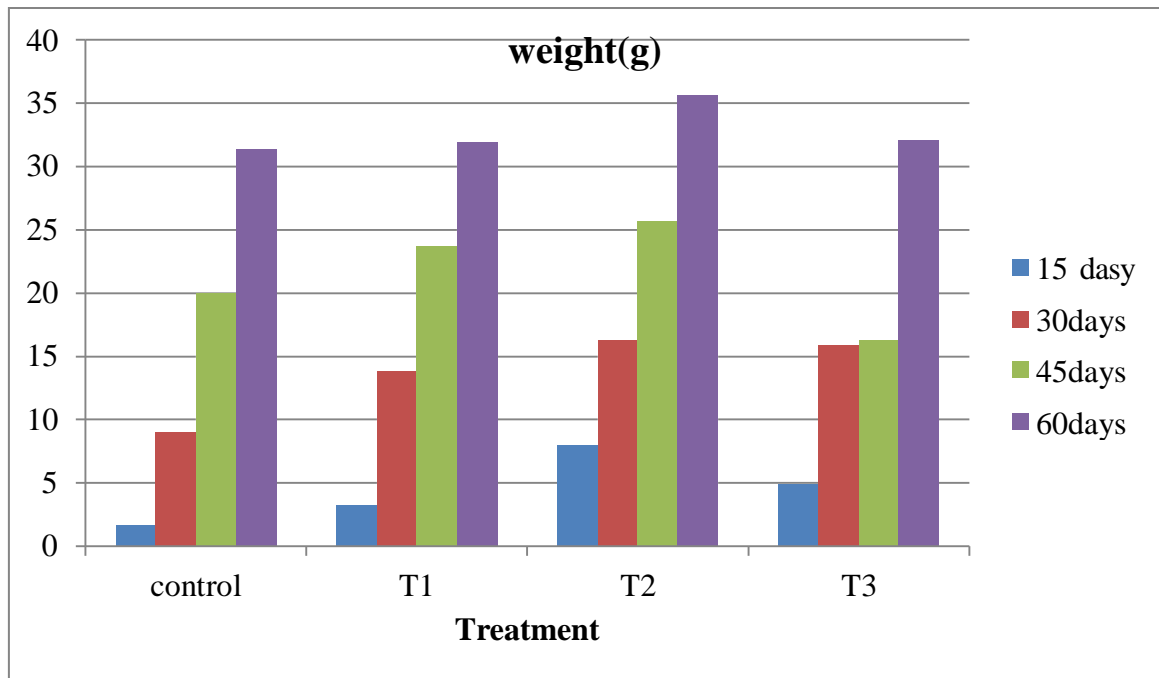


Figure-5

Weight gain (g) in *Catla catla* during different days of the experiment in control and three different treatments

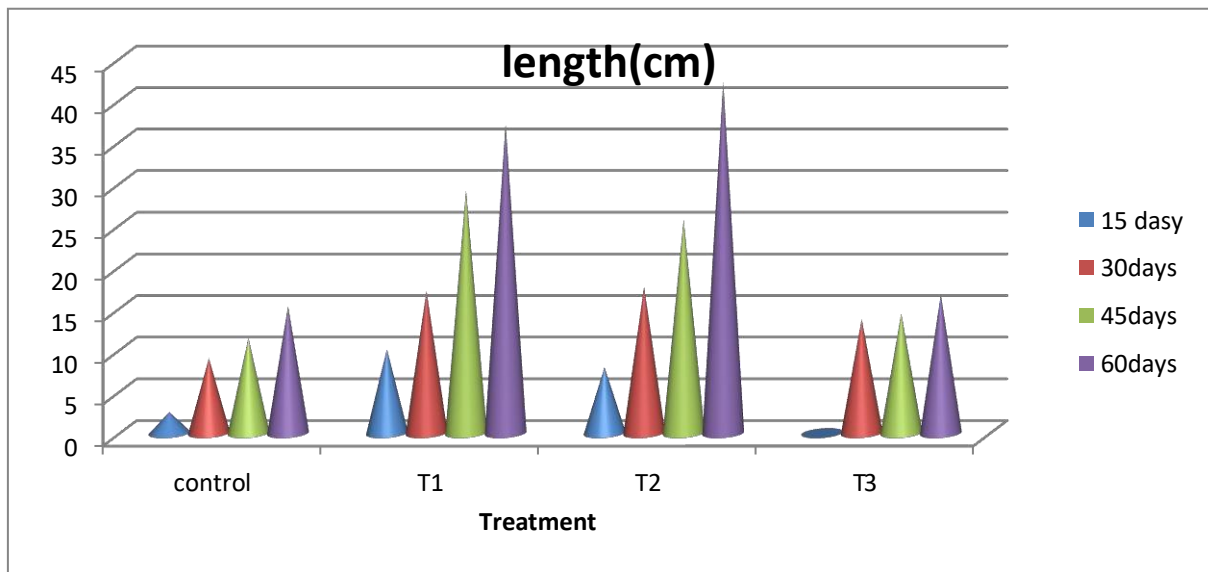


Figure-6

Length gain (cm) in *Catla catla* during different days of the experiment in control and three different treatments

