1. INTRODUCTION

1.1 General background

Aquaculture is the fastest growing food-producing sector, now accounts nearly 50% of the world's food fish, contributing significantly in the world economy (FAO 2015). Aquaculture includes farming of both plants and animals in both inland (freshwater) and coastal water (marine). It helps in the production of white meat at large scale for human consumption. Globally, around 567 (354 species with 5 hybrids) aquatic species are currently farmed with the net production of 158 millions tones and world per capita apparent has been found to be increased from an average 9.9 Kg in the 1960s to 19.2 kg in 2012 (FAO 2014). Aquaculture being an industry constantly seeking better well trained people to develop a sustainable future. It has been found that 54.8 million total employments (38.2 million people in capture fisheries and 16.6 million people in aquaculture practices) were engaged in aquaculture sector (Ababouch and Karunasagar 2013). FAO (2014) revelled that China is in the first rank in the world to contribute in the sector of aquaculture practices nearly 5.9% of the global production i.e. around 43.5 millions tones of food fish and 13.5 millions tones of aquatic algae up to 2012.

1.2 Aquaculture in Nepal

Nepal has no coastal area. So, Nepal has to depend on inland fisheries. Nepal has rich freshwater resources which constitute snows fed rivers, lakes, ponds where fisheries have been practiced for a long time. Aquaculture in Nepal is basically small but contributes 3% to the agricultural GDP. River is the major source of capture fishery covering 395000 ha. of the surface of natural water resources. Around 75000 people are engaged in aquaculture with net fish production of 64,900 Mt. (culture fisheries 43,400 Mt. and capture fisheries 21,500 Mt.) in the year 2014 (DoFD 2013/2014).

In Nepal, many fishermen, their families and others are engaged in capture fisheries, which represent nearly 0.28% of the total population of Nepal. Aquaculture in Nepal is continuously progressing. Here, numbers of people are increasing for fish farming in rivers, lakes, swamps, gholes, irrigated fields, and ponds. Different systems of fish farming techniques like: monoculture, polyculture, cage culture, pen culture, race way culture, integrated fish farming such as Rice-fish farming, Horticulture-fish farming, Livestock-fish farming, Pig-fish farming, Duck-fish farming, Poultry-fish farming and so on has been carried in the context of Nepal. The summary of fish production in Nepal 2013/2014 is shown below:

Table 1. Summary of fish production in Nepal

Resource details	Total area (ha.)	Fish production (Mt.)
Aquaculture practices	93,700	43,400
Rivers	395,000	7,110
Lakes	5,000	850
Reservoirs	1500	385
Marginal/Swamps/Gholes	11,100	5,990
Low land irrigated paddy fields	398,000	7,185
Total fish production (Mt.)	64,900.00	

Source: DoFD 2013/2014

1.3 Statement of the problem

Aquaculture is the main source of livelihood for many poor people in the developing countries including Nepal (Shakya and Labh 2014). The aqua culturist or farmers faces the challenges of disease outbreaks like bacterial, fungal, viral protozoans and so on. Diseases and pathogens are part of every intensive aquaculture production systems (Sivasankar et al. 2015). Farmers are in great loss due to farmed species are increasing exposed to pathogens where prophylactic treatment with antibiotics are no longer acceptable and ineffective for many pathogens (Wattes et al. 2001). Due to this reason, net production is low and is unable to fulfil the demands in the market.

In the context of Nepal, farmers usually treat the fish with antibiotics; thus producing unhealthy fish. Also, there is a research gap to analyse the efficacy of herbal plants product or extract in fish growth and its immune system. So the proper research is needed to evaluate the effects of herbal plants or extract in the field of aquaculture so that the net production can be enhanced with high profit.

Thus, in this research I plan to evaluate the effects of medicinal plant Lapsi on survival, growth performance and protein profile of Nile Tilapia during intensive aquaculture.

1.4 Lapsi as an herbal medicine

Lapsi *Choerospondias axillaris* (Roxb.) belongs to family Anacardiaceae, is a large, dioecious and deciduous fruit tree. The tree is native to Nepal (Roxburgh 1832) found growing in hills between 850-1900 m above the sea level and has also been reported from various countries like India, China, Thailand, Japan, Vietnam and Mongolia (Paudel et al. 2003). Nepal is unique for processing and use of Lapsi fruits (Paudel 2003). The fruits of Lapsi are rich in vitamin C content (Shah 1978) and are used as a medicinal plant to enhance the immune system of the body (Chunmei et al. 2013). The constituents of Lapsi fruits have been investigated chemically and shown to include phenolic compounds and flavonoid content (Zhou 2003). Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman et al.1998). The ability of phenolic compounds to serve as antioxidants has been recognized, leading to speculation about the potential benefits of ingesting phenolic rich foods (Shi et al. 1985). Lapsi fruit is commonly used for the treatment of cardiovascular diseases (Zhou 2003) and in Mongolia, it is used in the treatment of myocardial ischemia, calming nerves, ameliorating blood circulation and improving microcirculation (Dai et. al. 1992, Shi et al. 1985). The water extract of the bark of this plant is being used for the treatment of second degree burns in both children and adult in northern Vietnam (Nyugen et al. 1996).

1.5 Experimental fish

1.5.1 Systematic position (Source: Linnaeus 1758)

Kingdom- Animalia

Phylum- Chordata

Class- Actinopterygii

Order- Perciformes

Family- Cichlidae

Genus- *Oreochromis*

Species- *niloticus*

1.5.2 Introduction

Nile Tilapia *Oreochromis niloticus* (Linnaeus 1758) belongs to cichlidae family and the most important fish among other culture fishes in this 21st century (Shelton 2002). It is a major source of protein in many developing countries including Nepal. Two species of Tilapia, Nile Tilapia (*Oreochromis niloticus*) and Mossambique Tilapia (*O. mossambicus*) were introduced in Nepal for the first time from Thailand in 1985 (Pantha 1993, Shrestha 1994). Nile Tilapia is characterized by the presence of long spiny dorsal spines, interrupted lateral line and the presence of distinct black stripes on the body and tail. It is omnivorous which can attains sexual maturity and spawns within 6 months (Shrestha and Pandit 2012).

Nile Tilapia is rich in nutritional value like protein, selenium, Phosphorous, Potassium, B6, B12, Niacin and Pantothenic acid and high value of Omega-6 fatty acids to Omega-3 fatty acids and low fat, nearly 3%. Nile Tilapia contributed more than 75% in total Tilapia production in the world (FAO 1994).

1.6 Objectives of the study

The main objective of this research is to study the effects of Lapsi on growth and protein profiles of Nile Tilapia while the specific objectives are as:

- To study the effects of Lapsi on survival rate of Nile Tilapia fingerlings.
- To study the effects of Lapsi on growth performance of Nile Tilapia fingerlings.
- To study the effects of Lapsi on protein profile of Nile Tilapia) fingerlings.

1.7 Limitations

Every research studies face different kind of problems. So, this research faces the following challenges:

- The research has limitation regarding finance and time constrains.
- The research was carried with doses of Lapsi up to 400 mg/Kg.

2. LITERATURE REVIEW

The goals of the aquaculture industry are to optimize growth and to produce high-quality fish. As in all farming, the outbreak of diseases in fish farming can be a major concern. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced aqua culturists to concentrate their efforts on maintaining their fish in good health in order to achieve sustainable economic performance. For healthy fish growth; they must develop strong defence mechanisms against pathogen invasion. It is the nutritional quality of the feed that sustains healthy fish. Keeping these things in mind this chapter reviews the following topics for an appraisal of the work done on effects of immunostimulant plant Lapsi on survival, growth and immune status of Nile Tilapia during intensive aquaculture.

2.1 Tilapia culture in Nepal

Tilapia, an African fish is used in warm water aquaculture throughout the world. It is the major source of protein in many developing countries. In the context of Nepal, farmers can achieve much more benefits by Tilapia farming due to suitable climatic conditions (Yadav 2006). Nile Tilapia is cultured in limited scale in warm water where as Mossambique Tilapia was introduced unauthorizely by farmers (Shrestha and Bhujel 1999, Shrestha and Pandit 2012). Rai et al. (2007) had performed experiment by culturing Sahar and mixed sex Nile Tilapia in monoculture and co-culture system in Chitwan and found that high net yield in Sahar + Nile Tilapia polyculture where mean size of recruit of Nile Tilapia was significantly higher ($P<0.05$) in Sahar+ Nile Tilapia polyculture. He also concluded that introduction of Sahar is suitable species for polyculture to control excessive recruitment of Nile Tilapia. Mishra (2002) found higher production during the culture of African catfish (*Clarias gariepinus*) and mixed-sex Nile Tilapia in the ratio 1:1. Recently, culture practice of Tilapia has been carried out in cage-pond and cage culture in different Lakes.

2.2 Some medicinal plants as immunostimulants

Immunomodulation is the regulation and modulation of immunity either by enhancing or by reducing the immune response. Modulation of immune response may involve induction, expression or amplification of immune response. In other words, immunomodulation involves a change in the human body's immune system caused by agents that activate or suppress its function (Shivaprasad et al. 2006).

If the modulation in immune system results in enhancement of immune reaction, it is known as the immunostimulation. There are two main categories of immunostimulators; the specific and non-specific. The specific immunostimulators provide specific antigen in immune response, such as vaccines or any antigen; the non-specific immunostimulators stimulate components of the immune system without antigenic specificity such as adjuvants (Fenichet and Chirigo 1984).

The use of immunostimulants as an alternative to the chemotherapeutics and antibiotics currently being used to control fish diseases in fish culture is attracting the attention of many researchers. In this context, many have focused on the use of medicinal plants and animal originated products as potential therapeutic measures for modulating the immune response to prevent and control the fish diseases. The possible uses of naturally available herbal extracts such as *Ocimum sanctum* (Tulsi), *Phyllanthus emblica* (Amla), *Azadirachta indica* (Neem), *Solanum trilobatum* (Purple Fruited Pea Eggplant), *Eclipta alba* (Bhringraj), *Zingiber officinale* (Ginger), *Echinacea* (Purple coneflowers), *Allium sativum* (Garlic), *Camellia sinensis* (Green tea), *Aloe vera* (Ghyukumari), *Cynodon dactylon* (Bermuda Grass), *Achyranthes aspera* (Prickly Chaff Flower), *Nyctanthes arbortristis* (Night-flowering Jasmine), *Tinospora cordifolia* (Guduchi) and *Picrorhiza kurooa* (Kutki) and animal originated products like chitin, chitosan and fermented products of chicken egg (EF203) etc. have been discussed by Bairwa et al. (2012). Natural plant products promote various activities such as antistress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and antimicrobial properties due to the presence of active constituents such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids and essential oils.

2.2.1 Ocimum sanctum (Tulsi)

Leaves of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophylllene that may act as an immunostimulant. The acetone extract of *O. sanctum* was found to enhance the anti-sheep red blood cell (SRBC; sheep erythrocytes) antibody response. Leaves extract of *Ocimum sanctum* in Tilapia (*Oreochromis mossambicus*) affected both specific and non-specific immune responses and disease resistance against *Aeromonas hydrophila*. It stimulated both antibody response and neutrophil activity (Jayathirtha and Mishra 2004).

2.2.2 Phyllanthus emblica (Amla)

Phyllanthus emblica has antioxidant activity, anti-fungal activity, antimicrobial activity and anti-inflammatory activity. Amla fruit pulp contains large proportion of vitamin C, which has also been identified as an immunostimulant. An acetone extract of *P. emblica* enhanced the anti-SRBC antibody response in Tilapia (Jayathirtha and Mishra 2004) while both crude extract and a water-soluble fraction of *P. emblica* fruit had a stimulatory effect on the immune response of Tilapia (Balasubramani and Michael 2002).

2.2.3 Azadirachta indica (Neem)

Biomedical research has revealed that *Azadirachta indica* (Neem) possesses anti-human immunodeficiency virus, anti-tumour and antimicrobial activities. Azadirachtin, a triterpenoid derived from *A. indica*, enhanced respiratory burst activities, the leukocyte count and the primary and secondary antibody response against SRBC in Tilapia (Logambal and Michael 2001, Rao and Chakrabarti 2005).

