A PROJECT REPORT ON

MINOR RESEARCH PROJECT

Growth response of *Catla catla* and *Labeo rohita* to Plant Formulated Diets as Protein Source

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By

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Introduction

Aquaculture has gone through major changes from small-scale household activities to large-scale commercial farming. The last past decades, the sector has expanded, diversified, intensified and advanced technologically. As a result, its contribution to food production has increased significantly. A large production of global aquaculture production comes from small-scale producers in developing countries, especially in Asia. It significantly contributes to food security, poverty alleviation and social well-being in many countries. The contributions of aquaculture to trade, both local and international, have also increased over recent decades and its share in the generation of income and employment for national economic development has increased globally.

Aquaculture is fast growing food production sector of world. Compared with other food production sectors, aquaculture achieved 10% annual increase since 1984; while, production increase rate of meat is only 3% annually (FAO, 1997; Francesco et al., 2004). According to the Food and Agriculture organization, aquaculture is growing more rapidly than all other animal food- production sectors. Its contribution to global supplies of fish, crustaceans and mollusks increased from 3.9% of total production by weight in 1970 to 33% in 2005. This growth is at an average rate of9.2% per year since 1970, compared with only 1.4% for capture fisheries and 2.8% for terrestrial farmed-meat production systems. It is remarkable that one out of every three fish consumed in the world is now farm reared (FAO, 2007).

According to Yadava (2003), the fisheries sector occupies a very important place in the socio-economic development of India. This sector has been recognized as a powerful income and employment generator, as it stimulates growth of a number of subsidiary industries and is a source of cheap and nutritious food, at the same time it is an instrument of livelihood for a large section of economically

backward population of the country. More than 6.0 million fishermen and fish farmers in the country depend on fisheries and aquaculture for the major contributors of foreign exchange earnings through export.

Feed one of the main inputs in aquaculture production and a key element in intensive culture. Even in culture systems where the main source of nutrition for the cultured stock is natural food produced by fertilization, supplemental feeding is needed to achieve high production. However, nutrition and feed technology are relatively underdeveloped areas of aquaculture science, particularly in respect of warm-water species and their culture systems. Fish feed development in developing countries is hampered by the lack of necessary expertise to formulate, prepare and test appropriate feeds and demonstrate their nutritional and economic value.

The success of fish farming depends to a very large extent on the provision of adequate quantities of nutritionally balanced feeds in a form in which fish can utilize. Even when the natural feed forms the main source of nutrition, supplemental feeding with artificial feed is necessary to obtain increased production in ponds as has been shown by Sinha (1979). Fish feeds have been developed in some countries, for salmonoid fishes, cat fishes, eels, carps and shrimps. In most cases, the formulations are too expensive for use in developing countries. The high cost of some feed ingredients like fish meal has been a problem to use it in formulated diet. Considerable research is now underway to find suitable substitutes in order to formulate cheaper feeds.

Presently, fishmeal has traditionally been the principal source of protein, incorporated into many types of feeds used in aquaculture. Properly processed fishmeal is digestible, carries larger quantities of energy and high-quality protein per unit weight and contain little amount of carbohydrates. Fishmeal is produced principally from small marine fish not usually harvested for direct human

consumption. Although a renewable resource, fishmeal is expensive and the world-wide supply is limited, especially as the demand for aquaculture products expands and fishmeal is utilized in other animal industries.

The uncertain supply, high price and large demand of fishmeal lead to shortage of fishmeal. To sustain the fish growth it is needed to search alternative fish feed sources. Work on formulation, preparation and testing of feeds based on locally available ingredients is being investigated towards alternative inexpensive protein sources for fish feeds.

India produces enormous quantities of feed materials derived from crops. These include a wide variety of all cakes, mill byproducts of seeds and grains. Smaller quantities of byproducts from the meat, fish and dairy processing industries are also used in artificial feed formulation. Indian fish feed stuffs suitable for fish feed formulation are pods, seeds, leaves, fruits of certain plants, grains, oilcakes likes linseed, safflower, sunflower, soybean, roots and tubers of sweet potato, cassava cereals and cereals byproducts, rice polish, broken rice, sorghum, wheat bran, maize, etc, feed of animal origin are fish meal, prawn meal, meat meal, blood, bone meal, hydrolyzed poultry meal, silkworm pupae along with some miscellaneous feed stuffs. Almost all the feed stuffs consumed within the country are suitable for feeding the fish.

No serous attention was given towards the use of plants and byproducts as a fish feed. The use of plant products and byproducts in fish feed formulation helps to enhance the fish growth and to overcome the economical expenditure on fishmeal.

In order to contribute the use of the plant product in aqua-feed, the present work was undertaken. The present study deals with the

1. Utilization of terrestrial plant leaf (*Gliricidia maculata*), aquatic weed (*Eichhornia crassipes*) and a medicinal plant (*Asparagus racemosus*) in fish feed formulation
2. Determination of efficiency of plant based formulated feed in the diet of freshwater fish, *Catla catla* and *Labeo rohita*
3. Growth response and feed utilization efficiency of selected fishes to plant based diets.
4. Study of changes in biochemical components (total protein, lipid and glycogen) of fish tissues (muscle and liver) fed with plant protein diets.
5. Digestive enzymes (lipase, protease. Amylase and invertase) activity in fish intestine fed with plant based diets.

Fisheries play an important role in the world food economy. Fish is the primary source of the protein. The world average per capita consumption of fish has almost doubled in last five decades. Globally, fish provides 16% of the animal protein consumed by humans a valuable source of minerals and essential fatty acids. The reported production of fish for direct human consumption doubled between 1955 and 1975. An average of 10 kg to 12 kg of fish per capita. Fish consumption per person is expected to continue to rise. Supply will probably be limited by environmental factors and a likely range for demand is 160 to 165 million tons or between 19 to 20 kg per person in 2030. Global increases in consumption of food fish will take place largely in the developing countries, where population is growing and higher incomes are allowing purchase of high value fisheries products.

Fishmeal replacement

Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has

increased in balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture industry as it expands to satisfy increasing demand for affordable, safe and high-quality fish products.

Fish, like any other animal require nutrients to stay alive, healthy, and active growth. Nutrients are the building blocks of all tissues of the animals and they are involved in all the chemical reactions that make up “life”. The types of nutrient required by fish are the same as those required by other animals. Nutrients can be provided by various products of plant, microbial or animal origin or can be chemically synthesized. In nature, fish obtains nutrients from various types of food items, these being determined both by the feeding behaviour or preference of the animal and the availability.

From nutritional point of view, the nature or origin of the ingredients generally does not matter. Fundamentally, the animal is only looking at meeting its requirements for specific nutrients. To do so, it is equipped with a variety of digestive enzyme that allows it to harvest these nutrients from variety of sources. The use of a combination of ingredients is therefore, necessary to combine ingredients to obtain a mixture that fulfils all the requirements of the animals.

Feed is considered as the most critical input for fish production. The fish accepts a wide variety of agricultural by-products in the form of pelleted or dough feed. Several studies have been carried out on the development of the formulated feed for various species under controlled culture system (Mohanty et al., 1995; Mukhopadhyay & Ray, 1999, Khan et al., 2004; Biswas et al., 2006). In almost all the studies, ingredients like fishmeal, groundnut oilcake’ soybean meal were used. All these materials are costly and were not used continuously in aqua-feed by small scale farmers. This has necessitated search for alternative sources available locally in the country (Kalita et al., 2008).

Fishmeal is considered the most important protein source for aquaculture industry because of its high protein biological value. The long term availability of this resource is not possible in fish diet because of various reasons (Hardy, 1996; Sargent & Tacon, 1999). Variable, fluctuating supply and increasing cast of fishmeal, generated a need to replace this costly feedstuff by cheaper and alternative protein sources for fish feed. For both economic and practical reasons, fish feed should be prepared using locally available protein sources, mostly those which are unsuitable for human consumption (Hossain & Jauncey, 1989). The oilcakes are considered as good sources of protein for fish diets, as it is available in large quantity from edible oil industry. Soyabean, groundnut, sunflower, sesame and coconut are extensively used for partial or total replacement of fishmeal (El-Saidy, 1995; Mbahinzireki et al., 2001). These plant product incorporated meals have high protein levels and favorable essential amino acids. It is important to evaluate the nutritional value of combination of plant proteins in order to replace fishmeal in fish diets without any adverse effect on fish growth.

Diet formulation represents translation of nutrient and energy requirement of a given species for a given response into an acceptable diet using a balanced mixture of ingredients which is economically sustainable. For any feed formulator a reliable, updated database on chemical consumption, physical characteristics and bioavailability information on feed ingredients is necessary. Protein is usually the first nutrient considered, with the level of energy in the diet being adjusted to provide the optimum ratio. Formulation of low-cost balanced fish diets using locally available agro-industry by-products is one of the ways to reduce feed cost for commercial fish culture. Protein is the most expensive dietary component in fish feed so it is necessary to find out alternative source to replace dietary protein in fishmeal. Less expensive sources of protein which provide good growth is advantageous for diet manufacturers and aqua-feed producers (Coyle et al., 2004).

Inconsistent supply of fishmeal, environmental concerns of fishmeal usage and high demand, high price of fishmeal make evaluation of alternative protein sources a high priority for fish nutritionists. Developing countries have realized that, in the long run they will be unable to afford fishmeal as a major protein source in aqua-feed. Presently, one of the challenges that fish nutritionists face is to partially or totally replace fishmeal with less expensive, untraditional animal and/or plant protein sources. Total replacement of fishmeal is not possible, because it reduces growth of fish. Higher inclusion of plant protein meals reduce fish growth and feed utilization (Riche et al., 2001). Some authors reported that total replacement of fishmeal with plant protein is possible (Shiau et al., 1989; EI-Saidy & Gaber, 1997, 2002; Muzinic et al., 2006; Goda et al., 2007). These conflicting results may be due to variation in environmental culture conditions (eg. Controlled laboratory conditions vs. pond culture), water quality, salinity and feed processing (Mendoza et al., 2001; Chowdhury et al., 2006; Amaya et al., 2007).

Combination of animal and plant protein sources in fish feed gives promising results. Such type of feed improves fish growth and nutrient utilization (Brown et al., 1997; Webster et al., 1992, 2000). Plant protein sources, which are more consistently available and cheaper to produce than fishmeal and other animal proteins, have been extensively used in combination with fishmeal in aqua-feed (Afzal Khan et al., 2003).

At present, the high quality commercial fish feeds almost depends on fishmeal. The dependency has been driving the feed price unreachable to small farmers as the international market price of fishmeal has almost doubled in last few years. A priority area of research in aquaculture nutrition is the reduction and possible elimination of fishmeal and fish oil from practical diets (Craig, 2004; Samocha et al., 2004).

Formulation of balanced diet and their adequate feeding are important for successful aquaculture. Protein is the major dietary nutrient. According to fish species, fish size, dietary protein sources and environmental conditions, protein requirement of fish changes (National Research Council, 1993).

Groundnut oil cake is used traditionally with rice bran in a 1:1 ratio for carp culture in India. It is widely used as a dietary protein source by many authors (Swamy & Mohanty, 1990; Bazaz & Keshavnath, 1993; Mohanty & Samantray, 1996; Basavaraja & Antony, 1997) at 5-68% in formulating the diets for carps, murrels and mahseer. Murthy & Naik (2000) studied the effect of dietary protein and lipid in combination with groundnut oil cake on the growth of Indian major carp,Catla catla. The effect of partial and total replacement of fishmeal by groundnut oil cake along with other oil cake sources on *Labeo rohita* was examined by khan et al. (2003). The use of groundnut oil cake at higher levels in fishmeal formulation was not suited due to low lysine and methionine contents. Although groundnut oil cake is highly palatable and has better binding properties than other oil cake, its use in fish feed is restricted at higher levels because of its poor amino acid profile.