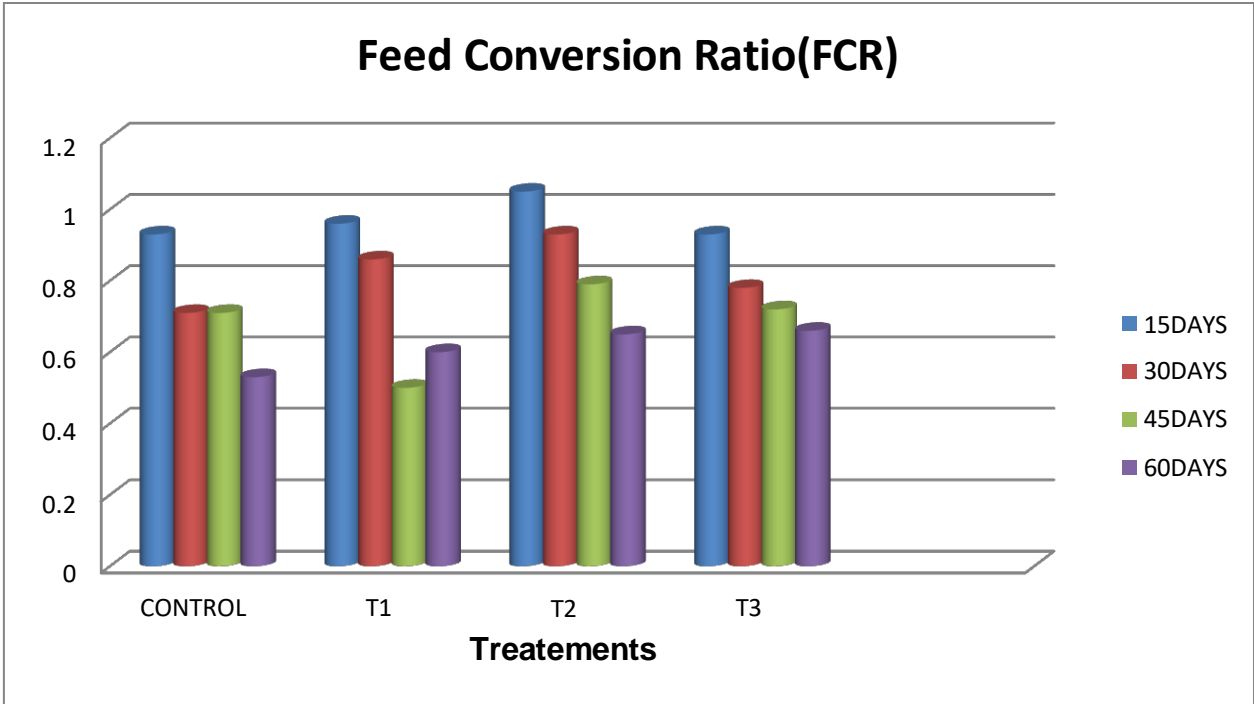


Figure-7

Feed conversion ratio (FCR (g g^{-1}) in *Catla catla* during different days of the experiment in the control and three different treatments