2.2.4 Solanum trilobatum (Purple Fruited Pea Eggplant)

The herbal extract of *Solanum trilobatum* contains compound like Sobatum, b-solamarine, solaine, solasodine, glycoalkaloid, diosogenin and tomatidine. *Solanum trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activities. A study aimed at assessing the effects of the water and hexane-soluble fractions of *S. trilobatum* the non-specific immune mechanisms and disease resistance of Tilapia found that all doses of the water soluble fraction significantly enhanced the production of reactive oxygen and decreased the percentage mortality following a challenge infection with *Aeromonas hydrophila* (Nya and Austin 2009).

2.2.5 Eclipta alba (Bhringraj)

Eclipta alba, an herb belonging to Asteraceae has been reported to possess several medicinal properties. The methanol extracts of the whole plant of *Eclipta alba* significantly increased the phagocytic index, antibody titer and WBC count in mice (Kirubakaran et al. 2010).Oral administration of aqueous extract of *Eclipta alba* leaf to *Oreochromis mossambicus* indicate that dietary intake of *E. alba* aqueous leaf extract enhances the non-specific immune responses and disease resistance in O. mossambicus against *A. hydrophila* (Christybapita et.al. 2007).

2.2.6 Zingiber officinale (Ginger)

Roots and the obtained extracts of *Zingiber officinale* contain polyphenol compounds (6-Gingerol and its derivatives), which have a high antioxidant activity. The use of Ginger @ 0.5g/110g of feed reduced mortalities to 0% compared with the controls (64%). Moreover, there was a significant increase in growth, feed conversion and protein efficiency. There was proliferation in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities compared with the controls (Hemapriya 1997**).**

2.2.7 Echinacea (Purple coneflowers*) and Allium sativum* (Garlic)

Echinacea and *Allium sativum* improve the gain in body weight, survival rate and resistance against challenge infection of *Aeromonas hydrophila.* Both compounds showed extended effects after withdrawal and improved resistance to cold stress during the winter season (Aly and Mohamed 2010**).**

2.2.8 Camellia sinensis (Green tea)

Green tea (GT) extracts contain a unique set of catechins that possess antioxidant, anti-angiogenesis, and anti-proliferative assays that are potentially relevant to the prevention and treatment of various forms of cancer. The inclusion of green tea in fish diet up to 0.5 g/kg diet enhanced the protein contents in fish body, while the lowest lipid contents were obtained at 0.0-0.5 g GT/kg diet. Haematological and biochemical parameters were improved in fish fed 0.25–2.0 g GT/kg diet, while the lowest values were obtained in the control. The survival of fish challenged with *A. hydrophila* increased with increasing GT level in fish diets. These results indicate that GT supplement is promising immunostimulant, which could improve fish performance and health (Yoshida et al. 1993).

2.2.9 Aloe vera (Ghyukumari)

Oral administration of *Aloe vera* in common carp can enhance some of specific and non-specific immune responses. This appears to be achieved primarily by increasing lysozyme activity, serum bactericidal power and the total protein and IgM levels. *Aloe vera* supplementation (0.5%) per feed can increase the resistance to *Aeromonas hydrophila* and *A. septicemia*. The relative percent survival (RPS) was found to be increased in the fish fed with *Aloe vera* (Alishahi et.al. 2010).

2.2.10 Cynodon dactylon (Bermuda Grass)

The antiviral activity of extract of *Cynodon dactylon was* examined on white spot syndrome virus (WSSV) in Black Tiger Shrimp *Penaeus monodon* by in vivo testing after administration through oral route. The results of the study showed that the plant extract of *C. dactylon* was found to be highly effective in preventing WSSV infection with no mortality and no signs of WSD (White spot disease) at 2% and 40% mortality at 1% in P. monodon, respectively (Balasubramanian 2008).

2.2.11 Achyranthes aspera (Prickly Chaff Flower)

When *Catla catla* was fed with *Achyranthes aspera* (0.5%), both specific and non-specific immunity were enhanced compared with the control fish fed with the normal diet (Sahoo and Mukherjee 1999**)**. This was revealed by higher serum antibody levels and higher serum anti-proteases in the test group fish than control groups. Serum globulin level and RNA/DNA ratio of the spleen were also significantly enhanced in the fish fed with the diet containing *Achyranthes aspera.*

2.2.12 Nyctanthes arbortristis (Night-flowering Jasmine)

Nyctanthes arbortristis (L) possesses hepatoprotective, antileishmanial, antiviral and antifungal activities. Feeding Tilapia for 2 weeks with selected doses of chloroform extract of *Nyctanthes arbortristis* seeds significantly enhanced serum lysozyme, alternate complement activities and cellular ROS (cellular reactive oxygen species), RNI (reactive nitrogen intermediate) and MPO production. It was evident from the disease resistance test that feed supplemented with *Nyctanthes arbortristis* seed extract at 0.1% or 1% level significantly reduced the mortality of *O. mossambicus* and a 3-week feeding with 0.1% extract-supplemented diet appears to be the optimal regimen for maximal disease resistance (Logambal and Michael 2000).

2.2.13 Choerospondias axillaris (Nepalese Hog Plum*)*

Nepal is unique for processing and utilization of Lapsi fruits. The fruits are rich in vitamin C content (Paudel 2003), and are consumed fresh, pickled and processed into a variety of sweet, sweet & sour, hot & sweet, hot & sour tasty food products locally called 'Titaura' (Paudel 2002). Lapsi distribution is not restricted to the Himalayas, however, the tree is also found in Thailand (Jackson 1994), Vietnam (Nguyen et al. 1996) and China (Hau et al.1997, Zhou et al. 1997, Feng et al. 1999, Zhou et al. 1999, Lin et al. 2000). *Choerospondias axillaris* (Roxb.) is a medicinal plant used in Mongolia (Wang et al. 2008), used to treat burns and has medicinal uses for women during labour or childbirth.

2.2.14 Fermented vegetable product (FVP)

The phagocytic activities and superoxide generation of peritoneal induced leukocytes were significantly higher in Japanese flounder fish (Paralichthy solivaceus) fed with the FVP supplemented diet than fish fed with the control diet. FVP feeding in fish had a significantly higher ($P < 0.05$) activity of lysozyme than in the control fish (Ashida and Okimasu 2005).

2.2.15 Other plant extract

Plant extract of *Cyanodon dactylon*, *Aegle marmelos*, *Tinospora cordifolia*, *Picrorhiza kurooa* and *Eclipta alba* were used to increase the immunity of shrimps against the WSSV. The mixed methanolic extracts of above plants was supplemented with various concentrations viz. 100 (A), 200 (B), 400 (C), and 800 (D) mg kg-1 through artificial diets individually (Citarasu et al. 2006, Divyagnaneswari et al. 2007). The shrimps fed on diet D (800 mg kg-1) significantly ($P < 0.0001$) had more survival (74%) and reduction in the viral load compare to other diet. It also showed better performance of haematological, biochemical and immunological parameters.

2.3 Antioxidant properties in fruits and herbs

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule (Velioglu et al.1998). Antioxidants have long been used for food preservation, but there has been concern that synthetic antioxidants are used for preservation such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluence (BHT) may cause liver damage and carcinogenicity (Gartner et al. 1997). Thus, the interest in natural antioxidants has increased considerably (Stahl and Sies 1992). In addition, high levels of natural antioxidants have been shown to have multiple benefits to human health (Larson 1988). Many fruits and herbs are capable to terminate free radical reaction. It has been stated that phytoconstituents have less side effect and compatible to body physiology (Sen et al. 2001). Dukachodal et al. (2011) found that, the extract prepared from lingonberry leaves (725.2 mg Trolox/g d.w.) exhibited the highest antioxidant activity.

Wang (2003) recorded that photochemical such as vitamin E, vitamin C, beta carotene, flavoneids, phenolic acid present in fruits and herbs are effective and potential source of natural environment. The antioxidant properties from the methanol extracted of 180 selected oriental herbs have been determined by Kim et al. (1994). Likely, Zheng and Wang (2001) studied the antioxidant capacity of 39 culinary and medicinal herbs and found that *Catharanthus roseus*, *Thymus vulgaris*, *Hypericum perforatum*, and *Mentha piperita* had highest antioxidant values. Rosmarinic acid and luteolin are the major constituents in the Sage (*Salvia officinalis*) which has medicinal properties for the treatment of various diseases (Areias et al. 2000). Prasad and Padhyoy (1993) concluded that herbal preparations consists antioxidant and antimicrobiological activities to control many diseases. Metwally (2009) recommended adding Garlic in any form increase the antioxidant activity in fish.

2.4 Effects of fruits and herbs on fish growth

In fish farming, the management is directed towards the realization of maximum fish biomass within a certain time (Verreth 1999, De-Franceso et al. 2007, Schuchard et al. 2008). Fish growth and survival rate correlates with the nature of feeds up on which they are fed. Use of fruits and herbs enhance the growth and survival rate of cultured fish. The possible use of naturally available herbal extract in aquaculture as growth promoter such as *Ocimum sanctum* (Tulsi), *Phyllanthus emblica* (Amla), *Azadirachta indica* (Neem), *Solanum trilobatum* (Purple Fruited Pea Eggplant), *Eclipta alba* (Bhringraj), Zingiber *officinale* (Ginger), *Echinacea* (Purple coneflowers), *Allium sativum* (Garlic), *Camellia sinensis* (Green tea), *Aloe vera*, *Cynodon dactylon* (Bermuda Grass), *Achyranthes aspera* (Prickly Chaff Flower), *Nyctanthes arbortristis* (Night-flowering Jasmine), *Tinospora cordifolia* (Guduchi) and *Picrorhiza kurooa* (Kutki) etc. has been discussed by (Bairwa et. al 2012).

Various herbal products such as *Hygrophila spinosa*, *Withania somnifera*, *Zingiber officinalis*, *Solanum trilobatum*, *A. paniculata*, *Psoralea corylifolia*, *Ecliptae recta*, *Ocimum sanctum*, *Picrorhiza kurooa*, *P. niruri*, *Tinospora cordifolia*, purified Silajit and cod-liver oil have the characteristics of growth promotion, anti-stress, immunostimulation, and anti-bacterial. These preparations had a good influence in the *Penaeus* larviculture (Citarasu et al. 1998, 2002). Sambhu and Jayaprakash (2000) recommended the use of 1% of Livol (IHF-1000) enhance maximum growth and improve nutrient digestibility of prawn. Pandey et al. (2012) showed the use of vitamin C and E as herbal drugs act as growth promoter and cure all kinds of diseases of fishes and aquatic animals.

Herbal products Stressol- I and Stressol- II enriched Artemia naupliifeed with *Peraeus indicus* postlarvae increased growth and efficiencies significantly and reduced the osmotic stress (Chitra 1995). Livol (IHF-1000) is herbal growth promoters which significantly improves digestion leading to maximum growth, net production and improve health in cultivable fishes (Shadakshari 1993, Unnikrishnan 1995, Jayaprakas and Euphrasia 1996). Ashraf and Goda (2008) found that the use of dietary Ginseng herb (Ginsana G115) greatly enhanced the growth performance, diet utilization in the Nile Tilapia (*Oreochromis niloticus*) fingerlings. The use of *Quillaja saponins* – a natural growth promoter for fish species and reduce their metabolic rate in Tilapia (Francis et al. 2005). Yakubu et al. (2012) suggested using intensive tank culture of Nile Tilapia in a flow- through system helps to obtain excessive reproduction of *Oreochromis niloticus* resulting maximum growth of fish. Abid and Ahmed (2009) did experiment and concluded that the survival rate was 100% and fish fingerlings fed with low cost based diet showed significantly higher ($P< 0.05$) weight gain.

The use of antibiotics can be replaced by optimized dose of Garlic to enhance growth performance and flesh quality (Shakya and Labh 2014). Metwally (2009) recommended addition of Garlic in any form to fish can promote growth rate and decrease mortality rate. Four doses of Garlic, 0 (control), 10g, 20g and 30g of fresh clove of Garlic explained by (Meghowon et al. 2013) fed for 12 weeks found significantly increased weight in all groups fed on Garlic and improve survival rate due to enhanced immune response resulting from improved defence mechanism. Priyadarshini et al. (2012) found that the fish fed with different doses of Immuplus promote growth and improve inflammatory response (increase protease and amylase activity). Salah et al. (2001) in their study about effect of growth on the survival, growth, resistance and quality of Nile Tilapia has shown the improvement in growth performance, fish quality and survival rate of fish.