Utilization of aquatic plants in aqua feed formulation helps in two ways. One is to control the aquatic weed and other to overcome fishmeal prices. There are number of herbivorous fishes which directly consume some aquatic weeds. Use of aquatic plants and weeds for fish feed formulation is a new trend in aquaculture economics to overcome the aqua feed manufacture cost. As compared to fresh or live aquatic plant feeding, the formulated feed containing aquatic weeds or plants showed better growth performance and feed utilization in various fish species.

Fewer attempts have been made to explore the possibilities of utilization of aquatic weeds as feed by the Indian major carps. The *Labeo rohita* and *Cirrhina*

mrigala can utilize some of the aquatic weeds to a limited extent (patra & Ray, 1988a; Patra et al., 1999; Vhanalakar et al., 2008)

The identification and utilization of non- conventional and lesser utilized plant protein sources to replace fishmeal either partially or totally in practical diets of fish has been an area of research in aquaculture nutrition (Davies et al., 1990; Gomes et al., 1995; Hossain et al., 2001 Ogunji & Wirth, 2001; Siddhuraju & Becker, 2003). Fish nutritionists have evaluated alternative sources of plant origin protein in fish diets as partial or total fishmeal replacement (Das et al., 1994; Ray & Das,1995; fernandes et al., 1999; Ramachandran & Ray, 2004; Olurin et., al., 2006; Stavros et al., 2006 ; Goda et al., 2007).

Ghatnekar et al., (1983) reported that diets with 30-65% Leucaena leaf meal had no adverse effects on growth or reproductive behaviour of tilapia (*Saretheredon mossambicus*) and Indian major carps (*Labeo rohhita, Cirrhina mrigala* and *Catla catla*). When Rahman et al. (1988) fed Nile tilapia (*T. nilotica*) a diet containing 25% Leucaena leaves; the fish grew more slowly than those on a standard diet. In evaluating *Leucaena leucocephala* leaf meal as a protein sources in Indian major carp' *Labeo rohita*, Hassan et al. (1994) observed the higher growth performance at 25% inclusion level.

Medicinal herbs are efficacious for growth, health management and for the immune systems of land mammals and humans. They originate mainly from vegetables and are popular medicines in eastern Asia including China, Korea, and Japan. In traditional Chinese medicine, several herbs are often administered at the same time. Studies have been done in which herbs, as dietary additives, were fed to fish. The focus of these studies includes their use as feeding attractants, and their effects on growth, survival, and immune system activity. With the shifting of attention from synthetic drugs to natural plant products, the use of plant extracts for enhancing growth performance in animals is now increasing. Plants that were

once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects.

There is a developing interest in using medicinal herbs as a kind of dietary supplement in aquaculture, and in showing the positive effects on growth and the immune response (Ji et al., 2007; Jung et al., 2002). Medicinal herbs can improve the quantity of micro-flora in the intestine by increasing some good microbes and inhibiting increased digestion capacity (Liu et al., 2004).

Many nutritionists tried to incorporate medicinal herbs in fish diet. They showed the positive results regarding growth performance and feed utilization of fish. Inclusion of medicinal plant material in fish diet showed better protein and lipid contents after long term feeding. Various medicinal plants like Obosan (Kim eet al., 1998), Chinese herb (wang et al., 2006) Quillaja (Francis et al., 2005), Glycyrrhiza glabra (Kumar et al., 2007). Winter cherry *Withania somnifera* and ginger (Immanuel et al., 2009) showed better growth results along with increased immune response in fishes.

Inclusion level of plant proteins above 50% of total diet often resulted in reduced fish growth. It is due to imbalance of amino acid, poor digestibility of protein, lipid and glycogen, presence of anti-nutritional factors or poor palatability (Balogun & Ologhobo, 1989 and Tacon, 1993).

Mixed feeding in which high protein diet was alternated by a low protein diet could result in improved nutrient utilization. The application of mixed feeding to reduce feed cost and improved nutrients utilization has been reported in Indian carps, *Caltla catla*, *Labeo rohita* and *Cirrhina mrigala*.

Complete replacement of fishmeal with individual plant protein source has generally resulted in a decrease in fish growth performance due to presence of anti-nutritional factors (Mbahinzirek et al., 2001; Sklan et al., 2004).Two strategies to overcome this limitation include fractionation of feed ingredient and replacement

of fishmeal with complex mixture of two or more than two different plant protein sources. This will enhance the fishmeal acceptance, growth and overcome anti-nutritional factors from fish feed (Borgeson et al., 2006).

Growth performance:

The continued expansion and improvements in efficiency of aquaculture production require continued improvements in nutritional formulation and feed technology. De Long et al., (1958) taking the body weigh as basic indicator are among the first to investigate dietary protein requirements of fish. Many authors use similar approaches. Protein contents on fish feeding are usually high. However, nutritional requirements of fishes can differ in pursuant to distinct to distinct factors as nutritional habits, life cycle, environmental temperature and health. Well-balanced protein diets are fundamental for optimize growth and development of fish particularly considering the offer of amino acids.

The dietary protein is an important aspect in achieving efficient fish production. The dietary protein should accommodate fish requirements due to age or size. This technique could significantly enhance the protein utilization leading to cost saving, and thereby reducing the nutrient loading into the aquatic ecosystem. The continuous feeding with a high-protein diet did not enhance feed intake or feed conversion ratio. Protein efficient ratio is used to assess protein utilization and turnover where they are related to the dietary protein intake and its conversion into protein gain.

To achieve good growth of fish, good quality artificial feed is essential with protein level ranging from 20 to 45% (Stickney, 1991).In the past, the studies were carried out to check the type of plant protein and inclusion level to achieve good fish growth. The utilization of plant protein and its conversion in the form of body protein determines the status of any plant source to use it in fish feed formulation.

Biochemical study:

Protein, carbohydrate and lipid which constitute a major components of the body, play an important role in body construction and energy metabolism. Biochemical composition determines the quality of flesh. Biochemical composition of fish is dependent on species, age, size, sex, environmental factors and most importantly quality of feed feeding habits.

The type and level of protein in the fish diet decides the whole body protein content in fish. There were many attempts to determine the relation between feed protein and fish protein. The nutritive value of the diet and morphology of i.e. toughness of the formulated diet affects the availability of protein in the compounded diet (Penaflorida et al., 1992).

Any change in feed and feeding practices is known to have its effect on fish growth performance and body composition. The carefully formulated fish feed always leads to best growth of fish, whereas the diet having low quality, poor protein content and higher anti-nutritional factors puts its impact in the way of reduced growth.

Dietary nutrients must be well balanced to achieve optimum protein-accumulation and growth rates both in individual tissues and in the whole animal. It was reported that there was a significant influence of diet upon protein concentration and turnover rates in the liver and muscle and fish and variations in fractional protein-synthesis values depending upon ration quantity and protein quality (Peragon et.al., 1994).

Dietary lipid provides essential fatty acids, phospholipids and energy to promote growth, health, metabolic pathways and is the precursors of eicosanoids (physiological functions). Dietary lipid, just as dietary carbohydrate, can act as an energy source to spare protein utilization for energy and also increase palatability to the diet. The development of high-energy diets (high lipid content) to reduce nitrogen and phosphorus loads from high-protein diets and improve feed

conversation ratios, among other factors, is rapidly gaining popularity (Sargent et al., 1999). Lipids are one of the major organic components of fish, and currently attract the attention of consumers due to the importance of their fatty acid profile.

Enzyme study:

Digestive enzymes have been investigated for understanding the nutritional requirements of the animal and the effect of dietary constituents on the enzyme activity (Schneider & Flatt, 1975; Divakaran et al., 1999). Different feeding habits and food diversity impact on digestive enzyme modification in fish. To achieve improved growth of fish, artificial diet plays important role. The digestive enzyme profile of fish helps to adjust the nutrient incorporation and diet composition so as to achieve better fish growth (Sabapathy & Teo, 1993; Hidalgo et al., 1999; Deguara et al., 2003). Chesley (1934) documented the relationship between structure of the digestive tract and digestive enzymes coexist in close correlation, e.g. the gastrointestinal tract of herbivorous fishes is longer than the carnivorous fish. Digestive functions capable of hydrolyzing a greater variety of feedstuffs have developed in herbivorous and omnivorous fish spices in contrast to carnivorous fish species (De Almeida et al., 2006). The study of digestive enzymes in fishes explains their nutritional physiology and helps to solve nutritional problems (Hidalgo et al., 1999).

Selection of plant protein sources:

In the present study, three plant origin protein sources were used to study the growth response of two freshwater fish spices, *Catla catla* and *Labeo rohita*. The three plant used in the study were a medicinal plant, *Asparagus racemosus*, tropical fodder plant, *Gliricidia maculata* and a aquatic weed, *Eichhornia crassipes*. The root of Asparagus and leaves of Gliricidia and Eichhornia were used in formulations of artificial feeds in the present work.

Asparagus racemosus is a medicinal plant grows all over Indian in tropical areas and is found in Himalayas up to an altitude of 1300-1400 meters. The plant is an armed climber, growing 1-2 meters in length. The roots of Asparagus are used mainly to promote milk secretion and as a demulcent, diuretic, aphrodisiac, tonic, alterative, antiseptic and anti-diarrhoeal. It is also used to treat debility, especially in women, and infertility, impotence, menopause, stomach ulcers, hyperacidity dehydration, lung abscess, haematemesis, cough, herpes, leucorrhoea and chronic fevers. It is also used in health tonics to increase appetite and immunity.

The use of Asparagus as a feed additive to study anti-diarrhoeal potential in various animals was performed by many scientists. There is less information available regarding use of Asparagus in animal diet to promote growth. An experiment was conducted to assess the effect of *Asparagus racemosus* root powder on the performance of broilers by Rekhate et al. (2004). It was found that the supplementation of *Asparagus racemosus* root power has beneficial effects on body weight gain and feed conversion efficiency indicating improvement in general health condition of broilers.

The use of Asparagus in *Cirrhina mrigala* at an inclusion level of 30% gives better growth results (Vhanalakar et al., 2008). The use of Asparagus in *Cyprinus carpio* diet at low level inclusion was studied by Vhanalakar & Muley (2009).

Gliricidia maculata is a fast-growing, tropical, leguminous tree up to 10-15 m. height. It is one of the commonest and best-known multi purposes trees in many parts of central America, where it probably originated, but it has also spread to West Africa, the West Indies, southern Asia and the tropical Americas. It is used for timber, firewood, medicinal purposes, charcoal, living fences, plantation shade and green manure, it has good potential as fodder for livestock. Gliricidia is most likely to be used as a green fodder/protein supplement to low-quality tropical forages and by-products for cattle, sheep and goats.

Gliricidia leaves are rich in protein and highly digestible for ruminants like goat and cattle, as they are low in fiber and tannin. There is evidence of improved animal production (both milk and meat) in large and small ruminants when Gliricidia is used as a supplement to fodder. Growth performance of goats fed with Gliricidia was studied by Richards et al. (1994) and Hao & Ledin (2001). Incorporation of Gliricidia in the diets of crossbred steers (Abdulrazak et al., 1997), Jersey cows (Juma et al., 2006), and layer bird (Ige et al., 2006) showed increased growth performance. Chadhokar & Kantharaju (1980) studied the effect of Gliricidia on the growth and breeding capacity of bannur ewes. Effect of Gliriacidia based diets on milk capacity was also revealed (Chadhokar & Lecamwaasam, 1982).

The inclusion of Gliricidia in fish diets was studied by Das et al.,(1994) and Adeparusi & Agbede (2005). Das et al. (1994) reported the inclusion of 30% Gliricidia in the diets of *Labeo rohita* and *Catla catla* showed good growth performance and feed utilization .Oreochromis niloticus fed bam-nut supplemented with Gliricidia was superior in growth, nutrient utilization, crude protein digestibility, carcass crude protein, least feed conversion ratio and best feed efficiency ratio (Adeparusi & Agbede, 2005).

Eichhornia crassipes, commonly known as Common water Hyacinth, is a wild freshwater fern belonging to the Family Pontederiaceae. This plant is native to South America, but has been naturalized in many tropical and subtropical regions of the world. It grows and reproduces at very high rates, yielding up to 100-400 mt/ha/year. Water hyacinth is listed as one of the most productive plants on earth and is considered the world's worst aquatic weed. It forms dense mats that interfere with navigation' recreation, irrigation and power generation. These mats competitively exclude native submersed and floating-leaved plants. Dried and

cleansed Eichhrornia plants can be used as fertilizer. Several studies have evaluated Eichhornia as a food source for domestic animals.