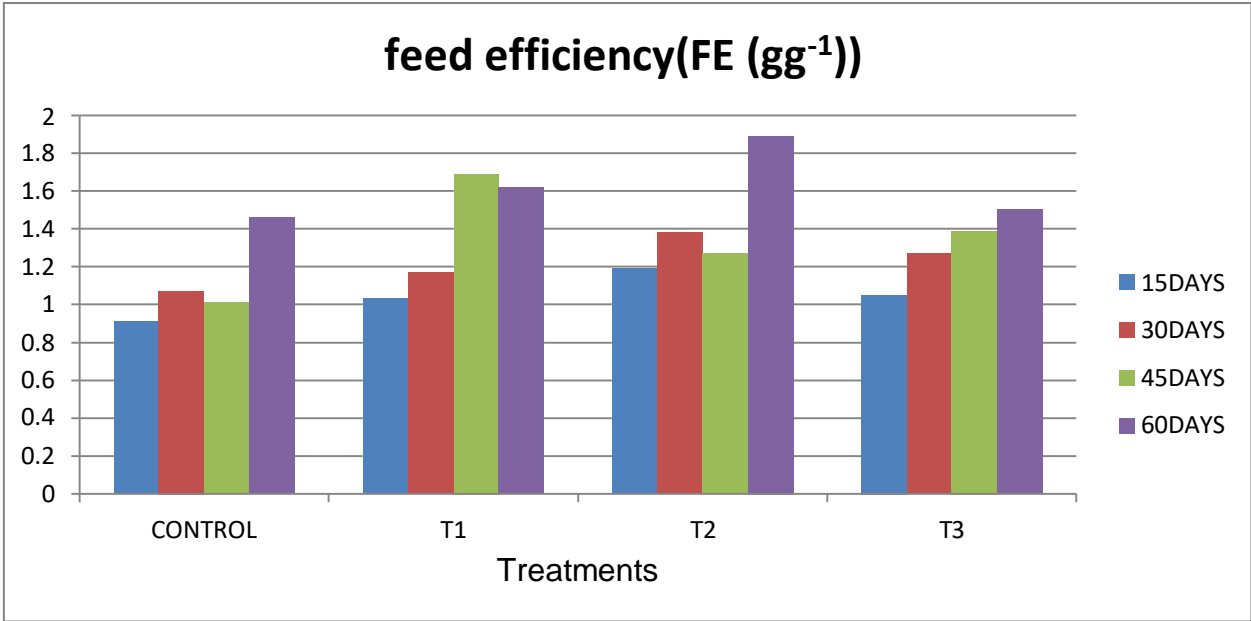


Figure-8

Feed efficiency (FE (gg⁻¹)) in *Catla catla* during different days of the experiment in the control and three different treatments

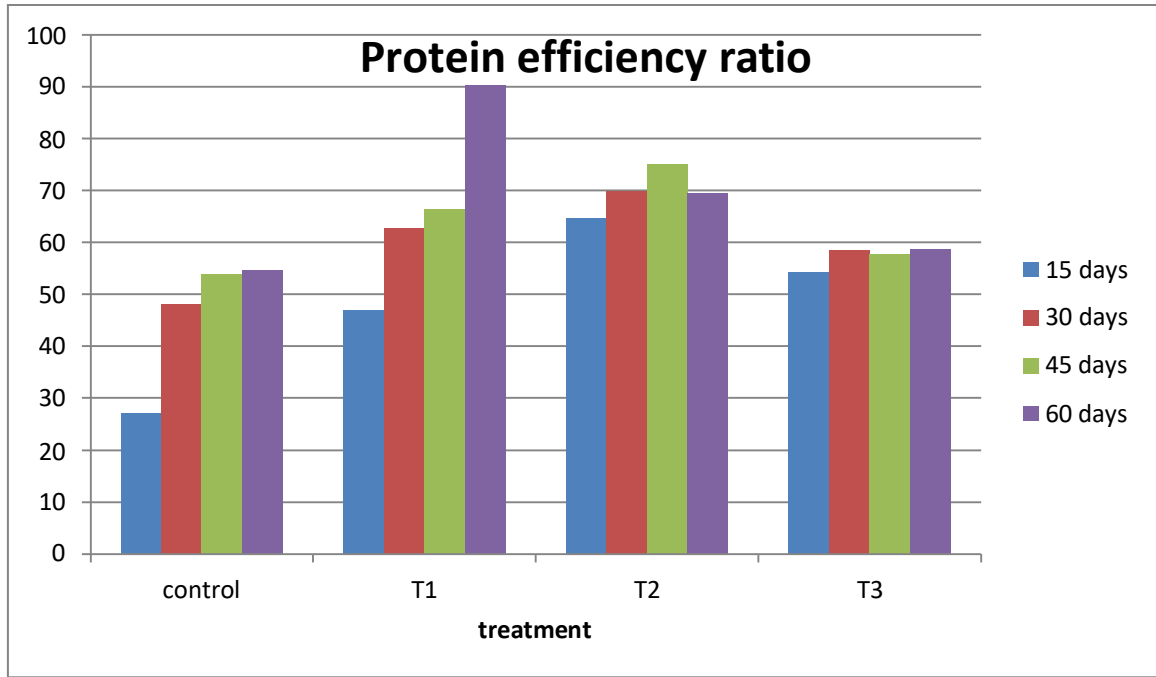


Figure-9

Protein efficiency ratio (PER (g gain/g protein intake)) in the muscle tissue of *Catla catla*

during different days of the experiment in the control and three different treatments