Implementation of *Phyllanthus emblica* in any doses to feed of fish results in maximum growth (Sivagurunathan et al. 2012). El-Sayed et al. (2014) concluded addition of

Echinacea (0.75 g/Kg) or Ginseng (0.6 g/Kg) or both to Nile Tilapia diets as alternative to oxytetracycline act as growth promoter and found that *Echinacea* extract is the most potent one of them. It has been recorded that administration of low amount of sulfated D-galactans (0.1 mg/Kg) extracted from the red marine alga; during sex-reversal treatment, might be a valuable tool to increase growth in fingerlings from the Nile Tilapia (Favias et al. 2004).

Labh and Chakrabarti (2011) had described the increment in average weight and specific growth rate of hybrid carp by the dietary addition of vitamin C at the rate of 400 mg Kg^{-1}. Higher growth rate in fish fed was seen with vitamin C than that of diet devoid of vitamin C (Lee and Dabrowski 2003). Pandey et al. (2012) showed the use of Vitamin C and E as herbal drugs act as growth promoter and cure all kinds of diseases of fishes and other aquatic animals. Mixture of Black Cumin Seed (*Nigella sativa*) and Turmeric (*Curcuma longa*) in the ratio 1:1 fed as a supplementary diet to Asian Sea Bass (*Lates calcarifer*) improved the growth performance but did not affect blood chemistry (Abdelwahab and El-Bahr 2012).

John et al. (2007) fed different four plants based immunostimulants (*Eichinacea purpurea* 0.25 and 1.0 ppt., *Allium sativum* 3%, *Nigella sativa* 3%, and *Origanum marjorana* 2 and 3% and mixture of *A. sativum*, *N. sativa* 3:1, 1:3 and 3:3 % respectively) as additives for their effect on survival and growth and the result suggested immunostimulants can enhance survival especially during winter stress. Significant increase in body weight and the total biomass production were seen with *Echinacea* (1.00 ppt.) Ayokanmi (2012) fed herbal powder (Superliv) to *Oreochromis niloticus* and found that specific growth rate (1.33 ± 0.33 % per day) and best FCR (0.85 ± 0.03) were obtained in the 10 g/ Kg herbal meal diet treatment.

2.5 Feeding fruits/ herbs and protein profile of fish

Protein is the most important nutrient for growth and plays a central role in the structure and functioning of all living organism. Carps like all other animals must consume protein to maintain a continuous supply of amino acids. The consumed protein is digested or hydrolyzed to release free amino acids that are absorbed from the intestinal tract of the animal and distributed by blood to various organs. According to Shang (1996) fish is an important component of total human food consumption and a principal source of animal protein for more than half of the world's population. Choo and Williams (2003) are of the view that fish and other aquatic animals are a good source of protein, vitamins and minerals. In recent years, fish has been acknowledged as a functional food and has important role in the prevention and management of heart disorders, neurological diseases and mood swings. Fish is an important component of human diet because fish food is not only easily digestible but is also rich in essential amino acids like methionine and lysine.

Recent research has revealed that fish meat carries anti cancerous properties which minimize the risk of heart ailment. Fish and other forms of aquaculture have many advantages. By adding extract of Ginger to fish diet increased the total protein level, the highest level of plasma protein was observed in those fishes fed with 1% Ginger extract (Dugenci et al. 2003). Hassan and Javed (2000) found higher concertration of sarcoplasmic protein in *Catla catla* corresponds with decrease of myofibrillar protein. Sambhu and Jayaprakash (2000) recommended the use of 1% livol (IHF-1000) enhance maximum growth and improved nutrient digestibility of Prawn. Pandey et al. (2012) showed the use of Vitamin C and E as herbal drugs act as growth promoter and cure all kinds of diseases of fishes and other aquatic animals.

Papaya Leaf Meal consists of an enzyme called papain which enhance the protein digestion, FCR, SGR and weight gain in the 16% unsoaked Papaya a meal dried to *P. monodon* postlarvae (Penaflorida 1995). Hamza et al. (2010) assessed the protein expression profile in the liver of 34 days old Pikeperch larvae fed with three isoprotic and isolipidic formulated diets varying by their phospholipids (PL) contents (0%, 1.4%, 4.7% and 9.5%) and found that there was significant modifications of protein expression in the liver of fish. Martin et al. (2003) recorded no differences in free amino acid pools in either liver or muscle between diets in Rainbow trout fed with control diet and soyabean meal containing fish meal and plant ingredients and synthetic amino acids. Binukumari and Vasanthi (2013) exposed fishes to different concentration of insecticide 'Encounter' for 24, 48, 72 and 96 hours and analysed protein content and found decrease value of protein content in all the tissues in comparison to control diet.

Fishes fed with diets containing Garlic in different forms; natural Garlic (40 g/Kg), Garlic oil capsules (250 mg/Kg) and Garlic powder tablets (32g/Kg) has shown significantly increased ($P<0.01$) total protein in blood serum compared to control diet (Metwally

2009). Khattab et al. (2004) found that the dietary of Biosen increased PER and crude protein in fish *Oreochromis niloticus*. Methanolic extracts of the herbals *O. sanctum*, *W. somnifera* and *Myristica fragrans* herbs significantly improved the immune parameters such as phagocyte activity, serum bactericidal activity, albumin–globulin (A/G) ratio and leukocrit against *Vibrio harveyi* challenge in juvenile grouper, *Epinephelus tauvina*, larviculture (Sivaram et al. 2004)

2.6 Feeding fruits/ herbs and fish immune system

The immune system is a system of many biological structure and process within an organism that protects against disease. The immune system of fish is physiologically similar to that of higher vertebrates, despite certain differences. Fish are free living organisms from early embryonic stages of life and depend on their innate immune system for survival (Rombout et al. 2005). So, to boost up immune system of fish, herbal medicines can be used as supplementary diet in the feed. An immunostimulant is a chemical drug, stressor or action that enhances the defence mechanisms or immune response (Anderson 1992) thus rendering the animal more resistant to diseases. Immunostimulants are known to activate non-specific defence mechanisms, cell-mediated immunity and specific immune responses (Siwicki et al. 1998). Bricknell and Dalmo (2005) defined immunostimulant as a naturally occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens. The biological effects of immunostimulants are highly dependent on the receptors of the target cells recognizing them as components of the immune system as well as non-specific immunity with consequent increase in the host defense against pathogenic stimuli. The herbal immunostimulants *Emblica officinalis, Cynodon dactylon* and *Adathoda vasica* improved the immune system and reduced microbial infection in the Goldfish *Carassius auratus* (Minomol 2005). Better performance of haematological, biochemical and immunological parameters were found in immunostimulant- incorporated diet fed to Shrimps (Citarasu et al. 2006).

A dose of 1.0% *of A. aspera* seed might be suitable as immunostimulatory properties for Rohu fry (Srivastav and Chakrabarti 2010). According to Labh and Shakya (2014) application of immunostimulants is an alternative approach to stimulate the innate system of farmed fish and is considered as attractive and promising agents for the prevention of diseases in fish and shellfish. The use of antibiotics can be replaced by optimized dose of Garlic in on growth performance, flesh quality, antimicrobial activity; as an immunostimulant and antiprotozoal agent in aquaculture (Shakya and Labh 2014). Four doses of Garlic, 0(control), 10g, 20g and 30g of fresh clove of Garlic fed for12 weeks. The result showed significantly increased weight and growth performance ($P<0.01$) in all groups fed on Garlic and improved survival may be due to enhanced immune response resulting from improved defence mechanism (Metwally 2009).

Sivagurunathan et al. (2012) studied and found increment in TEC, Hb, TLC, Lymphocyte, Neutrophils and monocyte count fed with 2% of P. emblica in fish feed as immunostimulator. Medicinal plants have been known as immunostimulant and show their main properties as growth promoters, immune enhancers where they act as antibacterial and antiviral agents to the host immune system (Van Hai 2015). In addition to this, addition of levamisole HCl (225 mg/Kg diet) significantly enhanced both cellular and humoral innate immune responses and increased resistance to *A. hydrophila* infection while addition of vitamin C and E needed for growth lead to maximum growth and little enhancement of the immune response as well as resistance to *A. hydrophila* infection (Abdelkhalek et al. 2008).

Labh and Chakrabarti (2011) noted marked antioxidant and immunostimulants properties of vitamin C (LATP-Ca) in fishes. Merchie et al.(1997) found a use of a level of 2000 mg ascorbic acid per kilogram promote the resistance of Shrimp postlarval to stress condition and bacterial infection *Penaeus vannamei*. Ndong and Fall (2011) demonstrated that juvenile hybrid Tilapia fed with Garlic (0.5g/Kg) enhanced TLC, respiratiory burst, phagocytic activity, phagocytic index and lysozyme activity as compared to diet D2 and control diet. According to El-Sayed et al. (2014) herbal compounds such as phenolics, polyphenols, alkaloids, quinines, terphenoids, lectines and polypeptides have shown to be very effective alternatives to antibiotics and other synthetic compounds as growth promotion, immunostimulation, antistress, antibacterial, antifungal, antiviral, appetite stimulators and aphrodisiac. To prevent fishes from diseases, Beta-glucans were found to be most promising immunostimulant to control diseases in aquaculture rather than plant products such as polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, muramyldipeptide and yeast derivatives (Mastan 2015). Mesalhy et al. (2008) suggested using *Echinacea* (0.25 ppt feed) as a growth enhancer, immunostimulator and a disease control agent in fish.

Medicinal plants are recognized to have great potential for preparing clinically useful drugs and use of their extracts as antiviral agents in aquaculture production may also be of eco-friendly as they are biodegradable (Sivasankar et al. 2015).

3. MATERIALS AND METHODS

3.1 Experimental design and setup

3.1.1. Selection of sites and study area

An experiment on Nile Tilapia was conducted in the Aquaculture Research Lab of Central Department of Zoology, Tribhuvan University, Kirtipur during June to September 2014.

3.1.2. Culture Tanks

Altogether twelve rectangular glass aquaria (100 litres; 18*10*12 inch) were kept in indoor culture conditions for the application of four different diets each with replicate forms (figure 1). Aquaria were filled with 80 litre dechlorinated jar water and covered with nylon net to prevent the escape of fish and entrance of insects. Adequate aeration facility was provided in each aquarium by the help of diffusers. Hence, fish were reared in indoor culture system and in natural atmospheric conditions.

Fig. 1. Experimental design for the culture of fish

3.1.3. Preparation of Lapsi supplemented artificial diets

Bhaktpur is the main centre for Lapsi production in Nepal. Lapsi *Choerospondias axillaris* (Roxb.) fruits collected from Bhaktapur were identified by plant taxonomist. Samples were taken to laboratory soon after their collection. Fine powder from the pulp of Lapsi fruit was prepared according to method described by (Arabshahi-Delouee and Urooj 2007).

Table 2. Ingredients of basal diet used during the experiment

Ingredients (g)	Control	D1	D2	D3
Fish meal	55.41	55.41	55.41	55.41
Wheat Flour	39.59	39.58	39.57	39.55
Cod Liver Oil	5	5	5	5
Lapsi Powder (LFPP)	0	0.01	0.02	0.04
	100	100	100	100

Three artificial diets (40% protein) containing 100 mg/kg, 200 mg/kg and 400 mg/ kg of Lapsi fruit pulp powder (LFPP) along with dry fish powder, wheat flour, cod liver oil and one control diet (0.0%) were prepared for the experiment (Table-2). Fish meal was dried well and ground in a grinder and then sieved (mesh size: 500µ). Then lukewarm water was added in required amount for the formation of dough. The powdered fishmeal was mixed thoroughly with wheat flour and recommended LFPP. Cod liver oil was added, mixed well so that all the ingredients were spread homogeneously. The prepared dough was passed through a feed maker using 1 mm die, the threads formed were dried. The dried threads were further chopped into small pieces of required sizes of pellets through a blender and then passed through a sieve to obtain equal sized particles. Diet was stored at 4ºC until used. A control diet with 40% protein was also prepared with same ingredients excluding LFPP. Wheat flour was reduced and replaced by Lapsi powder so as to make 100 % diet.