There have been numerous attempts to utilize aquatic weed, Eichhornia crassipes for animal feed in Southeast Asia. Eichhornia have potential as animal feed. Cattle have grazed floating water hyacinths when land forages were limited. Reddy & Moanrao (1979) supplemented Eichhornia with paddy straw as a feed for growing crossbred calves. Dominguer et al. (1996) tested Eichhornia with other two floating macrophytes as a pig feed. They found promising results with pig growth fed with Eichhornia. Utilization of Eichhornia as a supplementary feed for Bangladeshi bull calves (Rashid et al., 2001), growing goats (Dada, 2002) and duck (Luet al., 2008) showed better growth performance.

MATERIAL AND METHODS

Plant material collection and processing:

Fresh Asparagus roots were collected from the cultivated farms near Kolhapur region. Fresh Gliricidia leaves were collected from the campus of Shivaji University, Kolhapur and fresh Eichhornia leaves were collected from local water bodies of Kolhapur districts, Maharashtra, India. Plant parts collected were washed thoroughly under tap water to remove dirt and debris, drained properly and later sun dried. Dried root of Asparagus and leaves of Gliricidia and Eichhornia were milled using a kitchen mixer, packed in airtight polyethylene bags and kept in the freezer before use. Diets contained different combinations of dry root and leaf powder of Asparagus , Gliricidia and Eichhornia meal, groundnut oil cake, rice bran, fishmeal, guar gum binder and mineral vitamin mixture were formulated (table 1-4). A diet with groundnut oil cake, rice bran, fishmeal guar gum binder and mineral – vitamin mixture served as a control diet. Ingredients were mixed thoroughly in kitchen mixer till homogenous mass was obtained. With the help of mincer the 0.6mm pellets were prepared, which were immediately sun dried. After drying, the pellets were hand broken up into convenient pellet sizes and frozen in refrigerator until before feeding.

Experimental system and fish spices:

Experimental fish *Catla catla* and *Labeo rohita* fingerlings were obtained from governmental nursery ponds in Kollhapur Centre, M.S.(India) brought to the laboratory and divided among ten 100-L glass aquaria. The fish were acclimatized in glass tanks for 15 days with being fed on a commercial pelleted diet.

The experiment was conducted in the aquaria of 3’x 1’x 1’ size. Water from aquaria was changed every alternate day and continuous aeration was provided. Each aquarium was stocked with 10 fingerlings of uniform size and weight (in triplicate). Fishes were fed at the rate of 5% body weight in two equal rations daily.

At fortnightly intervals a minimum of 50% of fishes were sampled to record the growth.

Chemical analysis of formulated feed:

The chemical analyses of major feedstuffs and formulated diets were carried out according to the procedures of the AOAC (1990). Feed ingredients were analyzed for proximate composition such as moisture, crude protein, crude fat, crude fiber and total ash (Table 1-4).

Diet Performance Evaluation:

Growth performance of experimental fish was determined in terms of final individual fish weight (gm), weight gain (gm). These growth responses were calculated as follows:

Weight gain (%) = 0.100 (final body weight – initial body weight /initial body weight); SGR (% day 1) = 0. 100 ($\log_e$) final body weight ± 0. $\log_e$ initial body weight/ time days; FCR±0. [Dry weight of feed fed (g)]/ [fish weight gain (g)]; PER = 0. [Fish weight gain (g)] / [protein fed (g)]

Biochemical study

After the completion of experimental period (120 days) the fishes were sacrificed; liver and muscles tissues were dissected out as quickly as possible and stored to analyze total glycogen, total protein and total lipid contents of liver and muscles tissues. These tissues were weighed and used for the estimation of biochemical components like protein (Lowry et. Al., 1991), Glycogen (De Zwaan & Zandee, 1972) and lipids (Barnes & Blackstock, 1973).

Enzyme study

After the completion of feeding trial period, the fishes were sacrificed and whole intestine was removed out. It was homogenized in 0.9% NaCl solution so as to prepare homogenate. The homogenate was centrifuged at 5000 rpm, supernatant

was colleted, frozen in sample vials and stored in refrigerator until assayed for the digestive enzymes.

The estimation of lipase was carried out as per the method of Hayase & Tappel (1970), protease by Ishaya et al. (1971) and amylase and invertase by the method of Bernfeld (1955).

RESULTS

Fish in all dietary groups fed actively on the experimental diets. There was no rejection of feed till the end of experiment. No mortality was observed in any of the dietary groups during the study period.

Growth performance and feed utilization:

Catla catla

Asparagus diet:

The growth performance and feed utilization in terms of body weight gain (WG) of *Catla catla* fed with different levels of Asparagus diets are presented in table.4

After 120 days of feeding trial, it was noticed that there was excellent growth in fishes fed with 30% diet. This 30% diet group showed highest final body weight (24.60 ± 0.0.47gm), weight gain (23.27 ± 0.0.60 gm). The least growth and feed utilization was recorded from 70% diet group (Fig. 1). The lowest final body weight (11.85 ± 0.0.34), weight gain (9.65 ± 0.0.27) was recorded in 70% Asparagus diet.

Gliricidia diet:

The growth performance and feed utilization indices such as body weight gain (WG) of *Catla catla* fed with different levels of Gliricidia are presented in table-5

Fish groups fed with 40% Gliricidia diet showed better growth performance as compared to other diet groups. The final body weight (26.43 ± 0. 0.65), weight gain (27.66 ± 0.79 gm). The lowest weight gain was reported in control diet group (24.48 ± 0.59gm). There was significant increase in case of weight gain in all diet groups expect 20% compared with control (Fig. 2).

Eichhornia diet

The growth performance and feed utilization of fishes fed with Eichhornia diet was shown in table-6. 40% Eichhornia incorporated diet showed best growth performance in all the diets. The fish fed with 40% diet showed superior final body weight (22.60 ± 0.64), weight gain (22.32 ± 0.84). The control group showed lowest final weight (15.56 ± 0.42) and weight gain (13.26 ± 0.36) (Fig 3).

Labeo rohita

The growth performance and utilization in terms of body weight gain (28.74 ± 0.74). *Labeo rohita* fed with different levels of Asparagus diets are presented in table- 7 (Fig 4). Fishes fed with Gliricida diet was shown in table- 8 (Fig 5). Fishes fed with Eichhornia diet was shown in table-9 (Fig 6). 40% Eichhornia incorporated diet showed best growth performance in all the diets. 40% Eichhornia diet showed highest final body weight, weight gain. The fish fed with 40% diet showed significant final body weight (36.10 ± 0.99), weight gain (33.75 ± 0.93). The control diet fed group showed lowest final body weight (18.20 ± 0.49), weight gain (16.07 ± 0.43).

Biochemical alterations:

***Catla catla*: Liver:**

Changes in liver protein content of fish fed with different plant feeds are presented in table-10 (Fig 7). In most of the plant protein based diets there was increase in liver protein content. The Asparagus based diet showed the highest liver protein at the 30% level (mg/wet tissue), Gliricidia (mg/wet tissue) and Eichhornia (mg/wet tissue) at 40% level. As compared to control (mg/wet tissue) the 20%, 30% and 40% Asparagus diets showed more significant increase in liver protein content, whereas there was no significant difference between control with 50%, 60% and 70% diets. The Gliricidia diets showed significant increase in 20%,

30%, 40% and 50% and non significant decrease in 60% diet compared with control.

All Eichhornia based diets showed significant increase expect 70% as compared to others. Among all the plant protein diets the lowest liver protein content was recorded in 70% mixed diet (1.74±0. 0.01 mg/100 mg wet tissue).

Table 11(Fig 8) present the total lipid content of fish liver fed with Asparagus, Gliricidia, Eichhrnia . In most of the diets there was no difference in lipid content of liver as compared to control (8.40 ± 0.0.40 mg/100mg wet tissue). The highest lipid content was reported in 40% diet (11.70±0.0.06 mg/100mg wet tissue) and lowest in 70% Eichhornia diet (6.95 ± 0.0.34 mg/100mg wet tissue) (Fig. 10). As compared to control 30% Asparagus, 30% and 40% Gliricidia and Echhornia 20%, 30%, 40% and 50% diets showed more significant ($P<0.001$) increase in lipid content whereas 40% Asparagus, 40% Eichhornia ($P<0.01$) and 60% mixed diet ($P<0.05$) less significant higher lipid content than control.

The variation in glycogen content of liver fed with various plant protein diets was shown in table 12 (Fig. 9). In most of the diet fed groups there was no significant difference in glycogen content as compared to control (1.674 ± 0.0.23 mg/100mg wet tissue). The liver glycogen content was reported in the range of 1.74±0.0.01 mg/100mg wet tissue to 5.93 ± 0. 0.20 mg/100mg wet tissue. The highest glycogen content in Asparagus based diet was recorded at 30%diet (5.18 ± 0.0.09 mg/100mg wet tissue), in Gliricidia at 40% diet (5.93 ± 0.20 mg/100mg wet tissue), in Eichhornia at 30% diet (5.53 ± 0.0.18 mg/100mg wet tissue).(Fig. 11).

The lowest glycogen content was observed at 70% inclusion level in all the plant diets. 70% Asparagus (1.74±0.01 mg /100mg wet tissue), 70% Gliricidia (1.85 ±0.05 mg / 100mg wet tissue), 70% Eichhornia (1.926 ± 0.04mg/100mg wet tissue).

Mucles:

The influence of different dietary plant protein sources on protein content of fish muscle is shown in Table 13 (Fig 10). All the plant protein diets fed fish groups showed better muscle protein content. The highest muscle protein content was found in 40% diet (24.12±0.0.25mg /100mg wet tissue) and lowest in 70% Asparagus diet (10.69 ± 0.26 mg/100mg wet tissue). The highest muscle protein content for Gliricidia (24.12 ± 0.12mg/100mg wet tissue) and Eichhornia (22.42 ± 0.32 mg/100mg wet tissue) was observed at 40% diet level, while it was highest at 30% level (19.39 ± 0.0.08mg/100mg wet tissue) for Asparagus diet.

Most of the diets showed significantly higher ($P<0.001$) protein content of muscle compared with control. There was no significant difference of 50% Asparagus diet, 70% Gliricidia diet and 60% Eichhornia diet with control.

Dietary effect of various plant protein diets on lipid content of *Catla catla* muscle was presented in table 14 (Fig 11). The maximum lipid content (10.75 ± 0.29 mg/100mg wet tissue) in diet was found in fishes fed with 40% diet followed by 60% (8.57 ± 0.22mg/100mg wet tissue),30% (9.86 ± 0.13mg/100mg wet tissue) and 50%(8.57 ± 0.22mg/100mg wet tissue) diet. The minimum lipid content was found in control diet (6.08 ± 0.45mg/100mg wet tissue). In Asparagus fed diet groups, the maximum lipid content was reported at 30% inclusion level (10.02 ± 0.04mg/100mg wet tissue) while in Gliricidia (8.33 ± 0.23 mg/100mg wet tissue) and Eichhornia (10.20 ± 0.37 mg/100mg wet tissue) at 40% diet. The minimum lipid content was observed in 70% Eichhornia diet (5.36 ± 0.31mg/100mg wet tissue).

The alterations in muscle glycogen with response to various plant protein diets is given in table 15 (Fig 12). The data showed the highest glycogen content for Asparagus in 30% diet group (2.58 ± 0.02mg/100mg wet tissue), for Gliricidia in 40% diet group (2.79 ± 0.05mg/100mg wet tissue), for Eichhornia in 30% diet

group (3.11 ± 0.03mg/100mg wet tissue). The lowest glycogen content for 70% in Asparagus (1.47 ± 0.01mg/100mg wet tissue), Gliricidia (1.43 ± 0.01mg/100mg wet tissue), Eichhornia (1.46 ± 0.01mg/100mg wet tissue) (Fig. 14). Most of the plant protein diets showed significant increase (P<0.001) up to 50% inclusion level, when compared to control group.