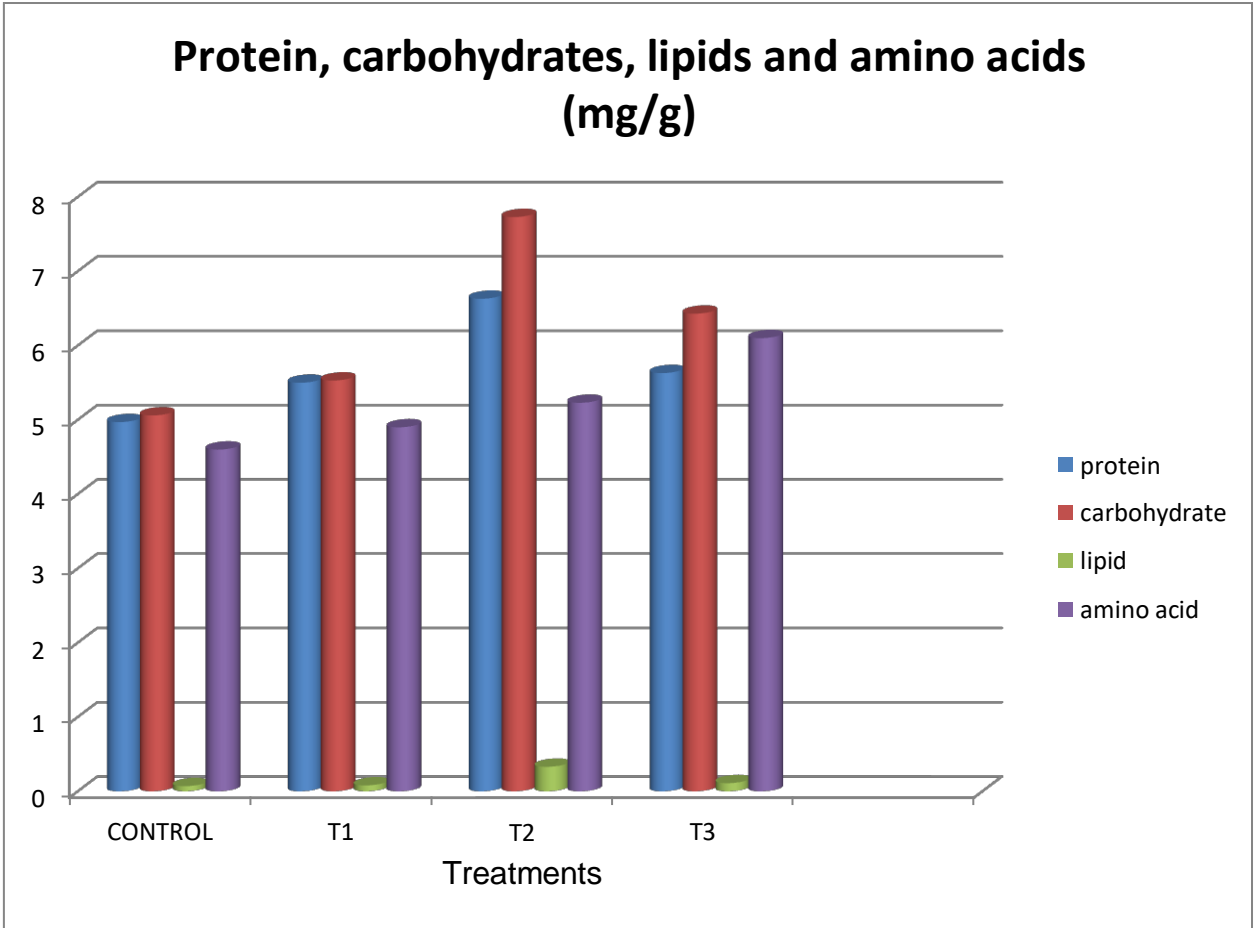


Figure-10

Protein, carbohydrates, lipids and amino acids (mg/g) in the control and three different feeds