3.1.4. Selection of Test Fish for Experiments

About three hundreds Nile Tilapia fingerlings (average 0.27 ± 0.3 g weights and 2.69 ± 0.11 cm length) were procured from the private hatchery farm of Pokhara Valley. Fish were packed in 10 Litres of polyethylene bags filled with oxygenated water and were transported to Kathmandu by bus. After arrival, Nile Tilapia fingerlings were kept in the stocking tank and acclimatized for 10 days in indoor conditions. After 10 days of acclimatization, Nile Tilapia was distributed randomly at the rate 21 fingerlings per aquarium into twelve aquaria equally.

3.1.5. Culture Period and Conditions

Dechlorinated jar water was used during experiment. Fingerlings were acclimated for 10 days and were fed with control diet without Lapsi during acclimatized period. After that, fingerlings were fed with test and control diet at the rate of 3% of their body weight once a day at 4:30 p.m. Temperature ranged from 25°C to 29°C and pH ranged from 7.53 to 7.92 throughout the study period. Dissolved oxygen was maintained above 5 mg/l with the help of aerators. Faecal matter was siphoned daily and two third of water was replenished at weekly intervals.

3.1.6. Sample Collection and Examination Procedure

After the end of experiment, fingerlings were collected from each experimental aquarium and individual length (cm) and weight (g) increments were recorded by the help of measuring scale and electric weighing machine respectively.

3.2. Experimental parameters

3.2.1. Fish survival:

The number of fish at harvest was determined for the estimation of survival. This was expressed in percent of the initial number of fish.

3.2.2. Growth parameters

3.2.2.1. Absolute growth

Change in absolute body weight was determined by recording the length and weight. At the time of harvest, fish was measured individually by the help of measuring scale and electric weighing machine.

3.2.2.2. Specific growth rate (SGR)

The specific growth rate (SGR) was calculated using the formula:

$SGR = 100 \times (\text{In}W_t - \text{In}W_i) / t$

Where, W_i and W_t are the initial and final body weight and 't' is the number of days.

3.2.2.3. Feed Conversion Ratio (FCR)

The FCR for each treatment was computed by the following equation:

$FCR = F/ (W_f - W_o)$, where F is the weight of food supplied to fish during the study period; W_o is the live weight of fish at the beginning of the study period; W_f is the live weight of fish at the end of the study period.

3.2.3. Biochemical Parameters

3.2.3.1. Collection of tissues

At the end of the experiment, five fingerlings from each treatment were collected and were anesthetized with (5 mg/l) tricaine methane sulfonate (MS-222) for 2-3 minutes. Fingerlings were dissected properly to obtain the required tissues. 100 mg of liver was collected through dissection and stored at -4ºC for further analysis. 100 mg of liver and 3 ml of lysis buffer (0.1 m Tris buffer at P^H 7.4) were homogenized and centrifuged at 3000 rpm for 5 min and supernatants were used for the following required assays.

3.2.3.2. Total protein

Total protein present in liver tissues was determined by biuret method developed by (Doumas 1975) using kit (Aspen laboratories Pvt. Ltd., India). 2ml of biuret reagent were added in 0.02 ml of supernatant solution and incubated for 5 min at room temperature. The absorbance was recorded at 546 nm against reagent blank. Concentration of total protein in test sample (g/dl) = (Absorbance of sample/ Absorbance of standard) × Concentration of standard.

3.2.3.3. Albumin

Albumin of liver tissues was determined by BCG (Bromo Cresol Green) method developed by (Doumas1971) using kit (Aspen Laboratories Pvt. Ltd., India). 2ml of BCG reagent were was added in 0.02 ml of supernatant solution and incubated for 5 min at room temperature. The absorbance was recorded at 630 nm against reagent blank. Concentration of albumin in test sample (g/dl) = (Absorbance of sample/ Absorbance of standard) × Concentration of standard.

3.2.3.4. Globulin and A-G Ratio

Globulin was calculated by the deduction of albumin from total protein while the ratio of albumin and globulin were calculated by division methods.

3.3. Statistical Analysis

Values for each parameter measured were expressed as mean ± standard error of mean. The results were analyzed with one-way analysis of variance (ANOVA) by the help of software 'R'. Differences at $P<0.05$ were regarded as statistically significant.

4. RESULTS

4.1 Growth Performance

4.1.1 Survival Rate (%)

The survival rate was found to be statistically significant (P<0.05) in fish fed with LFPP in comparison to control diet T1. A highest 93.65±3.27 % survival rate was observed in Tilapia fed with diet T3 followed by T2 (92.06±5.89 %), T4 (85.71±5.66 %) and control diet T1 (84.12±6.54 %) (APPENDIX I, Fig. 2).

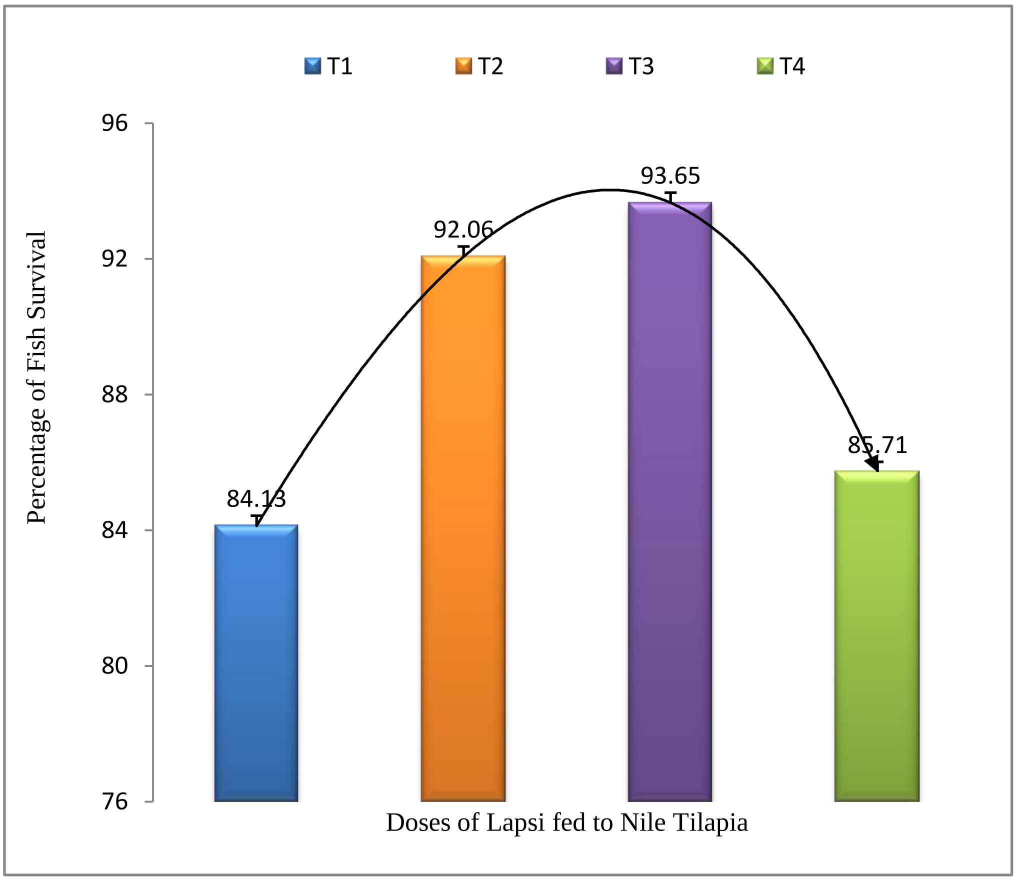

Figure 2. Survival rate of Nile Tilapia fed with diet containing four different doses of Lapsi

4.1.2 Final Average Length and Length Gain (%)

The initial average length of Nile Tilapia was 2.72±0.17 cm in the beginning of the experiment. After 10 weeks of feeding trial, final average length was found high in Tilapia fed with diet T3 (5.09±0.07cm) followed by T4 (4.75±0.18 cm), T2 (4.53±0.06 cm) and Control diet T1 (4.21±0.19 cm). A higher 19.44% length was increased in Nile Tilapia fed with diet T3 as compared to other groups (APPENDIX I, Fig.3). Thus, percentage of highest length gain was found in T3 diet (87.23±2.43 %) fed Nile Tilapia as compared to T4, T2 and T1 which were as 74.84±6.46, 66.60±2.37 and 54.84±6.93 % respectively (APPENDIX I, Fig.4)

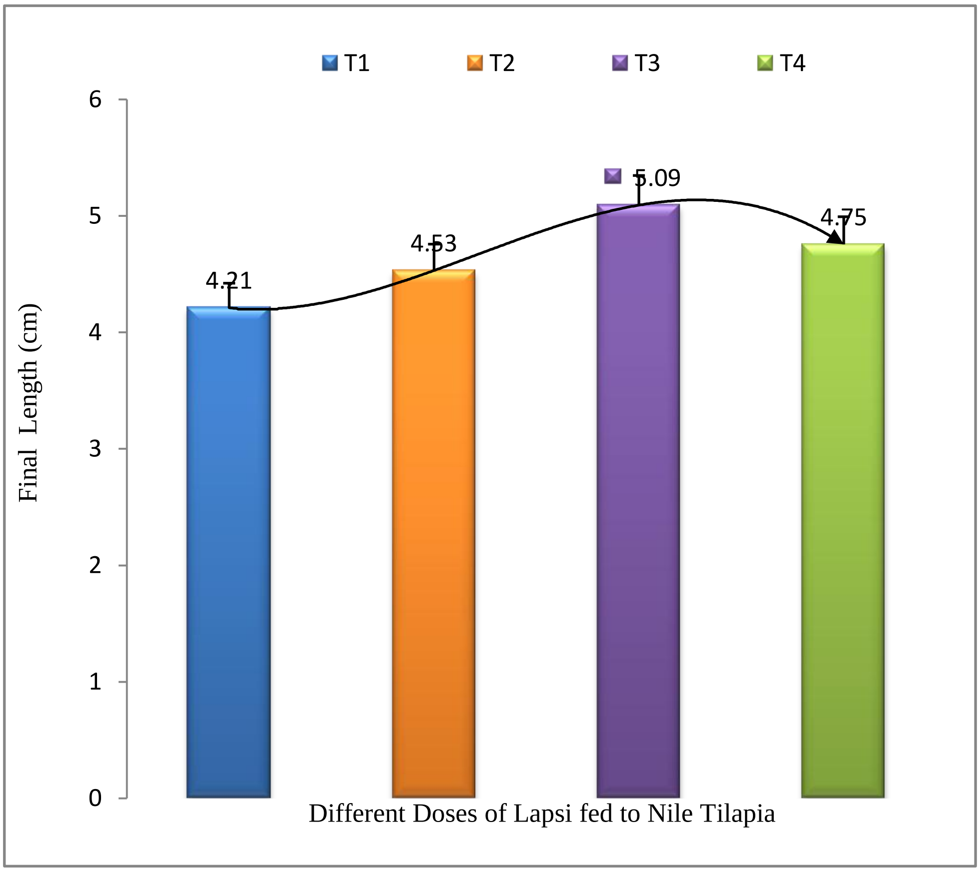

Figure 3. Final Length of Nile Tilapia fed with diets containing four different doses of Lapsi