Labeo rohita: **Liver**

Change in liver protein content of fish fed with different plant protein meals are presented in table 16 (Fig 13). The liver protein content of *Labeo rohita* showed the increasing patterns as compared to control diet. 70% inclusion of all the diet groups showed decreased liver lipid content compared with control. The Asparagus based diet showed the highest liver protein content at the 30% level (17.30 ± 0.90mg/100mg wet tissue), Gliricidia (16.86 ± 0.32mg /100mg wet tissue) and Eichhornnia (17.87 ± 0.52mg/100mg wet tissue) at 40% level.

The lowest liver protein content was observed in Eichhornia 70% diet (7.99 ± 0.13mg/100mg wet tissue). In the case of Asparagus, there was significant (P<0.001) increase in protein content with 20%, 30% diets and non-significant increases with 40%, 50%, 60% and 70% diet was observed.

The lipid content of fish liver fed with Asparagus, Gliricidia, Eichhornia table 17 (Fig 14). The Asparagus diet showed the peak of lipid at 30% diet level (16.37 ± 0.17mg/100mg wet tissue) and lowest value at 70% diet (9.69 ± 0.38mg/100mg wet tissue). The lowest lipid content for Gliricidia was 70% (10.52 ± 0.22mg/100mg wet tissue), for Eichhornia 40% (14.28 ± 0.12mg/100mg wet tissue) (Fig.16) In most of the diets there was difference in liver lipid content as compared to control (11.22 ± 0.54mg/100mg wet tissue).

The variation in glycogen content of liver fed with verious plant protein diets is shown in table 18. The glycogen content of *Labeo rohita* liver was highest in 40% Gliricidia (7.49 ± 0.26mg /100mg wet tissue). The lowest glycogen content

found in 70% Eichhronia (2.02 ± 0.03mg/100mg wet tissue) (Fig.15). Asparagus diets showed significant increase in glycogen content upto 40%. In Gliricidia based diet only 40% diet showed significant increase ($P<0.01$) as compared to control. In 30% ($P<0.001$) and 40% ($P<0.01$) Eichhornia based diets there were significantly higher glycogen content than control.

The lowest glycogen content was observed at 70% inclusion level in all the plant diets. 70% Asparagus (2.14 ± 0.05mg/100mg wet tissue), 70% Gliricidia (2.12 ± 0.06mg/100mg wet tissue), 70% Eichhornia (2.02 ± 0.03mg/100mg wet tissue).

Muscle:

Effect of various plant protein incorporated diets on muscle protein content of *Labeo rohita* is shown in table 19 (Fig 16). The highest protein content of muscles was reported from 40% Gliricidia diet (26.41 ± 0.26mg/100mg wet tissue) and lowest from fish fed with 70% Asparagus diet (11.65 ± 0.28mg/100mg wet tissue). Asparagus was seen effective to increase muscle protein up to 40% level (19.89 ± 0.35 mg/100mg wet tissue). The incorporation of Asparagus above 40% leads to decreased protein content. Gliricidia and Eichhornia diets showed their best performance regarding protein up to the inclusion level of 50% (Fig.18).

Moreover, all the diets showed significant increase ($P<0.001$) in protein content as compared to control. The highest muscle protein content with 30% Asparagus diet (22.31 ± 0.09mg/100mg wet tissue), in Gliricidia at 40 %(26.41 ± 0.14mg/100mg wet tissue), in Eichhornia at 40% (24.81 ± 0.35 mg/100mg wet tissue).

Dietary effect of various plant protein diets on lipid content of *Labeo rohita* muscle is presented in table 20. As compared to the other diet group.fishes showed better lipid content of muscle (Fig.17). In Asparagus diet fed fish groups, the highest lipid content was found in 30% diet group (13.23 ± 0.05 mg/100mg wet

tissue) and lowest in 70% diet group (7.09 ± 0.30mg/100mg wet tissue). In Gliricidia based diets, the highest and lowest lipid content was recorded from 40% (13.11 ± 0.48mg/100mg wet tissue) and 70% (6.74 ± 0.41 mg/100mg wet tissue) diet groups respectively. The diet group containing Eichhornia showed highest lipid content (13.52 ± 0.37 mg /100mg wet tissue) in 40% diet and lowest (6.82 ± 0.25mg/100mg wet tissue) in 70% diet group.

The fluctuations in muscle glycogen content of *Labeo rohita* with response to various plant protein diets was given in table 21 (Fig 18). The data showed the highest glycogen content for Asparagus in 30% diet group (2.96 ± 0.03mg/100mg wet tissue), for Gliricidia in 30% diet group (3.85 ± 0.08mg/100mg wet tissue), for Eichhornia in 40% diet group (3.46 ± 0.06mg/100mg wet tissue). The 70% diet group of all plant incorporated meals showed lowest glycogen content. It was 1.58 ± 0.01mg wet tissue for Asparagus, 1.61 ± 0.01mg/100mg wet tissue for Gliricidia, 1.59 ± 0.01mg/100mg wet tissue for Eichhornia. All the diets showed better glycogen content up to 60% inclusion level than control (Fig.20).

Enzyme study: Catla catla

The lipase activity from the intestine of fish, *Catla catla* fed with different plant based diets was presented in table 22 (Fig 19). In Asparagus diet, Gliricidia diet and Eichhornia diet the maximum lipase activity was observed at 40% (4.68 ± 0.11) (4.84 ± 0.06) (5.38 ± 0.19) inclusion level respectively (Fig.21) as compared to control (3.25 ± 0.05mg palmitic acid/gm protein /hr).

Effect of various plant protein sources on the protease activity of fish were shown in table 23 (Fig 20). The protease activity in Asparagus diet was reported high at 40% diet (18.74 ± 0.21mg tyrosine/gm protein/ hr) and lowest at 70% diet (13.52 ± 0.20 mg tyrosine/gm protein/hr.) The highest and lowest activity for Gliricidia diet was observed in fishes fed with 50% diet (22.34 ± 0.35 mg tyrosine/gm protein/ hr) and 70% diet (14.32 ± 0.09mg tyrosine/gm protein/hr)

respectively. The highest protease activity for Eichhornia was found in 40% diet (20.67 ± 0.22mg tyrosine/gm protein/hr) and control diet (16.76 ± 0.0.31mg tyrosine/gm protein/hr respectively) (Fig.22).

Asparagus diet show better amylase activity compared with control above 40% inclusions and lowest (22.98 ± 0.55mg maltose/gm protein / hr) table - 24 (Fig.21). All other diet showed better amylase activity.

The Invertase activity from *Catla catla* intestine was very low as compared to all other diets. The Invertase activity was seen in the range of 0.67 ± 0.09 mg glucose/gm protein/ hr to 1.82 ± 0.01 mg glucose/gm protein /hr (Table 25). The highest invertase activity in Asparagus diet was reported from 30% diet group (1.43 ±0.01mg gloucse/gm protein/hr). The gliricida (1.82 ± 0.01 mg glucose/gm protein/hr at 40%) and Eichhornia (1.74 ± 0.02 mg glucose/gm protein/hr at 40%) based diets showed highest invertase activity from fishes with 40% diet (Fig 22).

Labeo rohita

The lipase activity in *Labeo rohita* intestine fed with different plant protein diets was presented in table 26. Asparagus, Gliricidia and Eichhornia based meals showed highest lipase activity in 40% inclusion level (Fig.23). The highest lipase activity was recorded from 40% diet (6.33 ± 0.22mg palmitic acid/gm protein/hr) and lowest from 70% Gliricidia diet (3.35 ± 0.06mg palmitic acid/gm protein/hr). Other plant diets showed their higher lipase activity up to 40% diet than control.

The protease activity seems to be higher at 40% inclusion level of plant protein in most of the diets of *Labeo rohita* (table 27). As like lipase activity the protease activity in Asparagus, Gliricidia and Eichhornia based diets was highest at 40% inclusion level. The lowest activity of protease in all diets was observed at 70% inclusion level (Fig.24). Overall lowest protease activity was recorded from 70% Asparagus (17.49 ± 0.24 mg tyrosine/gm protein/hr).

Among the digestive enzymes in the present work , the activity of amylase was highest (Fig 25).The amylase activity was found in the range of 26.11 ± 0.63 mg maltose/gm protein/hr to 43.56 ± 0.25 mg maltose/gm protein /hr (Table 28).

Effect of diets containing various plant protein sources on the invertase activity of *Labeo rohita* was shown in table 29. The highest activity of Asparagus diet was observed in 30% diet (1.64 ± 0.02 mg glucose/gm protein/hr), in Gliricidia at 40% diet (2.04 ± 0.02 mg glucose/gm protein/hr), in Eichhorina at 40% (2.07 ± 0.03 mg glucose/gm protein/hr) inclusion levels. There was no significant difference in most of the diet groups when compared with control. The lowest invertase activity was found in 70% diet (0.80 ± 0.10 mg glucose/gm protein/hr) (Fig.26).

SUMMARY AND CONCLUSION

In the present investigation an attempt has been made to evaluate the three plants protein sources i.e *Asparagus racemosus, Gliricidia maculate* and *Eichhornia crassipes* as an effective fish feed ingredient for the fish species, *Catla catla* and *Labeo rohita*. The incorporation of selected plant sources in fish diet was kept in the range of 20% to 70%. In the present study the growth performance, feed utilization, biochemical composition and enzyme activity of fishes fed with formulated diet was studied. The effect of single plant protein as well as compound diet containing all the plant meals collectively in the form of mixed diet on the fish performance was evaluated.

After 120 days of feeding trial in *Catla catla* it was noticed that, both the selected species responded well to plant protein diets up to an optimum level. Each plant diet showed its own growth trend. The Asparagus based diet showed the best fish growth performance and feed utilization in 30% diet, Gliricidia and Eichhronia in 40% diet.

The fish group feed with Asparagus meal at the inclusion level of 30% showed superior weight gain among all other Asparagus containing diets. The Gliricidia, Eichhornia and mixed diet, recorded their best use for fish growth (weigth gain) having inclusion level of 40% and 50%.

In *Catla catla* ,peaks of growth performance and feed utilization were same as like *Labeo rohita* .The 30% Asparagus diet , 40% Gliricidia diet, 40% Echhornia diet and 50% mixed diet fed group has highest final body weight , weight gain.

The present work revealed that, all the growth performance indices i.e final body weight gain showed positive correlation among them. In most of protein fed diet growth and weight gain value was observed higher in control diet rather than plant based diet.

The biochemical composition of *Catla catla* and *Labeo rohita* was greatly affected by dietary plant protein treatment. The biochemical composition of fish liver and muscle varies as per the dietary protein level. The highest liver protein content of *Catla catla* was recorded from 30% Asparagus, while muscle protein content was found in 50% mixed diet and lowest in 70% Aspargus diet. The highest liver and muscle lipid content was reported in 40% diet whereas, the glycogen content showed highest deposition in liver and muscles in 40% diet.

The liver and muscle protein content of *Catla catla* was reported highest in 40% Eichhornia. The highest liplid content of liver was recorded from 40% diet and that of muscle in 40% diet fed fish groups. The biochemical composition of *Catla catla* was seen well than *Labeo rohita.*

Both the fish species used for the present work showed the higher amylase enzyme activity among all selected digestive enzymes as a response to plant diets. The amylase activity was followed by protease, lipase and least activity was recorded with invertase enzyme.

The maximum amylase activity in *Catla catla* was seen from the fish fed with 50% mixed diet. The protease activity was higher in the 40% mixed diet fed fish group, while maximum lipase and invertase activity was observed in the diet groups having 50% mixed meal.

The 50% mixed meal showed the marked digestive enzyme activity in *Labeo rohita.* The maximum amylase, protease, lipase and invertase activity was related with the fish group fed with 50% mixed meal.