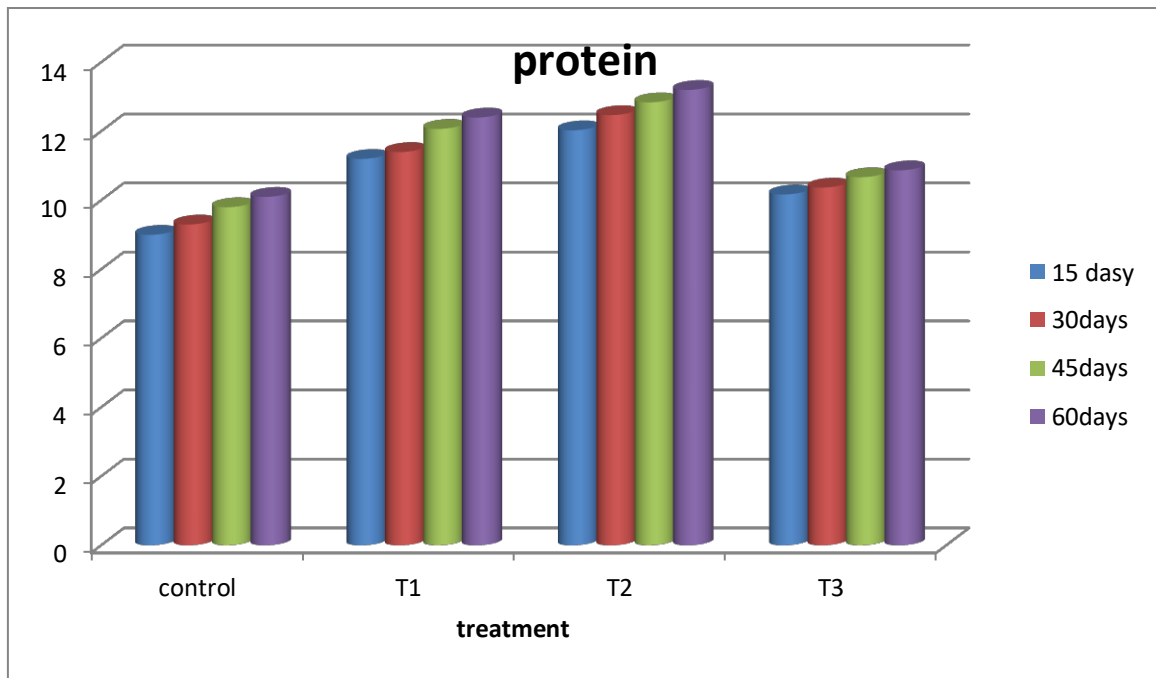


Figure-11

Protein (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

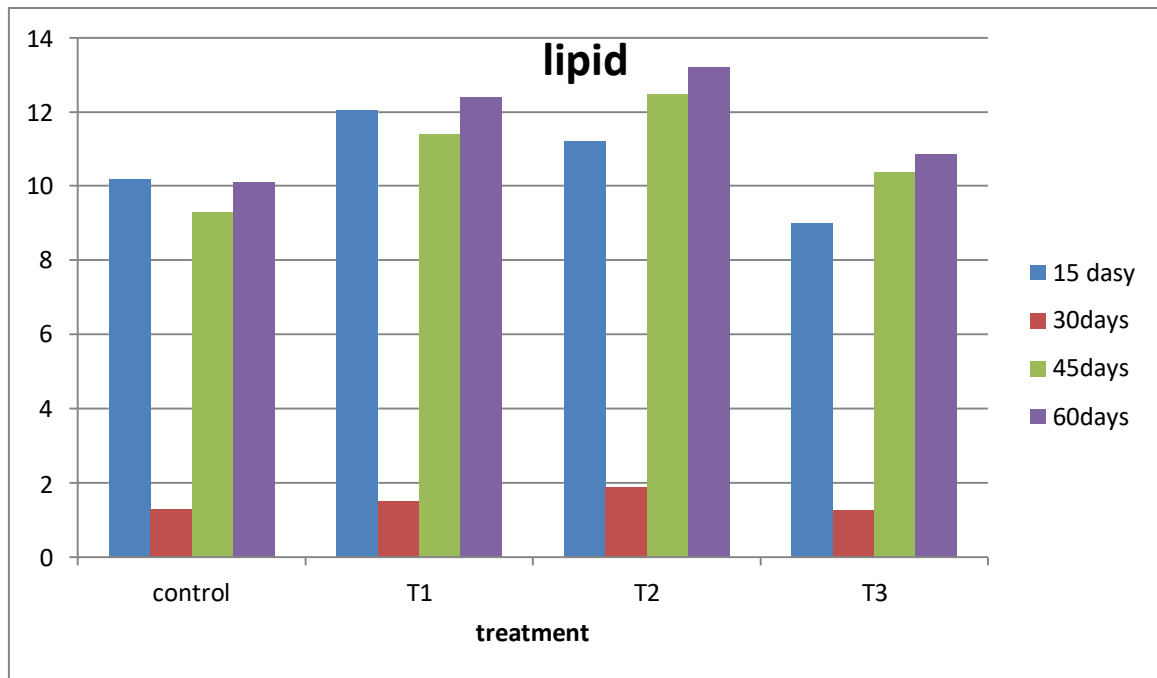


Figure-12

Lipid (mg/g) in the muscle tissue of *catla catla* during different days of the experiment in the control and three different treatments