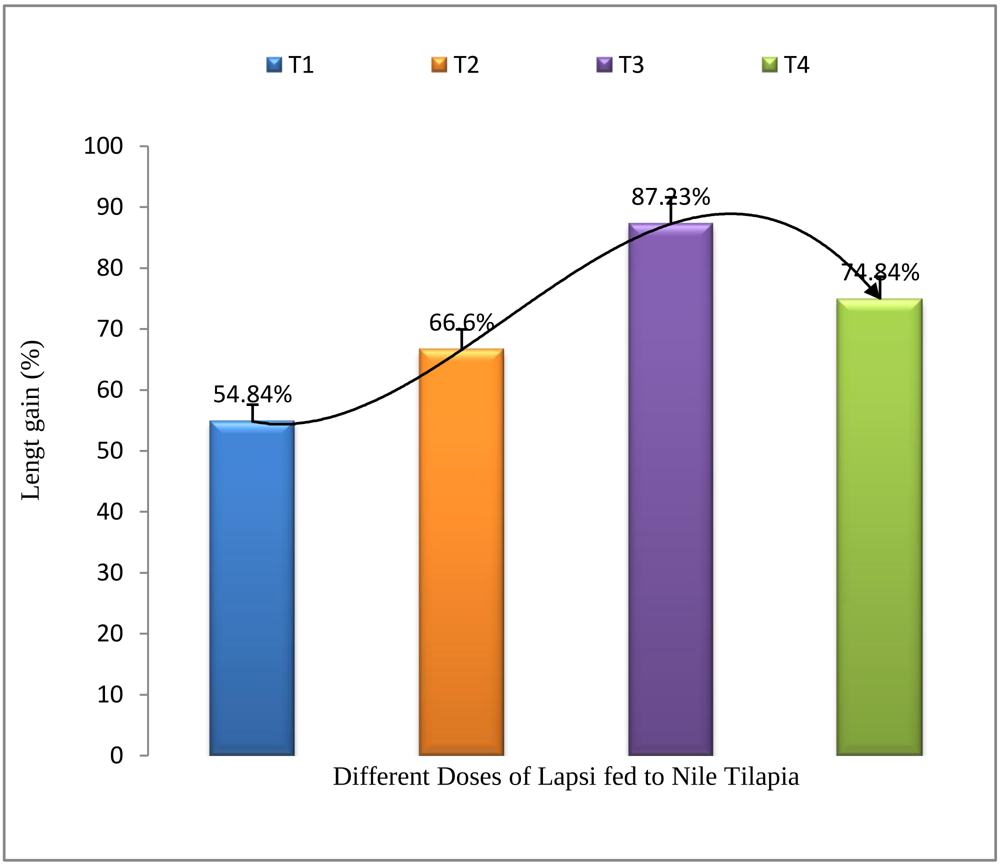

Figure 4. Percentage Length Gain of Nile Tilapia fed with diets containing four different doses of Lapsi

4.1.3 Final Average Weight and Weight Gain (%)

The average weight of Tilapia in the beginning was 0.284±0.37 g. After 70 days of culture, a direct relationship was found between the final average body weight of Tilapia and diet containing different doses of Lapsi fruit pulp powder (APPENDIX I, Fig.5). The final average weight of Tilapia fed with diet T3 was found high (1.75±0.08 g) followed by diet T4 (1.42±0.18 g), T2 (1.14±0.06 g) and control diet T1 (0.89±0.14 g). The final average weight was 63.53% higher in the Nile Tilapia fed with D2 diet compared to the Tilapia fed with other three diets.

Similarly, percentage of highest weight gain was found in T3 diet (517.07±26.79 %) fed Tilapia as compared to T4; T2 and T1 which were as 403.37±61.93, 301.78±20.78 and 214.21±47.87 % respectively (APPENDIX I, Fig.6).

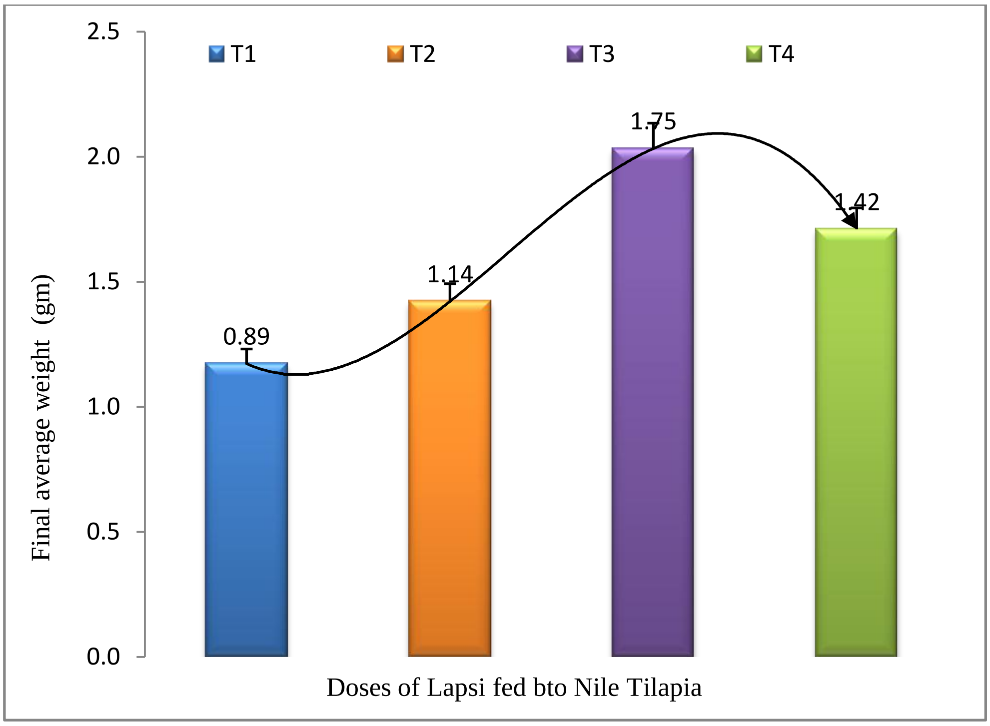

Figure 5. Final average weight of Nile Tilapia fed with diets containing four different doses of LFPP.

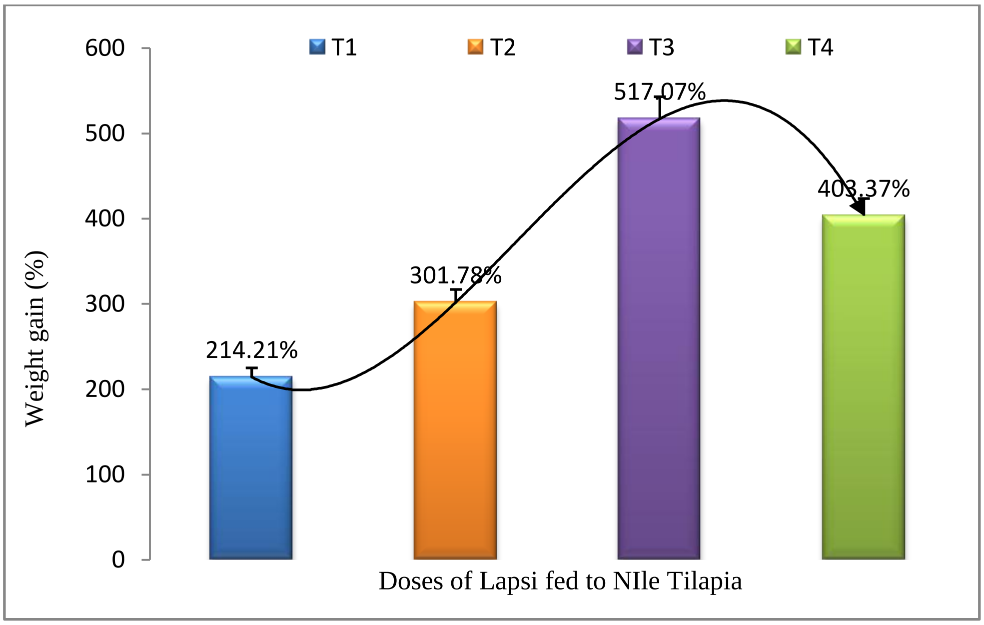

Figure 6. Percentage Weight Gain of Nile Tilapia Fed with diets containing four different doses of LFPP in diet.

4.1.4 Specific Growth Rate (SGR)

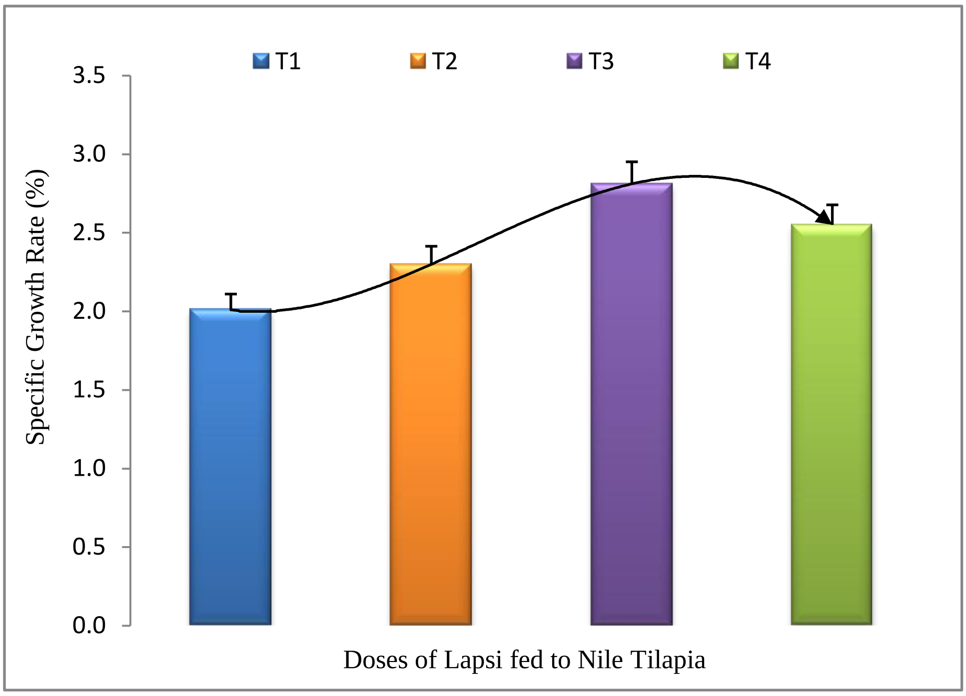

Figure 7. Specific growth rate (SGR) of Nile Tilapia fed four different doses of LFPP in diets.

A direct relationship was found between the Lapsi fruit pulp powder (LFPP) contained in diets and the specific growth rate of Nile Tilapia (Fig. 6). However, the specific growth rate was significantly (P<0.05) high in the Nile Tilapia fed with diet T3 (2.80±0.05) followed by diets T4 (2.54±0.16), T2 (2.29±0.06) and control diet T1 (2.0±0.16). The specific growth rate was 16.39 to 34.01% higher in the Nile Tilapia fed with T3 diets compared to the Tilapia fed with other diets ((APPENDIX I, Fig.7)).

4.1.5 Feed Conversion Ratio (FCR) and Feed Conversion Efficiency (FCE)

An inverse relationship was found between feed conversion ratio and the dose of Lapsi fruits contained in the diets. The FCR level was found high in Tilapia fed with control diet T1 (14.67±0.91) followed by diet T2 (11.07±0.58) T4 (0.43±0.06) and finally in diet T3 (7.18±0.31) (APPENDIX I, Fig.8). Whereas, as the dose of Lapsi increased in the diets FCE level also increased and it was significantly high (P<0.05) in T3 (13.96±0.61) diet fed fish followed by T4 (12.72±0.17), T2 (9.07±0.47) and T1 (7.09±0.09) respectfully (APPENDIX I, Fig.9).