Conclusions:

It may be concluded from the present study that-

1. The Asparagus meal can be incorporated in the fish up to 30% inclusion level without any negative growth effect.
2. The Gliricidia and Eichhornia diets can replace the fish meal upto 40% inclusion level.
3. The mixed diet having all the three plants proteins in equal proportions can be incorporated at highest level i.e. 50% among all the diets used in the study without any harm or adverse effect on fish growth.
4. The results obtained in the study an important conclusion that the use of mixed or compound feed will be beneficial and economic rather than to use single plant protein source.

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Table 1: Formulation and proximate composition of fish diets contain *Asparagus rascemosus* root protein

	Diet						
	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Ingredient (%)							
Groundnut oilcake	43	35	29	24	19	13	8
Rice bran	36	27	23	18	13	9	4
Fishmeal	10	9	9	9	9	9	9
Guar gum binder	10	8	8	8	8	8	8
Mineral-Vitamin mixture	1	1	1	1	1	1	1
A.racemosus root powder	0	20	30	40	50	60	70
Nutrient content (%)							
Moisture	7.05	8.48	7.21	7.30	7.17	6.36	6.19
Total ash	11.13	5.82	6.61	6.48	6.13	6.93	7.20
Protein	27.24	24.67	27.62	28.98	27.14	24.85	23.47
Fat	4.81	4.08	4.86	5.72	6.38	7.19	7.49
Fiber	9.54	9.32	11.58	13.58	15.52	16.20	16.15

Table 2: Formulation and proximate composition of fish diets contain *Gliricidia maculata* leaf meal protein

	Diet						
	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Ingredient (%)							
Groundnut oilcake	43	35	29	24	19	13	8
Rice bran	36	27	23	18	13	9	4
Fishmeal	10	9	9	9	9	9	9
Guar gum binder	10	8	8	8	8	8	8
Mineral-Vitamin mixture	1	1	1	1	1	1	1
G. maculata root powder	0	20	30	40	50	60	70
Nutrient content (%)							
Moisture	7.05	6.32	6.93	7.27	7.75	8.18	8.65
Total ash	11.13	11.26	10.59	10.38	9.89	9.62	9.02
Protein	27.24	29.30	30.93	31.42	32.10	32.16	31.38
Fat	4.81	8.33	7.40	7.26	6.56	6.02	6.89
Fibre	9.54	8.21	9.78	10.76	10.60	11.02	13.31

Table 3: Formulation and proximate composition of fish diets contain *Eichhornia crassipes* leaf meal protein

	Diet						
	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Ingredient (%)							
Groundnut oilcake	43	35	29	24	19	13	8
Rice bran	36	27	23	18	13	9	4
Fishmeal	10	9	9	9	9	9	9
Guar gum binder	10	8	8	8	8	8	8
Mineral-Vitamin mixture	1	1	1	1	1	1	1
E. crassipes root powder	0	20	30	40	50	60	70
Nutrient content (%)							
Moisture	7.05	5.54	5.86	6.87	6.89	6.63	7.09
Total ash	11.13	9.90	10.80	12.08	10.47	11.54	12.26
Protein	27.24	25.12	26.61	29.47	30.75	30.45	29.32
Fat	4.81	9.24	9.00	8.63	7.38	8.20	6.32
Fibre	9.54	7.16	9.54	9.79	11.26	11.78	12.67

Table 4: Growth performance and feed utilization in *C. catla* fed diets containing *Asparagus rascemosus* root protein

	Control	20%	30%	40%	50%	60%	70%
Initial body weight (gm)	2.32 ±0.05	3.5±0.02	1.33±0.06	1.44±0.05	1.65±0.04	2.10±0.05	2.85±0.05
Final body weight (gm)	15.56±0.42	18.73±0.47	24.60±0.67	21.32±0.59	17.30±0.52	16.10±0.40	13.40±0.34
Weight gain (gm)	13.26±0.36	15.23±0.40	23.27±0.60	19.88±0.52	15.65±0.46	14.00±0.34	10.55±0.27

Table 5: Growth performance and feed utilization in *C. catla* fed diets containing *Gliricidia maculata* leaf meal protein

	Control	20%	30%	40%	50%	60%	70%
Initial body weight (gm)	0.97±0.05	1.05±0.02	1.95±0.05	1.56±0.05	1.24±0.04	2.00±0.05	2.77±0.05
Final body weight (gm)	15.40±0.42	20.02±0.49	26.43±0.65	23.64±0.86	20.43±0.76	18.40±0.59	15.87±0.50
Weight gain (gm)	14.43±0.36	18.97±0.42	24.48±0.59	22.08±0.79	19.19±0.70	16.40±0.52	13.01±0.44

Table 6: Growth performance and feed utilization in *C. catla* fed diets containing *Eichhornia crassipes* leaf meal protein

	Control	20%	30%	40%	50%	60%	70%
Initial body weight (gm)	2.3±0.05	3.5±0.02	1.33±0.06	1.44±0.05	1.65±0.04	2.10±0.05	2.85±0.05
Final body weight (gm)	15.56±0.42	19.73±0.52	23.60±0.64	22.32±0.84	16.30±0.66	15.10±0.64	13.40±0.49
Weight gain (gm)	13.26±0.36	16.23±0.46	22.27±0.58	20.88±0.78	14.65±0.60	13.00±0.57	10.55±0.43

Table 7: Growth performance and feed utilization in *L. rohita* fed diets containing *Asparagus rascemosus* root protein

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Initial body weight (gm)	1.36±0.02	1.41±0.05	1.84±0.05	2.26±0.06	2.04±0.05	2.42±0.05	2.74±0.04
Final body weight (gm)	16.23±0.49	19.60±0.55	28.74±0.74	25.23±0.70	22.87±0.61	15.56±0.47	14.90±0.40
Weight gain (gm)	14.87±0.43	18.19±0.48	26.9±0.72	22.97±0.63	20.83±0.55	13.14±0.41	11.16±0.33

Table 8: Growth performance and feed utilization in *L. rohita* fed diets containing *Gliricidia maculata* leaf meal protein

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Initial body weight (gm)	1.22±0.02	1.55±0.05	1.56±0.05	2.11±0.06	2.54±0.05	3.23±0.05	4.09±0.04
Final body weight (gm)	16.40±0.49	21.74±0.57	28.23±0.77	37.62±0.01	33.90±0.90	25.67±0.69	20.87±0.59
Weight gain (gm)	15.18±0.43	20.24±0.50	26.67±0.70	35.51±1.01	31.36±0.84	22.44±0.63	16.78±0.53

Table 9: Growth performance and feed utilization in *L. rohita* fed diets containing *Eichhornia crassipes* leaf meal protein

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Initial body weight (gm)	2.13±0.02	2.55±0.05	2.45±0.05	2.35±0.06	2.33±0.05	2.4±0.05	2.2±0.04
Final body weight (gm)	18.20±0.49	22.68±0.62	28.51±0.76	36.10±0.99	29.16±0.78	27’09±0.75	20.21±0.58
Weight gain (gm)	16.07±0.43	20.13±0.55	26.06±0.69	33.75±0.93	26.83±0.72	24.69±0.69	18.01±0.51

Table 10: Liver protein content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	6.13±0.039	10.49±0.24	13.54±0.70	12.23±0.16	8.38±0.31	7.43±0.50	6.67±0.27
Gliricidia	6.13±0.39	9.14±0.12	12.27±0.30	13.48±0.39	11.10±0.23	7.47±0.29	6.17±0.09
Eichhornia	6.13±0.39	9.56±0.14	10.89±0.27	12.73±0.24	11.47±0.25	9.87±0.28	6.93±0.40

(Value expressed in mg/100mg wet tissue)

Table 11: Liver Lipid content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	8.40±0.40	9.49±0.21	12.37±0.12	10.99±0.25	10.04±0.31	9.02±0.23	7.43±0.20
Gliricidia	8.40±0.40	10.12±0.01	11.30±0.04	11.70±0.06	10.03±0.26	9.28±0.13	8.32±0.17
Eichhornia	8.40±0.40	9.90±0.06	10.63±0.14	11.36±0.10	10.67±0.42	9.54±0.19	6.95±0.34

(Value expressed in mg/100mg wet tissue)

Table 12: Liver Glycogen content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	1.67±0.23	3.32±0.27	5.18±0.09	4.36±0.37	3.14±0.34	2.65±0.44	1.74±0.01
Gliricidia	1.67±0.23	3.37±0.24	4.38±0.29	5.93±0.20	4.39±0.56	3.06±0.69	1.85±0.05
Eichhornia	1.67±0.23	3.02±0.10	5.53±0.18	5.01±0.75	3.14±0.31	2.86±0.01	1.92±0.04

(Value expressed in mg/100mg wet tissue)

Table 16: Liver protein content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	8.59±0.50	13.33±0.31	17.30±0.90	15.69±0.21	10.11±0.40	9.37±0.64	8.37±0.35
Gliricidia	8.59±0.50	12.56±0.19	14.36±0.36	16.86±0.32	15.16±0.33	12.95±0.37	9.09±0.53
Eichhornia	8.59±0.50	12.01±0.17	13.22±0.40	17.87±0.52	14.65±0.31	9.74±0.39	7.99±0.13

(Value expressed in mg/100mg wet tissue)

Table 17: Liver Lipid content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	11.22±0.54	12.47±0.28	16.37±0.17	14.51±0.34	13.21±0.42	11.84±0.21	9.69±0.38
Gliricidia	11.22±0.54	13.01±0.02	14.56±0.06	15.08±0.08	12.89±0.35	11.89±0.18	10.52±0.22
Eichhornia	11.22±0.54	12.41±0.08	13.34±0.18	14.28±0.12	13.40±0.54	11.95±0.25	8.63±0.44

(Value expressed in mg/100mg wet tissue)

Table 18: Liver Glycogen content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	2.06±0.29	3.5±0.12	3.64±0.23	5.94±0.92	3.98±0.39	3.31±0.02	2.14±0.05
Gliricidia	2.06±0.29	4.12±0.32	5.45±0.39	7.49±0.26	5.46±0.47	3.72±0.90	2.12±0.06
Eichhornia	2.06±0.29	4.19±0.37	6.73±0.13	5.60±0.51	3.93±0.46	3.26±0.60	2.02±0.03

(Value expressed in mg/100mg wet tissue)

Table 13: Muscle Protein content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	13.98±0.53	17.25±0.29	19.39±0.08	17.67±0.32	12.55±0.31	11.26±0.24	10.69±0.26
Gliricidia	13.98±0.53	19.02±0.37	20.56±0.17	24.12±0.12	21.49±0.23	19.93±0.58	16.37±0.24
Eichhornia	13.98±0.53	17.41±0.11	20.49±0.27	22.42±0.32	19.08±0.03	15.89±0.29	12.84±0.11

(Value expressed in mg/100mg wet tissue)

Table 14: Muscle Lipid content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	6.08±0.45	7.93±0.15	10.02±0.04	8.33±0.24	7.90±0.08	6.86±0.15	5.51±0.22
Gliricidia	6.08±0.45	9.24±0.18	9.86±0.13	10.75±0.29	8.57±0.22	7.25±0.22	5.54±0.19
Eichhornia	6.08±0.45	9.04±0.15	9.59±0.11	10.20±0.37	8.33±0.01	7.43±0.03	5.36±0.31

(Value expressed in mg/100mg wet tissue)

Table 15: Muscle Glycogen content of *Catla catla* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	0.88±0.08	2.27±0.08	2.58±0.02	2.50±0.03	2.18±0.08	1.88±0.07	1.47±0.01
Gliricidia	0.88±0.08	2.03±0.04	2.70±0.06	2.79±0.05	1.98±0.04	1.46±0.01	1.43±0.01
Eichhornia	0.88±0.08	2.28±0.05	3.11±0.03	2.83±0.11	2.21±0.12	1.95±0.02	1.46±0.01

(Value expressed in mg/100mg wet tissue)

Table 19: Muscle Protein content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	15.36±0.59	18.86±0.32	22.31±0.09	19.89±0.35	15.89±0.34	14.47±0.26	11.65±0.28
Gliricidia	15.36±0.59	19.20±0.03	22.42±0.42	26.41±0.14	23.52±0.31	21.80±0.64	17.98±0.27
Eichhornia	15.36±0.59	19.23±0.13	22.74±0.74	24.81±0.35	21.09±0.04	17.54±0.33	14.16±0.12