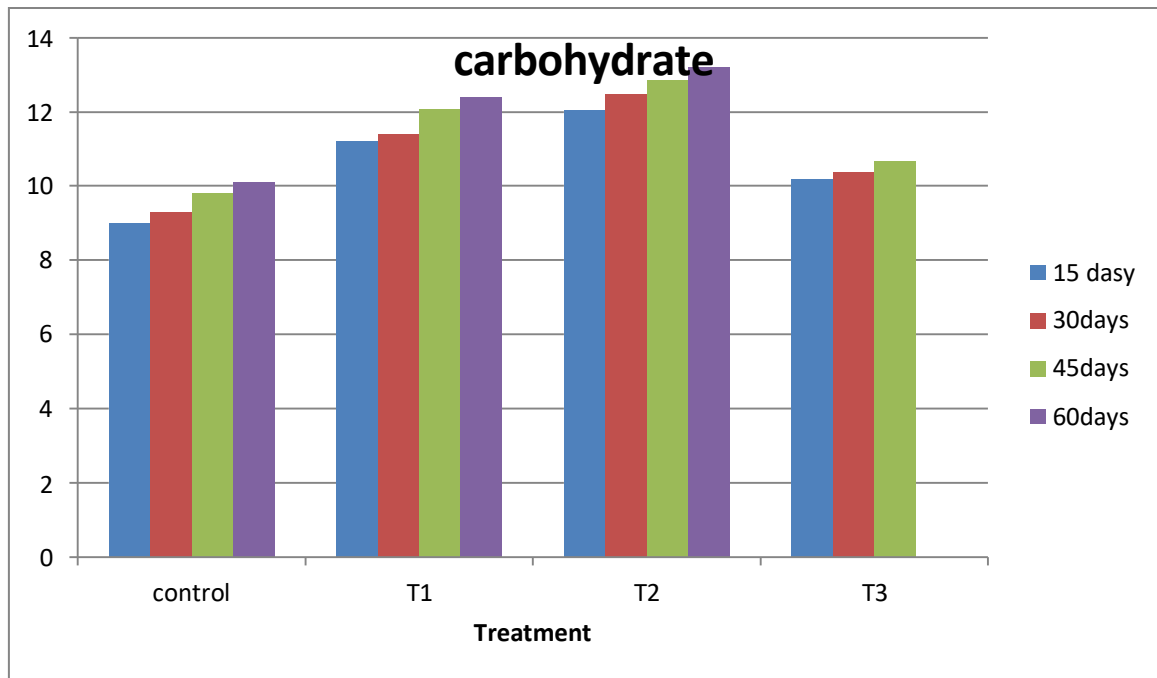


Figure-13

Carbohydrates (mg/g) in the muscle tissue of *catla catla* during different days of the experiment in the control and three different treatments

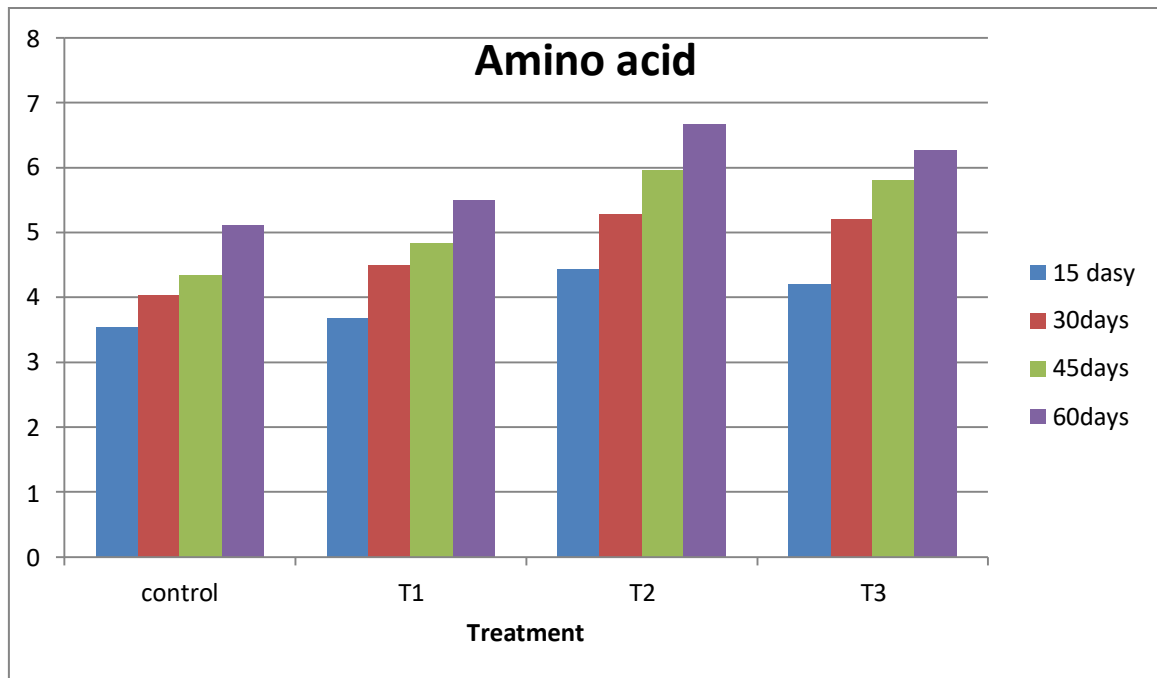


Figure-14

Amino acids (mg/g) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments.