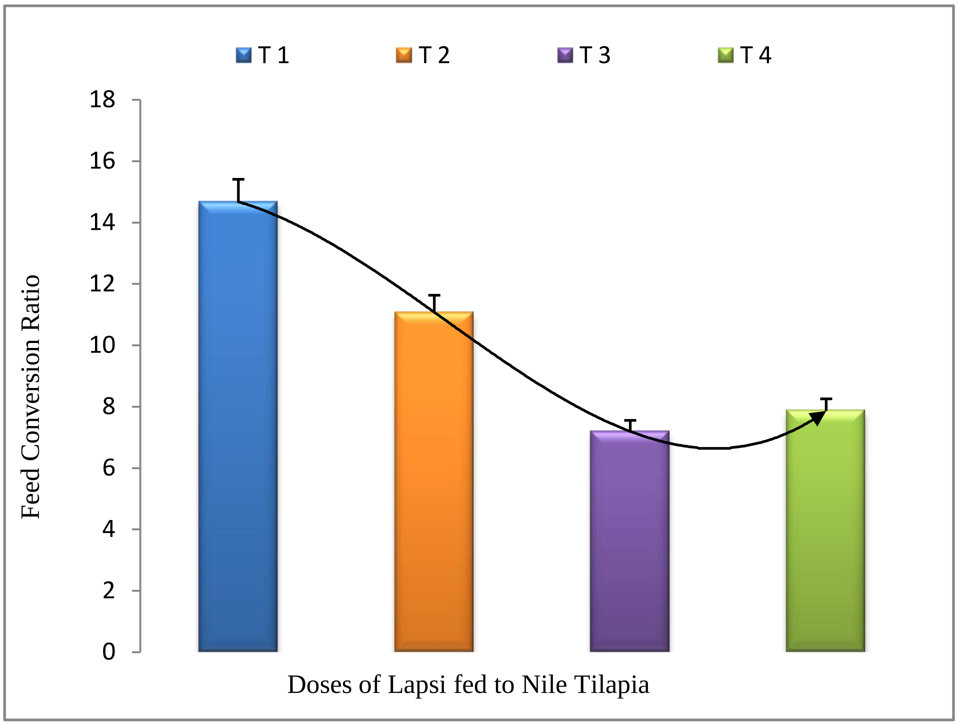

Figure 8. Feed Conversion Ratio (FCR) of Nile Tilapia fed with four different doses of LFPP in diets.

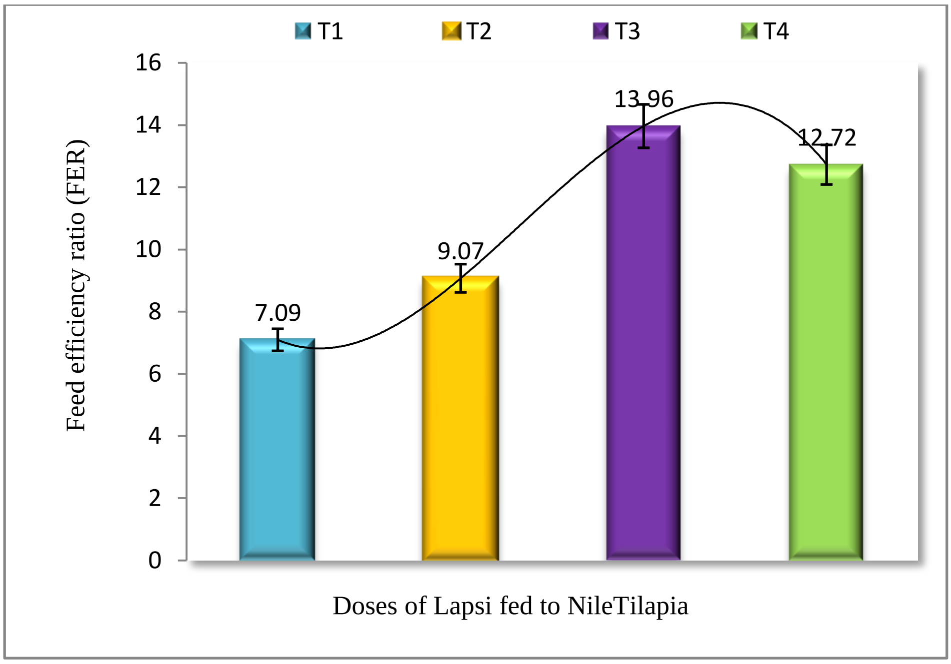

Figure 9. Feed Conversion Efficiency (FCE) of Nile Tilapia fed with four different doses of LFPP in diets.

4.2 Biochemical Parameters

4.2.1 Total Protein in liver of Nile Tilapia

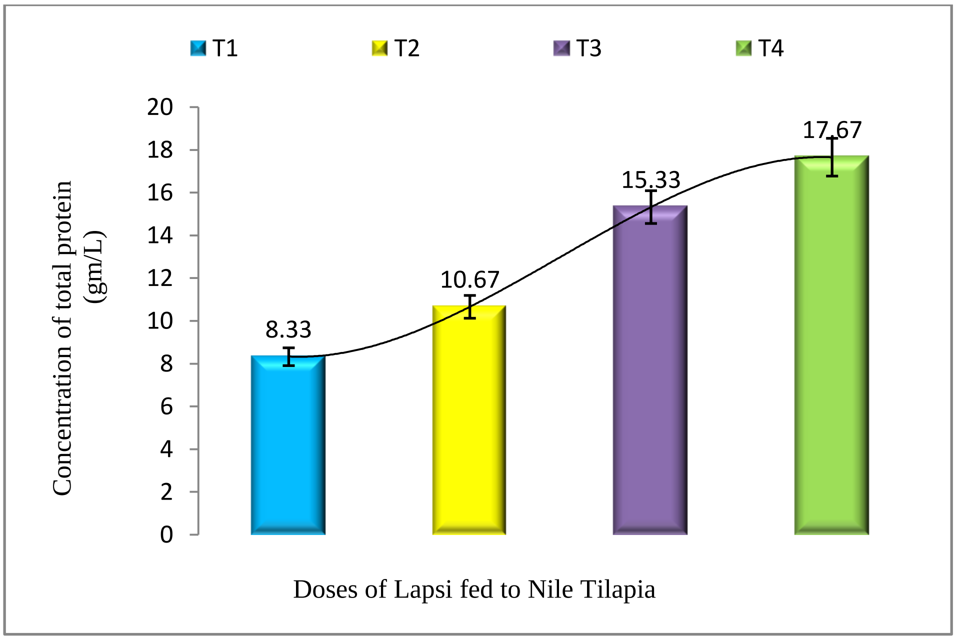

Figure 10. Concentration of total protein in the liver of Nile Tilapia fed with four different doses of LFPP in diets.

A direct relation was observed between the dose of LFPP in the diets of Nile Tilapia and the concentration of total protein concentration in the liver of Nile Tilapia (APPENDIX I, Fig.10). The concentration of total protein was found significantly ($P<0.05$) high in the liver of Nile Tilapia fed with diet T4 (17.67 ± 0.88 gm/L) followed by diet T3 (15.33 ± 0.33 gm/L), diet T2 (10.67 ± 0.88 gm/L) and control diet T1 (15.33 ± 0.33 gm/L). The concentration of total protein was two folds higher in the Nile Tilapia fed with diet T4 as compared to the Nile Tilapia fed with control diet T1.

4.2.2 Albumin in liver of Nile Tilapia

Similar trend of average albumin concentration was recorded from Nile Tilapia fingerlings after the feeding trials of 70 days with different concentration of LFPP. The quantitative estimation of albumin in the liver tissue of Nile Tilapia showed that the concentration of albumin in the liver increased as the dose of Lapsi in the diet increased (APPENDIX I, Fig.11). The concentration of albumin level was significantly ($P<0.05$)

high in the fish fed with diet T4 (15.33 ± 0.33 gm/L) followed by diet T3 (12.33 ± 0.67 gm/L), diet T2 (8.67 ± 0.33 gm/L) and control diet T1 (6.67 ± 0.33 gm/L). Albumin level was 15.34 to 28.47% higher in the Nile Tilapia fed with diet T4 compared to control diet T1.

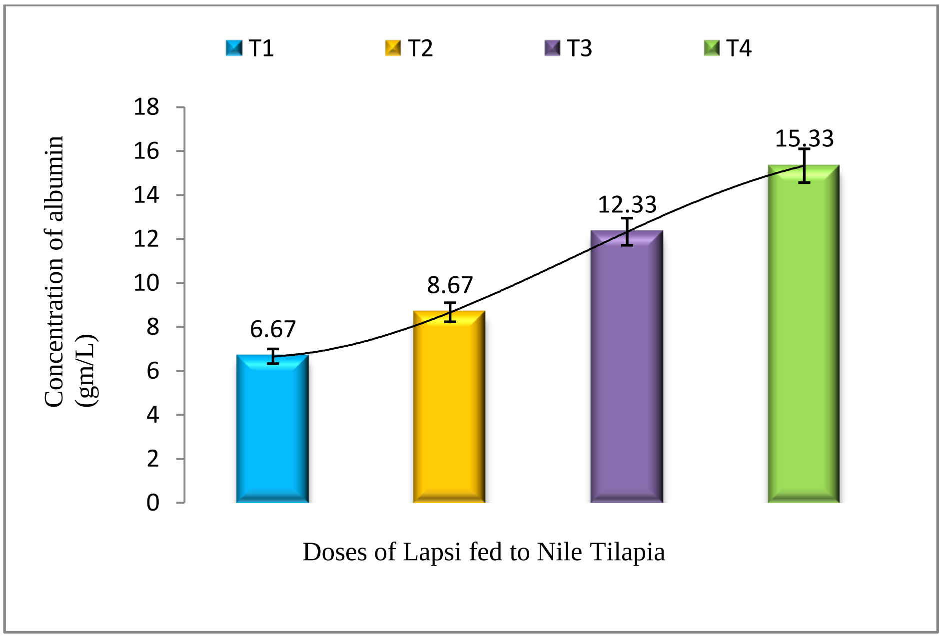

Figure 11. Concentration of albumin in the liver of Nile Tilapia fed with four different doses of LFPP in diets.

4.2.3 Globulin concentration

Like in albumin, the concentration of globulin in the liver was found to be increased as the dose of Lapsi in the diet increased (APPENDIX I, Fig.12). The concentration of globulin level was significantly ($P<0.05$) high in the fish fed with diet T3 (3.0±0.6 gm/L) followed by diet T4 (2.33±0.9 gm/L), diet T2 (2.0±0.6 gm/L) and control diet T1 (1.66±0.3 gm/L).

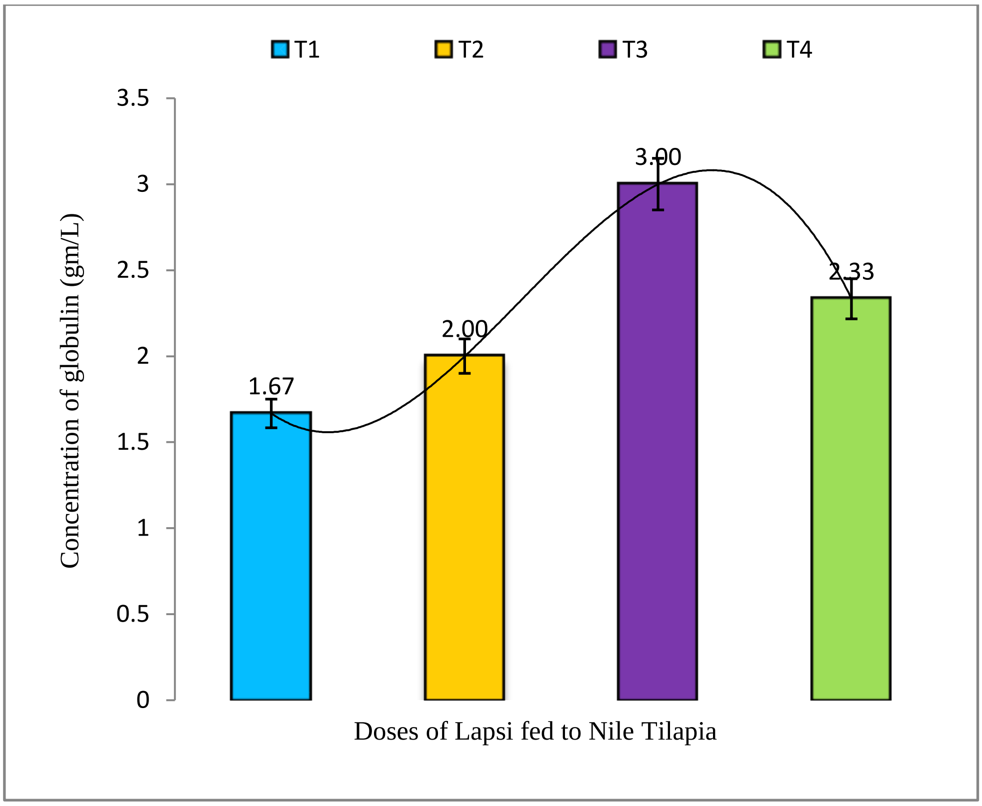

Figure 12. Concentration of globulin in the liver of Nile Tilapia fed with four different doses of LFPP in diets.