(Value expressed in mg/100mg wet tissue)

Table 20: Muscle Lipid content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	8.22±0.60	10.36±0.20	13.23±0.05	10.91±0.32	10.33±0.11	8.92±0.20	7.09±0.30
Gliricidia	8.22±0.60	11,59±0.19	12.30±0.14	13.11±0.48	10.65±0.01	9.47±0.04	6.74±0.41
Eichhornia	8.22±0.60	11.56±0.23	12.36±0.17	13.53±0.37	10.70±0.29	9.01±0.28	6.82±0.25

(Value expressed in mg/100mg wet tissue)

Table 21: Muscle Glycogen content of *Labeo rohita* fed with different levels of plant protein diets

	Control	20%	30%	40%	50%	60%	70%
Asparagus	1.08±0.10	2.57±0.10	2.96±0.03	2.86±0.03	2.46±0.10	2.17±0.03	1.58±0.01
Gliricidia	1.08±0.10	2.69±0.06	3.85±0.08	3.41±0.15	2.34±0.16	2.16±0.09	1.61±0.01
Eichhornia	1.08±0.10	2.42±0.06	3.32±0.09	3.46±0.06	2.34±0.05	1.64±0.07	1.59±0.01

(Value expressed in mg/100mg wet tissue)

Table 22: Lipase activity in *Catla catla* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	3.72±0.04	4.31±0.11	4.65±0.08	4.68±0.11	3.99±0.08	3.47±0.02	3.35±0.09
Gliricidia	3.72±0.04	4.23±0.17	4.66±0.06	4.84±0.06	3.89±0.03	3.57±0.01	3.36±0.01
Eichhornia	3.72±0.04	4.16±0.02	4.83±0.01	5.38±0.19	4.34±0.14	3.74±0.15	3.25±0.05

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 23: Protease activity in *Catla catla* with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	16.76±0.31	17.30±0.44	17.54±0.09	18.74±0.21	17.80±0.24	16.51±0.28	13,52±0.20
Gliricidia	16.76±0.31	17.40±0.12	18.66±0.34	20.49±0.46	22.34±0.35	17.59±0.24	14.32±0.09
Eichhornia	16.76±0.31	17.93±0.13	18.97±0.12	20.67±0.22	17.80±0.10	16.77±0.26	15.74±0.35

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 24: Amylase activity in *Catla catla* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	28.06±0.46	28.51±0.12	30.36±0.27	31.89±0.43	27.22±0.41	26.49±0.43	22.98±0.55
Gliricidia	28.06±0.46	29.47±0.25	31.65±0.43	34.97±0.13	34.13±0.29	31.34±0.13	28.36±0.27
Eichhornia	28.06±0.46	30.29±0.16	30.41±0.22	34.34±0.40	31.67±0.27	29.09±0.79	27.03±0.23

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 25: Invertase activity in *Catla catla* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	1.25±0.11	1.20±0.02	1.43±0.01	1.17±0.05	1.09±0.01	0.96±0.03	0.69±0.01
Gliricidia	1.25±0.11	1.32±0.06	1.47±0.02	1.82±0.01	1.46±0.01	1.23±0.04	0.76±0.01
Eichhornia	1.25±0.11	1.20±0.08	1.39±0.01	1.74±0.02	1.44±0.10	1.12±0.06	0.67±0.09

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 26: Lipase activity in *Labeo rohita* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	4.37±0.05	5.07±0.10	5.47±0.10	5.50±0.13	4.69±0.09	4.09±0.02	3.52±0.11
Gliricidia	4.37±0.05	4.89±0.02	5.69±0.01	6.33±0.22	5.11±0.16	4.40±0.17	3.35±0.06
Eichhornia	4.37±0.05	4.87±0.20	5.36±0.08	5.57±0.07	4.47±0.03	4.10±0.01	3.86±0.02

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 27: Protease activity in *Labeo rohita* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	20.20±0.38	20.84±0.53	21.14±0.10	22.59±0.26	21.44±0.29	19.89±0.33	17.49±0.24
Gliricidia	20.20±0.38	20.84±0.26	23.37±0.34	26.60±0.20	25.92±0.25	21.42±0.30	20.30±0.24
Eichhornia	20.20±0.38	21.34±0.16	22.58±0.14	24.61±0.26	21.19±0.12	19.95±0.31	18.74±0.42

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 28: Amylase activity in *Labeo rohita* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	31.88±0.52	32.41±0.14	34.51±0.31	36.24±0.49	30.93±0.46	30.11±0.49	26.11±0.63
Gliricidia	31.88±0.52	33.12±0.28	35.56±0.44	39.29±0.15	38.35±0.33	35.23±0.15	31.87±0.30
Eichhornia	31.88±0.52	33.29±0.18	43.56±0.25	39.03±0.45	35.99±0.30	33.04±0.90	30.72±0.26

(Value expressed in mg palmitic acid/gm protein/ hr)

Table 29: Invertase activity in *Labeo rohita* fed with different levels of plant protein diets

	Control	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**
Asparagus	1.44±0.12	1.38±0.02	1.64±0.02	1.53±0.05	1.25±0.01	1.10±0.03	0.81±0.01
Gliricidia	1.44±0.12	1.49±0.07	1.66±0.02	2.04±0.02	1.65±0.01	1.38±0.05	0.86±0.03
Eichhornia	1.44±0.12	1.43±0.10	1.65±0.01	2.07±0.03	1.72±0.12	1.34±0.07	0.80±0.10

(Value expressed in mg palmitic acid/gm protein/ hr)

Figure 1: Growth performance and feed utilization in *C. catla* fed diets containing *Asparagus rascemosus* root protein

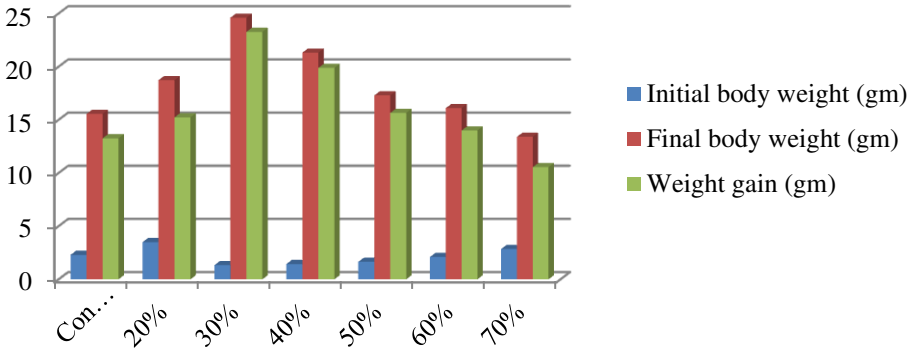

Figure 2: Growth performance and feed utilization in *C. catla* fed diets containing *Gliricidia maculata* leaf meal protein

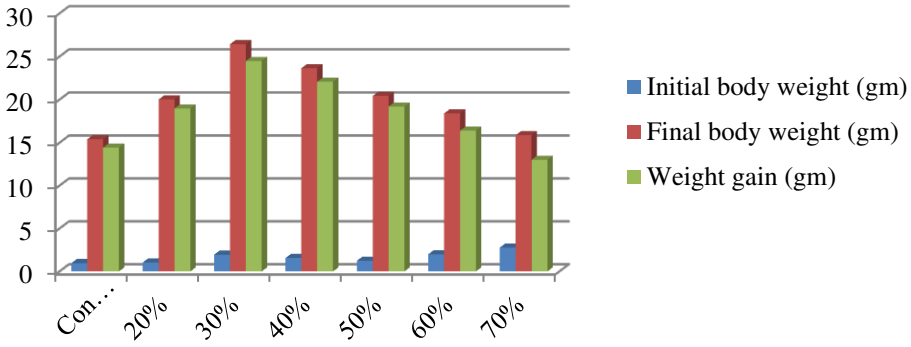

Figure 3: Growth performance and feed utilization in *C. catla* fed diets containing *Eichhornia crassipes* leaf meal protein

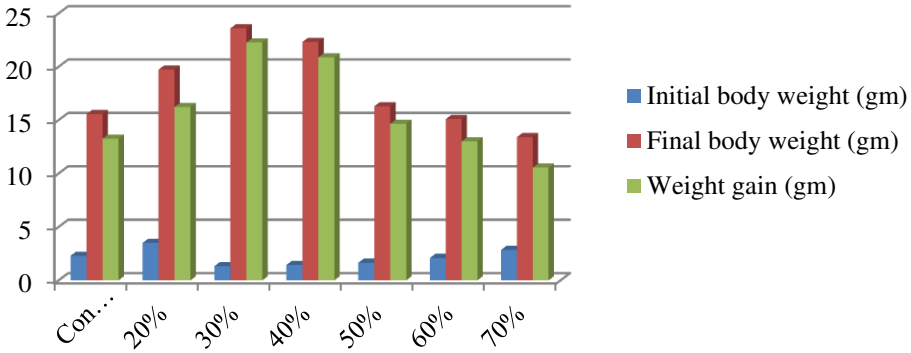

Figure 4: Growth performance and feed utilization in *L. rohita* fed diets containing *Asparagus rascemosus* root protein

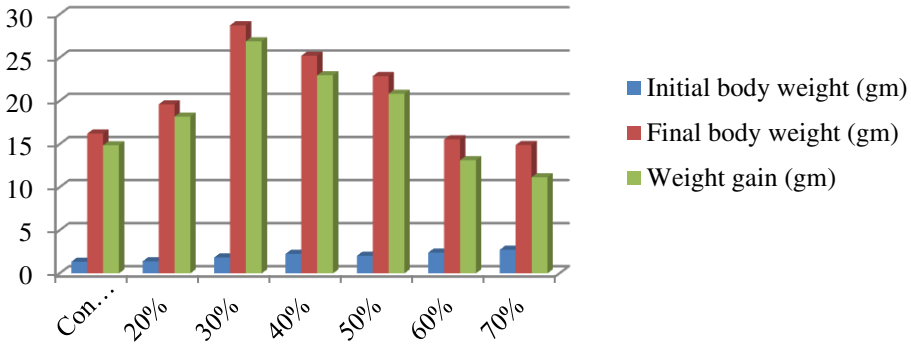

Figure 5: Growth performance and feed utilization in *L. rohita* fed diets containing *Gliricidia maculata* leaf meal protein

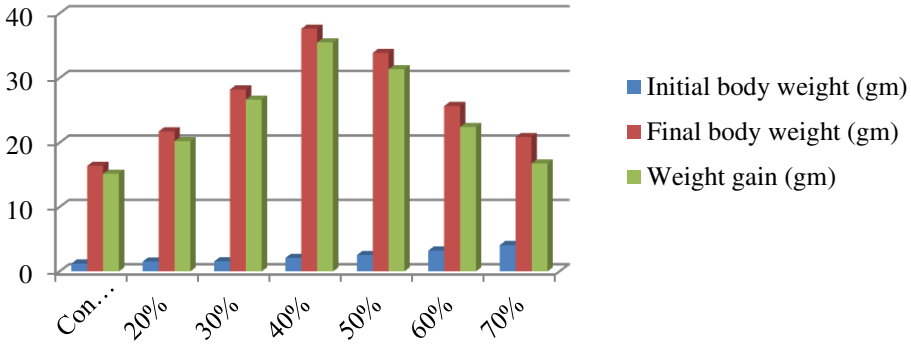

Figure 6: Growth performance and feed utilization in *L. rohita* fed diets containing *Eichhornia crassipes* leaf meal protein