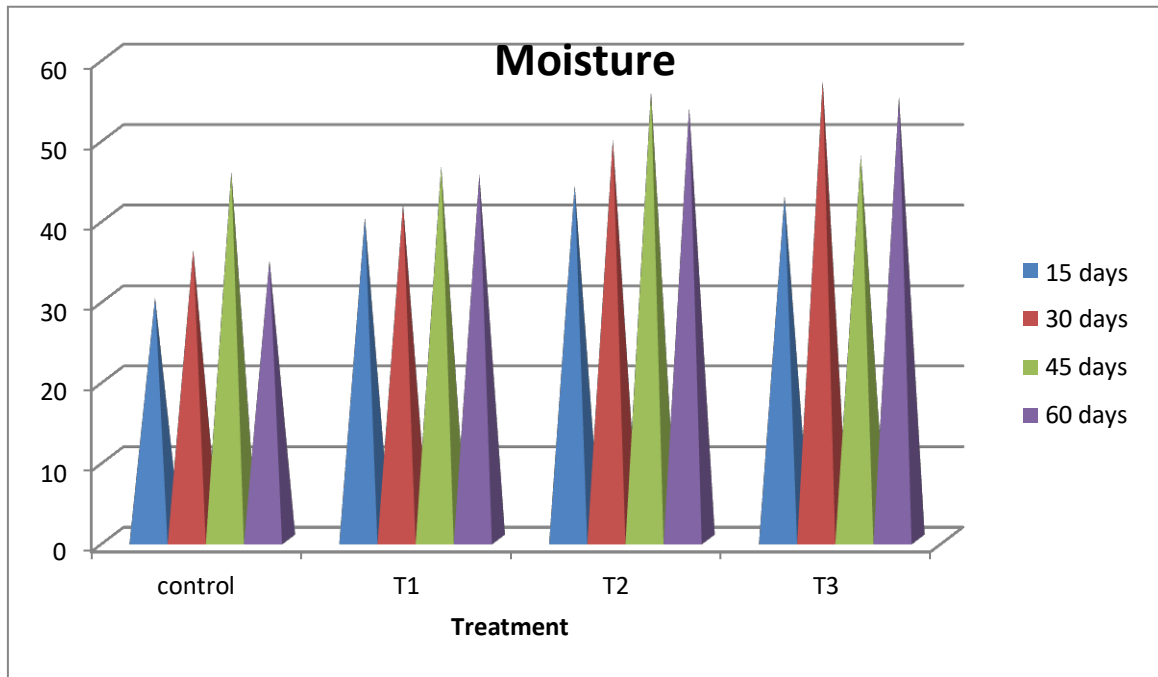


Figure-15

Moisture (%) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

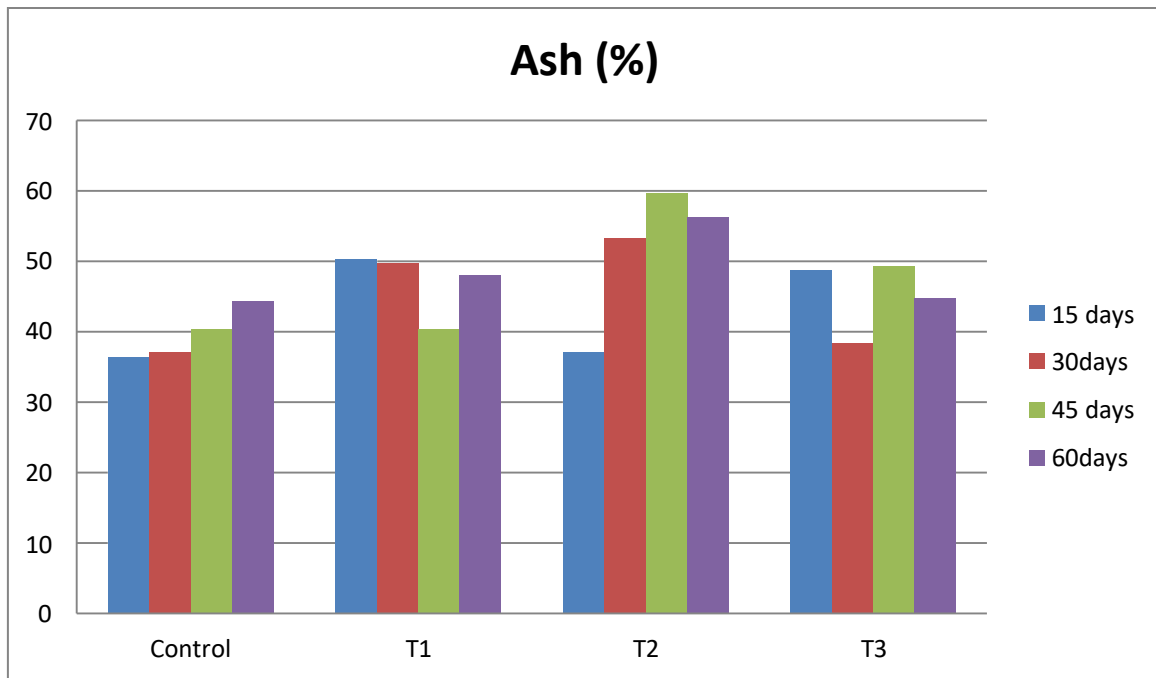


Figure-16

Ash (%) in the muscle tissue of *Catla catla* during different days of the experiment in the control and three different treatments

SUMMARY AND CONCLUSION

Efficacy of curry leaf as formulated feed on the growth ,nutritional status and feed utilization efficiency of Indian major carp, *Catla catla* has been studied .In the present investigation fishes were fed with the feed prepared from different concentration of curry leaf as replacement diet for 60 days and the result were observed for 15,30,45and 60 days.