4.2.4 Ratio of albumin and globulin in liver of Nile Tilapia

The ratio of albumin and globulin in liver of Nile Tilapia fingerlings fed with different treatments was found to highest in fish fed with diet T4 (8.92 ± 3.28) followed by diet T3 (4.52±1.1), diet T2 (5.16±1.5) and control diet T1(4.5±1.3) (APPENDIX I, Fig.13).

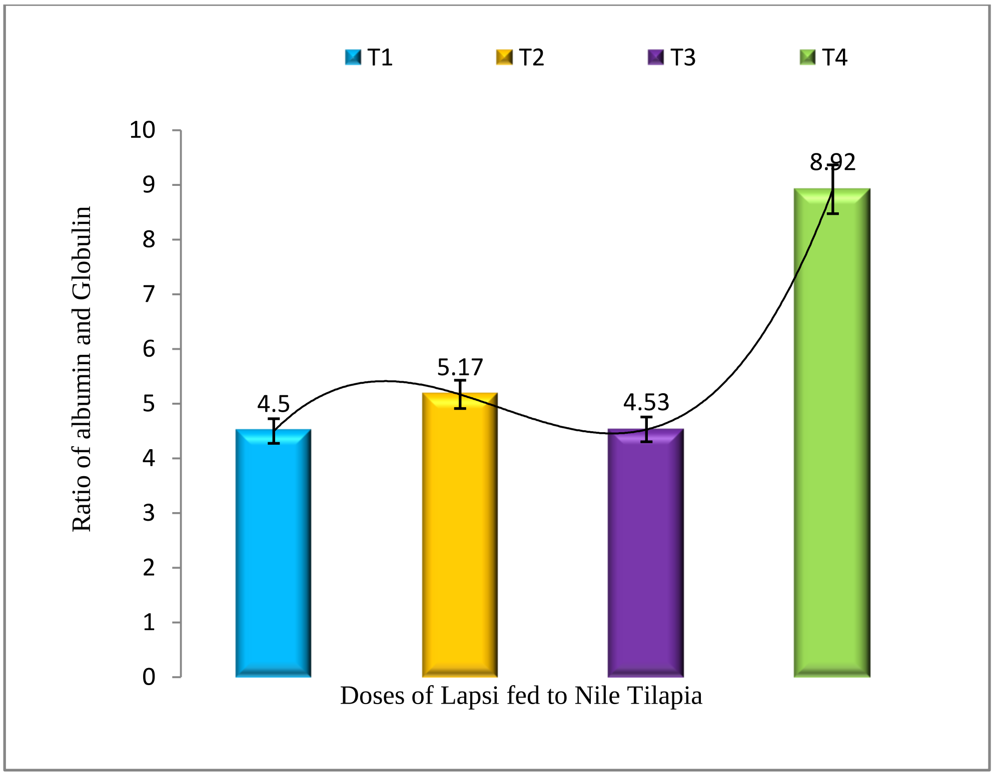

Figure 13. Concentration of ratio of albumin and globulin in the liver of Nile Tilapia fed with four different doses of LFPP in diets.

5. DISCUSSION

Fish is an important part of diet for a large proportion of the people living in the developing world. Fish food represents the primary source of animal protein for a billion of people in the 58 countries worldwide (FAO 2007). The contribution of fisheries is very promising and important for creating job opportunities for unemployed people, earning foreign exchange, alleviating poverty and improving nutritional status of the people (Subasinghe 2005).

Today's world population is estimated to be about 7 billion, which by 2030, 2 billion more people will be added in the world population mean that aquaculture will need to produce nearly double that, 85 million tons of fish per year just to maintain current consumption levels (FAO 2007). It is estimated that the total feed cost in culture accounts for 30 to 70% of production cost, depending upon the type of culture and intensity of feeding (De Silva and Anderson 1995).

Tilapia is native to Africa (El-Sayed 2006) and Egypt is the first largest producer of farm-raised Tilapia in Africa (Fagbenro et al. 2010). Tilapia is not only the second most important farmed fish globally next to carps but is also described as the most important aquaculture species of the 21st century (Shelton 2002). In 1950, annual Tilapia production was only 1.5 tons, it increased to 1.5 million tons in 2002; increased by 1 million fold and in the year 2010 it has surpassed even 3 million tons (Bhujel 2011). There are about 80 species of Tilapias worldwide (Fortes 2005, Grafman and Chriswaterguy 1998) and the most important Tilapias in aquaculture are the Nile Tilapia *Oreochromis niloticus* and the Red Belly Tilapia *Tilapia zillii* (Hepher and Pruginin 1981, Mair 2001, FAO 2002). They are widely cultured in about 100 countries in the tropical and subtropical regions. These species account for 99.5% of global Tilapia production. The total world Tilapia landings from capture and culture have been estimated at 1.16 million tons with cultured Tilapia accounting for 57% of the total (659,000 tons) (Fortes 2005).

O. niloticus are principally herbivorous, although occasionally omnivorous. The fish is an efficient converter of waste foodstuff and appears to thrive well on artificial supplemental feed (Omoregie et al. 2009). Tilapia farming is socially more acceptable and technically and economically more viable and sustainable. Tilapia culture is a profitable enterprise and even small farmers of Nepal can afford to culture Tilapia for their income. Tilapia is consumed by poor people as it is relatively low priced commodity. Thus an experiment was conducted with the objectives to determine the effect of dietary supplementation of economically viable and locally available herbal plant Lapsi *Choerospondias axillaris* (Roxb.) on survival, growth and immune response of Tilapia *O. niloticus* fingerlings.

5.1 Growth performance of Nile Tilapia

From the present study, the result suggest that the dietary supplementation of Lapsi fruit pulp powder at all concentrations enhanced significantly ($P<0.05$) the growth and nutrient utilization, which is shown in improved weight gain, length gain, SGR, FCR and FER of Nile Tilapia (*O. niloticus*) fingerlings. In overall, growth performance obtained was high as compared to control diet T1 (0.0 mg/Kg of Lapsi powder). The optimal growth was obtained in fish fed in treatment T3 (200mg/Kg Lapsi powder) in comparison to others. Similar result was shown by Turan (2006) who used Red Clover *Trifolium pretense*a herbal medicine as a growth promoting agent for Tilapia *O. niloticus*. Megbowon *et al*. 2013 found better performance in terms of growth, nutrient utilization, high survival rate in the fish fed with different composition of Garlic than control diet (A); agreeing with Metwally (2009) and Shalaby et al. (2006) who reported significant increased weight gain, FE, PER and SGR in the *O. niloticus* when fed with diet consisting of 30 g/Kg and32 g/Kg Garlic powder respectively. In addition, the use of 1% Livol (IHF 1000) as an herbal medicine enhances maximum growth and improved nutrient digestibility of Prawn (Shadakshari 1993; Unnikrishnan 1995; Jayaprakas and Euphrasia 1996). Administration of low amount of sulfated D- galactans (0.1 mg/Kg) extracted from the red marine alga; during sex-reversal treatment, might be a valuable tool to increase growth in fingerlings from the Nile Tilapia (Favias et al. 2004).

Generally, the results of the present study are in favour with previous studies in which feeding feed with vitamin C (Ascorbic acid) promote the weight gain and growth rate in several fish species (Dabrowski et al. 1990, 1996; Lee et al. 2001). These results were also supported strongly by Pandey et al. (2012). He demonstrated the use of vitamin C as an herbal drug act as growth promoter and cure all kinds of diseases of fishes and other aquatic animals.

Kim et al. (1998) suggested that the presence of unknown factors in various medicinal herbs led to outstanding results in fish growth trails. The present findings may indicate the presence of vitamin C (Ascorbic acid, AA) in Lapsi fruit pulp powder stimulated the growth and nutrients utilization in Nile Tilapia. It has been shown that vitamin C requirement for normal growth of trout range from 10 to 20 mg (Merchie et al.) although the NRC recommended 50mg vitamin C/ Kg diet for an optimal performance of trout. Likely, the present study has shown 200 mg/Kg of Lapsi powder containing vitamin C gives the optimal growth in Nile Tilapia. Similarly, Gammanpila et al. 2007 resulted that female Nile Tilapia exhibited significantly lower weight gain due to vitamin C deficient in the diet compared to diet supplemented with higher vitamin C. He also found the higher survival rate in the fish fed with high dose of vitamin C. Ayokanmi (2012) fed herbal powder (Superliv) to *Oreochromis niloticus* and found that specific growth rate (1.33 ± 0.33 % per day) and best FCR (0.85 ± 0.03) were obtained in the 10 g/ Kg herbal meal diet treatment whereas present result shows maximum SGR to be 2.81 ±0.05% per day and best FCR (7.15 ±0.3) in the fish fed with 200 mg/kg of Lapsi powder. The best result obtained was due to presence of vitamin C (AA) in the supplemented diet.

In the present research, the SGR was high in the fish with treatment T3 than in other treatments. This may be due to the positive effect of Ascorbic acid in the formation of collagen, which is necessary for normal growth as explained by Masumoto et al. (1991). The present research was also satisfied with the result of (Soliman et al. 1986a). He recorded significant lower (P<0.05) SGR in the O. niloticus juvenile fed without Ascorbic acid than in the fish fed with 125 mg of AA/100 gm. Shiau and Hsu (1995) fed 90 mg/Kg of L-Ascorbic acid and obtained highest weight gain of juvenile hybrid Tilapia *O. niloticus* x *O. aureus*. Supplementation of vitamin C at 1000 mg/kg diet enhanced the growth performance and feed utilization of experimental fingerlings of *B. sharpeyi* (Yousefi et al. 2013). Tewary and Patra (2008) obtained maximum growth in *Labeo rohita* fingerlings fed with 1000 mg AA/Kg of supplemented diet. Alam et al. (2009) reported a similar result in *Heteropneusts fossilis*.

However, vitamin C requirement varies in different fish species. Utilization of vitamin C is different in different fish species (Dabrowski et al. 1994; Ai et al. 2004). Labh and Chakrabarti (2011) had described the increment in average weight and specific growth rate of hybrid carp by the dietary addition of vitamin C at the rate of 400 mg Kg^{-1}. From the present research, 200 mg/Kg of Lapsi powder shows the statistically significant (P<0.05) increase in growth performance of Nile Tilapia *O. niloticus.*

5.2 Protein profile in Nile Tilapia

From the present study, the result suggest that the dietary supplementation of Lapsi fruit pulp powder in different concentration had significant (P<0.05) higher protein profile i.e. total protein, albumin and globulin in all treatments in comparison to treatment T1. This herbal medicine boosts the immune system of Nile Tilapia. This may be due to the presence of vitamin C (AA) in the Lapsi fruit pulp powder which acts as antioxidant and immunostimulant. In agreement with present findings, Ajeel and Al-Faragi (2012) found that the use of Garlic (Allium sativum) and Ginger (Zingiber officinale) or mixture increased total plasma protein, albumin and globulin concentration significantly (P<0.05) and concluded that they protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. The same result was also agreed by Metwally (2009). By adding extract of Ginger to fish diet increased the total protein level, the highest level of plasma protein was observed in those fishes fed with 1% Ginger extract (Dugenci et al. 2003). Hassan and Javed (2000) also found higher concertration of sarcoplasmic protein in *Catla catla* corresponds with decrease of myofibrillar protein. Hamza et al. (2010) assessed the protein expression profile in the liver of 34 days old Pikeperch larvae fed with three isoprotic and isolipidic formulated diets and found that there was a significant modification of protein expression in the liver of fish. Sivaram et al. (2004) used methanolic extracts of the herbals *O. sanctum*, *W. somnifera* and *Myristica fragrans* herbs and found significantly improved immune parameters such as phagocytic activity, serum bactericidal activity, albumin–globulin (A/G) ratio and leukocrit against *Vibrio harveyi* challenge in juvenile grouper, *Epinephelus tauvina* larviculture. Likely, Sivagurunathan et al. (2012) studied and found increment in TEC, Hb, TLC, Lymphocyte, Neutrophils and monocyte count fed with 2% of *P. emblica* in fish feed as immunostimulator.