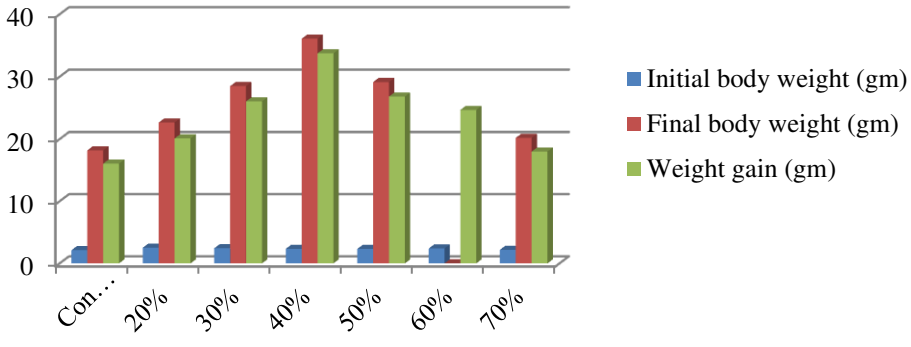

Figure 7: Liver protein content of *Catla catla* fed with different levels of plant protein diets

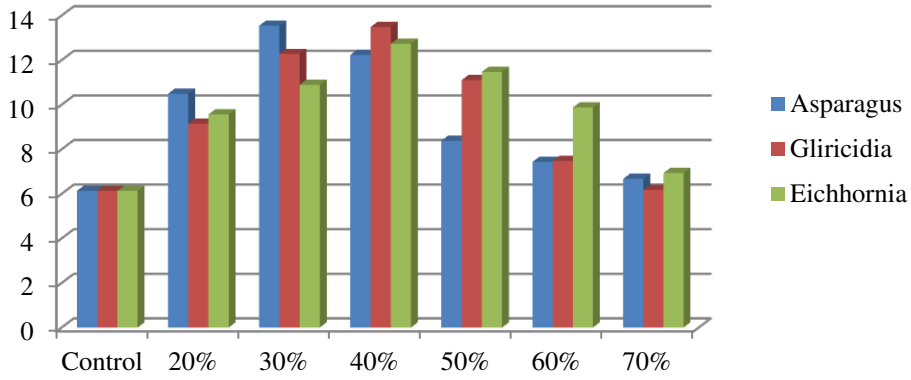

Figure 8: Liver Lipid content of *Catla catla* fed with different levels of plant protein diets

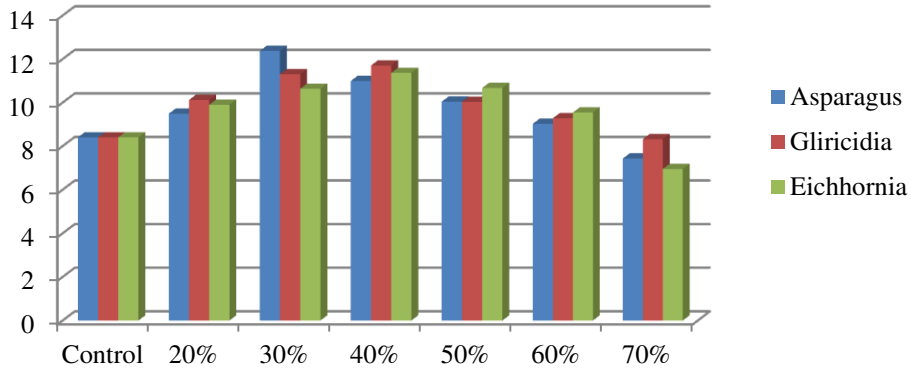

Figure 9: Liver Glycogen content of *Catla catla* fed with different levels of plant protein diets

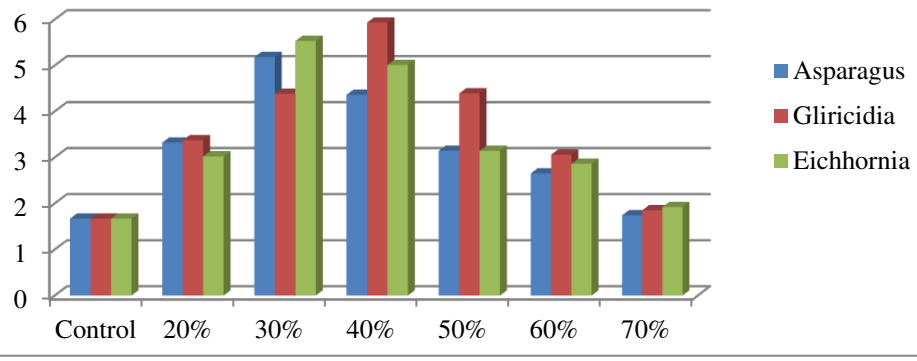

Figure 10: Muscle Protein content of *Catla catla* fed with different levels of plant protein diets

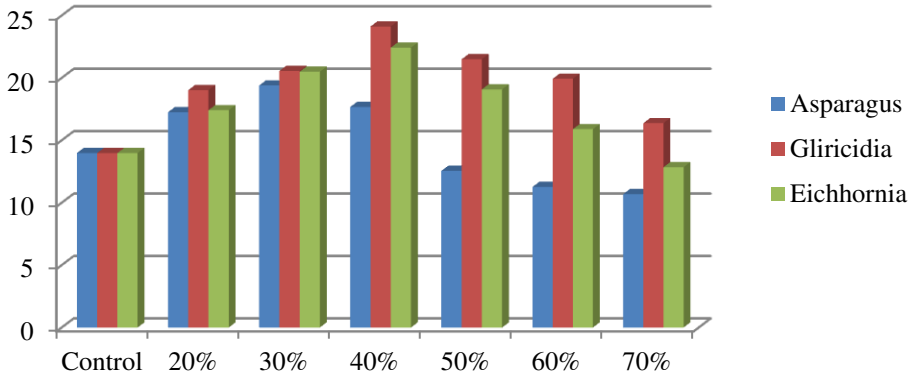

Figure 11: Muscle Lipid content of *Catla catla* fed with different levels of plant protein diets

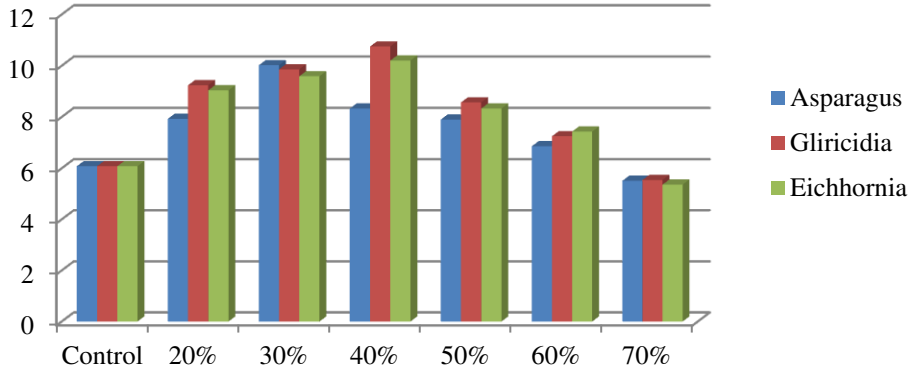

Figure 12: Muscle Glycogen content of *Catla catla* fed with different levels of plant protein diets

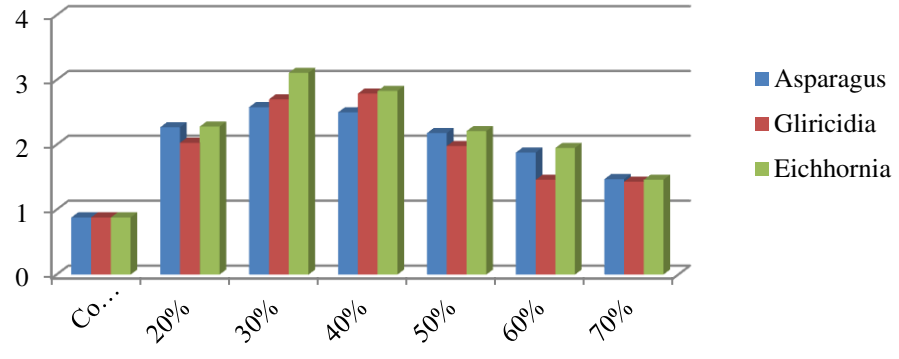

Figure 13: Liver protein content of *Labeo rohita* fed with different levels of plant protein diets

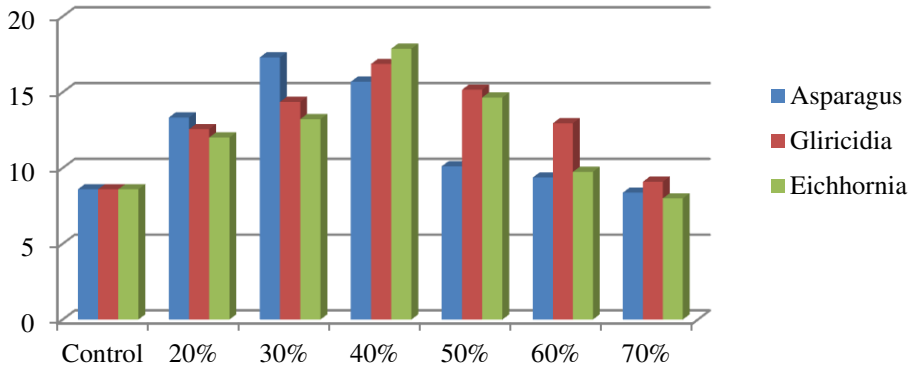

Figure 14: Liver Lipid content of *Labeo rohita* fed with different levels of plant protein diets

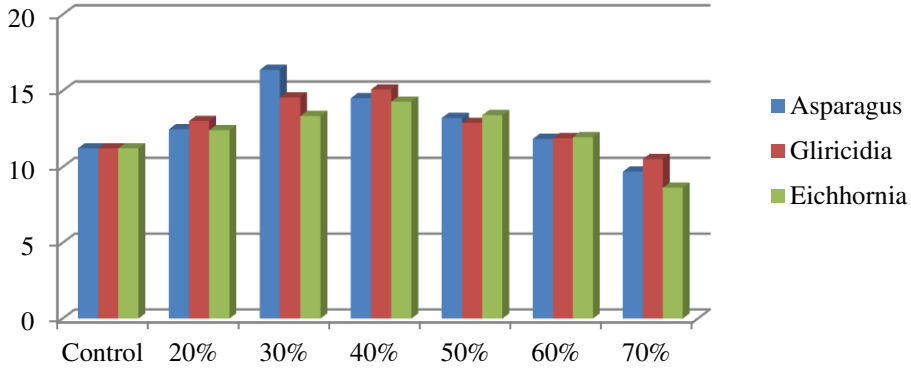

Figure 15: Liver Glycogen content of *Labeo rohita* fed with different levels of plant protein diets

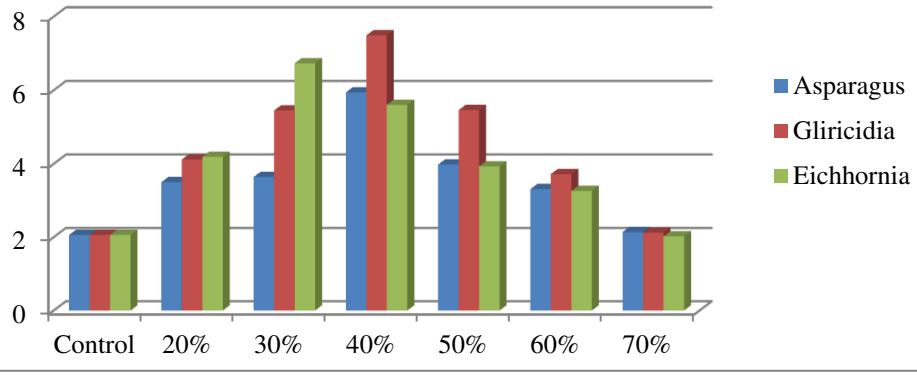

Figure 16: Muscle Protein content of *Labeo rohita* fed with different levels of plant protein diets