The bio-growth parameters such as weight gain, length gain feed utilization efficiencies such as feed efficiency (FE), feed conservation ratio (FCR) in *Catla catla* for 15,30,45and 60 days were studied. The bio chemical composition such as protein, carbohydrates, lipids, amino acids moisture and ash content were analyzed in the control and three different treatments. Biochemical composition includes protein, carbohydrates, lipids, amino acids, moisture and ash in the control and three different treatments.

The detail of the study is summarized below

1. Curry leaf incorporated feed improved the growth performance, feed utilization efficiency and biochemical composition in the *Catla catla*.
2. During the experiment period (15, 30, 45 and 60 days), the maximum weight gain (8.02%) was observed in T2 fishes and minimum weight gain (0.81%) was recorded in the control fishes.
3. The length gain (%) in *Catla catla* , was maximum in T1 fishes during 15 (10.11%),45 day (29.14%) ,30 day (17.58%) and 60 day (42.22%) of the experiment followed by fishes grown in T2. The minimum length gain was recorded in the control (15.25%) fishes.
4. Among all the treatments maximum weight gain (35.68%) and length gain (42.22%) was observed in fishes grown in T2 treatments.
5. Maximum protein contents (38%), carbohydrate contents (12%) and fat contents (17%) were found in T2 feed and minimum protein (30%), carbohydrate (4%) and fat (8%) were recorded in the control feed.
6. On 15 , 30 , 45 and 60 day of the experiment maximum protein content was found in T2 fed fishes (18.10% to 18.77%) followed by T1(17.87% to 18.77%) and T3 (17.10% to 17.80%). Minimum protein content was observed in the control fishes (16.27% to

17.30%). During the experiment period the maximum protein content (19.57%) was recorded in T2 feed fishes.

7. On 15, 30, 45 and 60 day of the experiment maximum carbohydrate content was found in T2 (12.03% to 13.20%) followed by T1 (11.20% to 12.40 %) and T3 (10.17% to 10.87%) and minimum carbohydrate content was recorded in control feed fishes(9.00% to 10.10%) .
8. During 15, 30, 45 and 60 days of the experiment, maximum fat content was recorded in T2 feed fishes (1.30% to 2.50%) followed by T1 (1.23% to 2.10 %) and T3 (1.10 % to 1.70%) and minimum fat content was in the control feed fishes (1.10% to 1.70%).
9. The feed utilization efficiency such as feed efficiency (FE), feed conversion ratio (FCR) in *Catla catla* was analyzed in the control and three different treatments.
10. Feed conversion ratio (FCR) showed significant variations. The maximum FCR (1.09g) was obtained in T2 feed and minimum (0.90g) was recorded in the control feed.
11. Feed efficiency (FE) showed significant variations. The maximum FE (1.09g) was obtained in T2 feed and minimum (0.91g) was recorded in the control feed.
12. Maximum protein content (38%), carbohydrates content (12%) and lipid content (17%) was found in T2 feed and amino acid (6.1%) was observed in T2 feed. Minimum protein content (30%), carbohydrates content (4%) and lipid content (8%) was found in T2 feed and amino acid (3%) was recorded in the control feed.
13. On 15, 30, 45 and 60 days of the experiment, maximum moisture content (44 to 53.67%) was observed in T2 fishes followed by T3(42.64 to 55%),T1(40 to 45.33%).minimum moisture content(30.00 to 34.67%) was observed in the control feed.
14. On 15, 30, 45 and 60 days of the experiment ,maximum ash content(37 to 56.33%) was observed in T2 fishes followed by T3(48.67 to 44.67%),T1(50.33 to 48.00%).minimum ash content(36.33 to 44.33%) was observed in the control feed.
15. The result was subjected to two-way ANOVA to show the significant $n=$ value of biochemical parameter, feed utilization efficiency and biochemical composition in *Catla catla* grown in T2 feed.

In conclusion of the present study, it was observed that 50% supplementation of prawn waste had significantly improved the growth rate in terms of weight gain, length gain and feed utilization efficiency (FCR and FE). Furthermore the supplementation of

curry leaves also increased the biochemical composition which facilitated digestion process. In coherence to the obtained results, supplemented nutrients from curry leaves provided sustainable growth for the fish, *Catla catla*.

The present investigation provides adequate information on the formulation of fish feed with curry leaves in different concentration to feed the fish, *Catla catla*. Hence in aquaculture practices it is preferable to use curry leaves as fish feed for the enhancement of growth nutrient level and feed utilization efficiency. The finding would be formulating a cost effective and eco-friendly farm made feed.

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