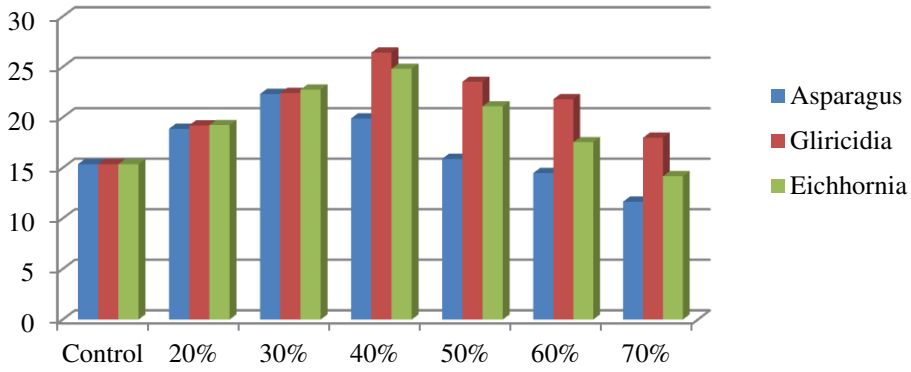

Figure 17: Muscle Lipid content of *Labeo rohita* fed with different levels of plant protein diets

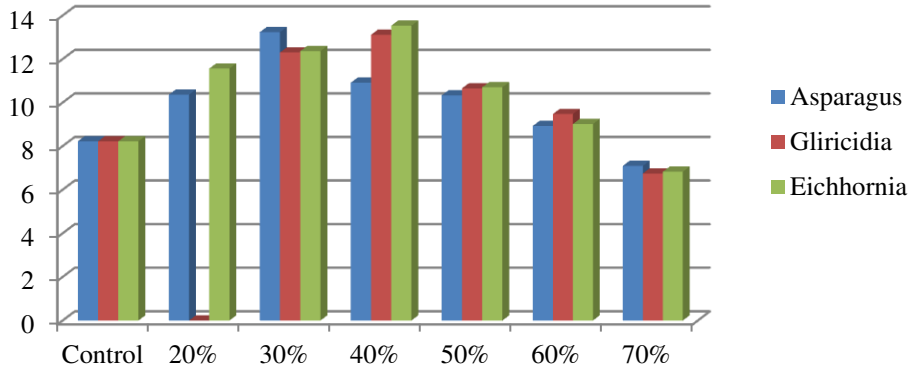

Figure 18: Muscle Glycogen content of *Labeo rohita* fed with different levels of plant protein diets

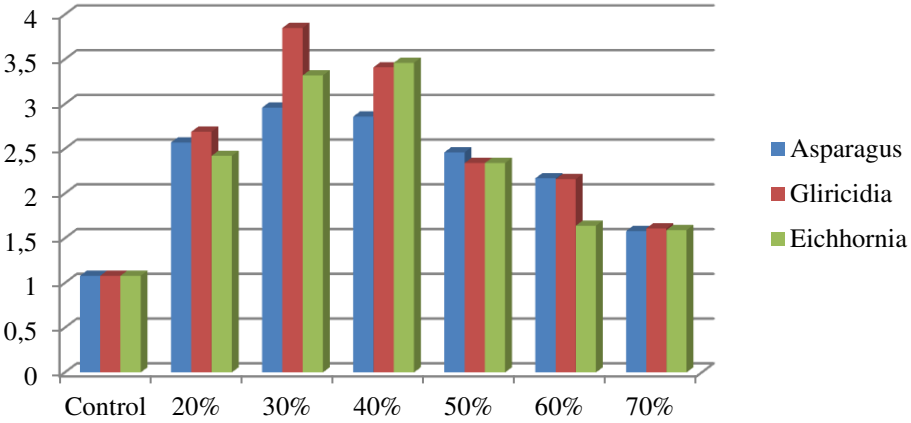

Figure 19: Lipase activity in *Catla catla* fed with different levels of plant protein diets

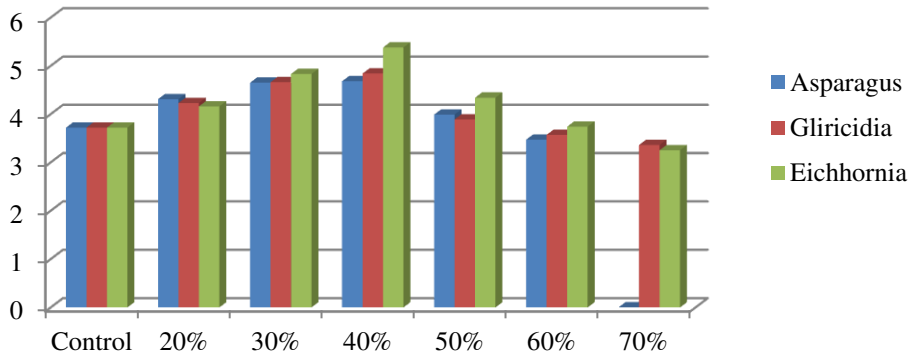

Figure 20: Protease activity in *Catla catla* with different levels of plant protein diets

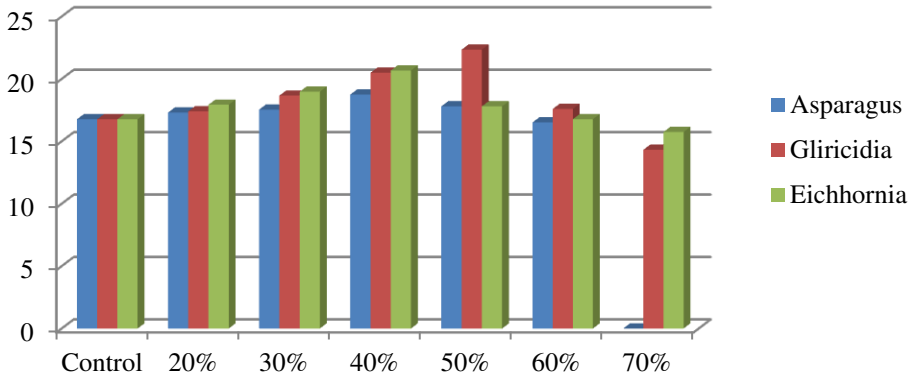

Figure 21: Amylase activity in *Catla Catla* fed with different levels of plant protein diets

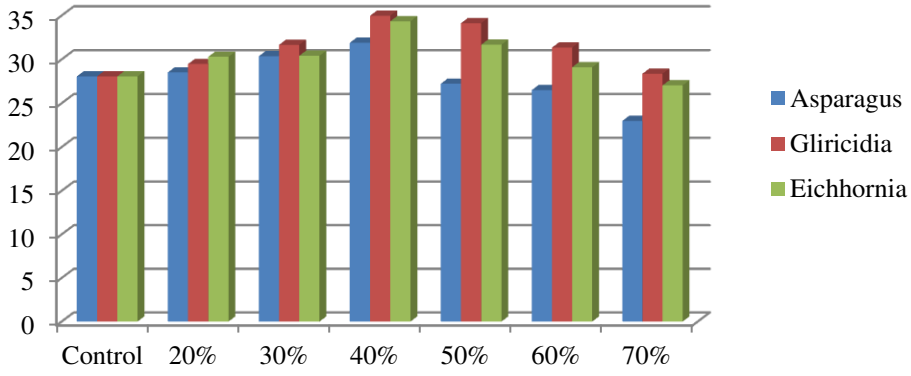

Figure 22:Invertase activity in *Catla catla* fed with different levels of plant protein diets

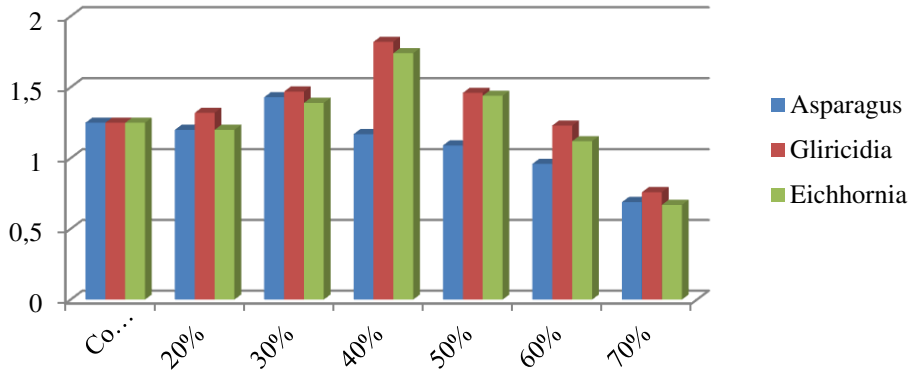

Figure 23: Lipase activity in *Labeo rohita* fed with different levels of plant protein diets

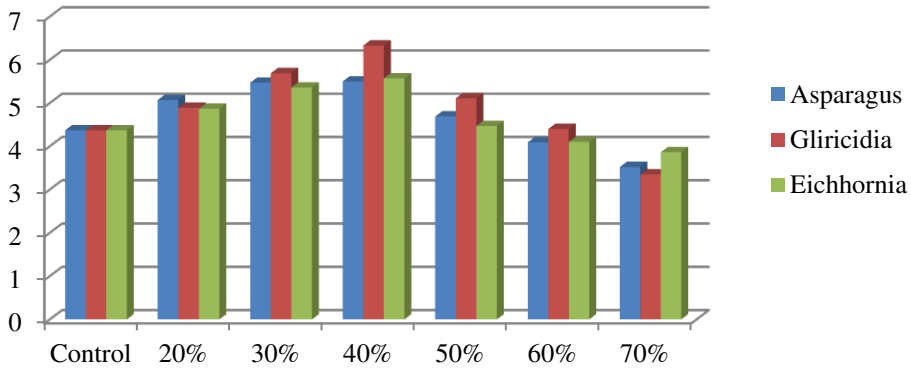

Figure 24: Protease activity in *Labeo rohita* fed with different levels of plant protein diets

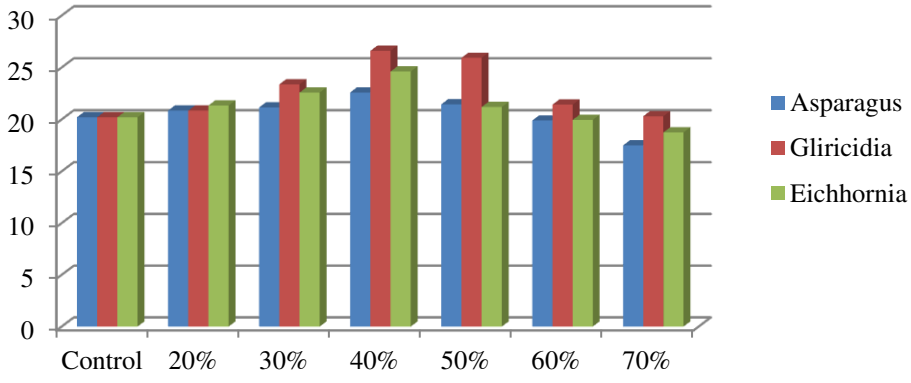

Figure 25: Amylase activity in *Labeo rohita* fed with different levels of plant protein diets

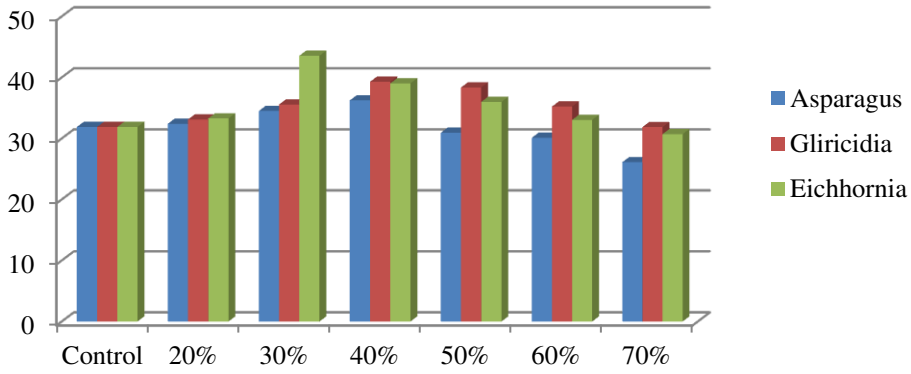

Figure 26: Invertase activity in *Labeo rohita* fed with different levels of plant protein diets