Higher concentration in protein profile of liver in the present study may be due to antioxidant property of Lapsi fruit pulp powder. Pandey et al. (2012) concluded the use of Vitamin C and E as herbal drugs act as growth promoter and cure all kinds of diseases of fishes and other aquatic animals. Ibrahem et al. (2010) suggested that the vitamin C at the rate of 500 mg/Kg for one month could be a potential, less expensive and positively affect innate immunity and resistance of Nile Tilapia (*O. niloticus*) in aquaculture. Lovell (1991) reviewed use of vitamin C reduces the deficiencies sign in fishes whereas supplementation of vitamin C ranged from 25 to 50 mg/Kg as explained by Lim and Lovell (1978) improved wound healing three times in channel catfish. Kumari and Sahoo (2005) recommended increasing the concentration of vitamin C ten times in the diet explained by Lim and Lovell (1978) stimulates the immunity against bacterial infection in Asian catfish *Clarias batrachus*. Such improvement was also observed in O. niloticus (Soliman et al. 1994). Labh and Chakrabarti (2011) also noted marked antioxidant and immunostimulants properties of vitamin C (LATP-Ca) in fishes. This result was also supported by Merchie et al.(1997). They found a use of 2000 mg ascorbic acid per kilogram promote the resistance of shrimp postlarval to stress condition and bacterial infection *Penaeus vannamei.* Ndong and Fall (2011) demonstrated that juvenile hybrid Tilapia fed with Garlic (0.5g/Kg) enhanced TLC, respiratiory burst, phagocytic activity, phagocytic index and lysozyme activity as compared to diet D2 and control diet. According to El-Sayed et al. (2014) herbal compounds such as phenolics, polyphenols, alkaloids, quinines, terphenoids, lectines and polypeptides have shown to be very effective alternatives to antibiotics and other synthetic compounds as growth promotion, immunostimulation, antistress, antibacterial, antifungal, antiviral, appetite stimulators and aphrodisiac.

6. CONCLUSION AND RECOMMENDATIONS

Lapsi *Choerospondias axillaris* (Roxb.) is a wild, large, deciduous and dioecious fruit tree of the family Anacardiaceae and native to Nepal (Roxburgh 1832). The tree is largely known for its medicinal value in Vietnam and Mongolia and delicious fruit (rich in vitamin C) in Nepal. Several immunostimulants have been proved to stimulate the immunity in both mammals and fishes. Similarly Lapsi has been found to enhance growth and stimulate immunity in Nile Tilapia in this experiment. 10 weeks of feeding Tilapia with Lapsi fruits pulp powder (LFPP) enhances protein level in them. Total protein, albumin and globulin levels in the liver of Nile Tilapia increased as compared to control diet fed fish.

Farmers involved in the aquaculture are in great loss due to pathogens (bacterial, viral, fungal and so on) exposed to the farmed fish species. So, they use expensive vaccines and antibiotics for controlling pathogens which have negative impact on fish as well as consumers and environment. This problem can be solved by using herbal medicines either in natural form or in extract form as growth promoter and immunostimulant. Among them, one is Lapsi fruit pulp powder.

Finally, it can be concluded that Lapsi fruit pulp powder (LFPP) enhance the growth of fish and strongly stimulates the immune system of Nile Tilapia; protect them from infection in adverse conditions and reduced mortality and increased growth rate. This result may be a guideline to the aqua farmers in relation to healthy fish production and proper growth.

Thus, from the result obtained from this study, some of the suggestions regarding the improvement in aquaculture sector and socio-economic status of farmers are listed below:

a) Use of herbal medicines (200 mg/Kg of LFPP) in the fish diet should be carried out to enhance the growth rate of Nile Tilapia. This helps to improve the socio-economic status of farmers and the nation.

b) Nile Tilapia should be fed with Lapsi fruit pulp powder to stimulate the non-specific immune system of fish. This helps to get high quality of fish meat.

c) Use of expensive artificial vaccines and antibiotics should be replaced by herbal medicines which do not have negative impact on fish, other aquatic organisms, consumers and environment i.e. eco-friendly.

d) To secure the food security problems in the nation, herbal medicines that are acceptable by fish body are to be applied in the fish diet.

e) Further research should be carried to know the need and importance of the herbal medicines in the field of aquaculture.

7. REFERENCES

Ababouch, L. and Karunasagar, I. 2013. Proceedings of tenth World Seafood Congress Saint John's, 29 September – 3 October. Food and Agriculture Organization, United States

Abdelkhalek, N.K.M., Zaki, V.H. and Yousef, M.A.A. 2008. Effect of some immunostimulants on health status and disease resistance of Nile Tilapia (*Oreochromis niloticus*). International Journal of research in Fisheries and Aquaculture **4**(1): 22-30.

Abdelwahab, A.M. and El-Bahr, S.M. 2012. Influence of black cumin seeds (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) mixture on performance and serum biochemistry of Asian Sea Bass, *Lates calcarifer*. World Journal of Fish Marine Science **4:** 496-503.

Ai, Q. H., Mai, K. S., Li, H. T., Zhang, C. X., Zhang, L., Duan, Q. Y. et al. 2004. Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese sea bass, *Lateolabrax japonicus*. Aquaculture **230:** 509-516.

Ajeel, S.G. and Al-Faragi, J.K. 2012. Effect of Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) to enhance health of common carp *Cyprinus carpio L* . The Iraqi Journal of Veterinary Medicine **37**(1): 59 – 62.

Alam, M.J., Ghulam, Mustafa, M.M. and Khaleque, M.A. 2009. Evaluations of the Effects of Different Dietary Vitamin C levels on the Body Composition, Growth Performance and Feed Utilization Efficiencies in Stinging Catfish, *Heteropneustes fossilis* (Bloch, 1792). Journal of American Science **5**(3): 31-40.

Alishahi, M., Ranjbar, M. M.M., Ghorbanpour, M., Mesbah, M. and Razi Jalali, M. 2010. Effects of Dietary *Aloe vera* on Some Specific and Nonspecific Immunity in the Common Carp (*Cyprinus carpio*). International Journal of veterinary researches **4:** 189-195.

Aly, S.M., and Mohamed, M.F. 2010. *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile Tilapia (*Oreochromis niloticus*). International Journal of veterinary researches **5:** 31-39.

Anderson, D.P. 1992. Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. Annual Review of Fish Diseases **2:** 281–307.

Arabshahi-Delouee, S. and Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chemistry **102**(4): 1233–1240.

Ashida, T. and Okimasu, E. 2005. Immunostimulatory effects of fermented vegetable product on the non-specific immunity of Japanese flounder *Paralichthys olivaceus.* Fisheries Science **71:** 257–262.

Ashraf, M.A. and Goda, S. 2008. Effect of dietary Ginseng herb (Ginsana® G115) supplementation on growth, feed utilization and haematological indices of Nile Tilapia *Oreochromis niloticus* fingerlings. Journal of World Aquaculture Society **39**(2): 205-214.

Bairwa, R., Sodha, R. and Rajawat, B. 2012. Trachyspermumammi. Pharmacogn Reviews **6**(11): 56–60.

Balasubramanian, G., Sarathi, M., Venkatesan, C., Thomas, A.S. and Hameed, S. 2008. Oral administration of antiviral plant extract of *Cynodon dactylon* on a large scale production against White spot syndrome virus (WSSV) in *Penaeus monodon*. Aquaculture **279:** 2-5.

Balasubramanian, S.P. and Michael, .D. 2002. Immunomodulation by the fruit extract of Indian Gooseberry, *Phyllanthus emblica* (Linn). in *Oreochromis mossambicus* (Peters). M.Sc. Thesis.The American College, Maduria, India.

Bhujel, R.C. 2011. How to produce billions of high quality Tilapia fry. *In:* Liping, L. and Fitzsimmons, K. eds. Better Science, Better Fish, Better Life proceedings of the ninth international symposium on Tilapia in aquaculture, Aqua, 22-25 April 2011, Shanghai, China. Fish- Collaborative Research Support Program: 123-131.

Binukumari, S. and Vasanthi, J. 2013. Changes in protein content of the freshwater fish, *Labeo rohita* due to the effect of an insecticide ‘Encounter’ (Herbal extract plant). International Journal of Current Microbiology and Applied Sciences **2:** 150-153.

Bricknell, I. and Dalmo, R.A. 2005. The use of immunostimulants in fish larval aquaculture. Fish and Shellfish Immunology **19:** 457-472.

Chitra, S. 1995. Effects of feeding supplemented stresstol bioencapsulated *Artemia franciscana* on growth and stress tolerance in *Penaeus indicus* postlarvae. M.Phil Thesis. MS university, Tirunelveli, India.

Choo, P.S. and Williams, M.J. 2003. Fisheries production in Asia: Its role in food security and nutrition. The World Fish Centre Quarterly **26**(2): 11-16.

Christybapita, D., Divyagnaneswari, M. and Michael, R.D. 2007. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish & Shellfish Immunology **23:** 840-852.

Chunmei, L., Jie, H., Yonglin, G., Yanil, X. And Jingwei, T. 2003. Preventive effect of total flavones of *Choerospondias axillaris* onIschemial reperfusion-induced myocardial infarction –related MAOK signalling pathway. Cardiovascular toxicology **14**(2): 45-52.

Citarasu, T., Sivaram, V., Immanuel, G., Rout, N., Murugan, V. 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in Black Tiger Shrimp, *Peneaus monodon* with reference to haematological, biochemical and immunological changes. Fish Shellfish Immunology **21:** 372-384.

Citarasu, T.K., Babu, M.M., Sekar, R.J.R. and Marian, M.P. 2002. Developing *Artemia* enriched herbal diet for producing quality larvae in *Penaeus monodon*. Asian Fisheries Science Journal **15:** 21-32.

Citarasu, T.K., Immanuel, G. and Marian, M.P. 1998. Effect of feeding *Artemia* enriched with stresstol and cod- liver oil on growth and stress resistance in the Indian White Shrimp *Peaneus indicus* postlarvae. Asian Fisheries Science Journal **12:** 1-7.

Dabrowski, K. 1990. Absorption of ascorbic acid and ascorbic sulfate and ascorbate metabolism in common carp (*Cyprinus carpio L.*). Journal of Comparative Biochemistry and Physiology B **160:** 549–561.

Dabrowski, K., Ciereszko, A., Ramseyer, L., Culver, D. and Kestemont, P. 1994. Effects of hormonal treatment on induced spermation and ovulation in the Yellow Perch (*Perca flavescens*). Aquaculture **120:** 171-180.

Dabrowski, K., El-Fiky, N., Köch, G., Frigg, M. and Wieser, W. 1990. Requirement and utilization of AA and ascorbic acid sulphate in juvenile Rainbow trout. Aquaculture **91:** 317-337.

Dabrowski, K., Matusiewicz, K., Matusiewicz, M., Hopp, P.P. and Ebeling, J. 1996. Bioavailability of vitamin C from two ascorbyl monophosphate esters in Rainbow Trout *Onchorynchus mykiss* (Walbaum). Aquacultural Nutrition **2:** 3–10.

DADA, A.A. 2012. Effects of herbal growth promoter feed additive in fish meal on the performance of Nile Tilapia (*Oreochromis niloticus* (L.)). Egyptian Academic Journal of Biological Science **4**(1): 111-117.

Dai, H.Y., Li, Q. A., Chen, L. F., and Deng, H. W. 1992. Protective effect of extract from *Choerospondias axillaris* fruit on myocardial ischemia of rats. Chinese Traditional and Herbal Drugs **23:** 641-643.

De Silva, S.S. & Aderson, T.A. (1995). Fish Nutrition in Aquaculture. Chapman and Hall, London. 319p.

De-Francesco, M., Parisi, G., Perez-sanchez, J., Gomez-requeni, P., Medale, F., Kaushik, S.J., et al. 2007. Effect of high- level fishmeal replacement by plant proteins in gilthead sea bream (*Sparusaurata*) on growth and body fillet quality traits. Aquaculture Nutrition **13**(5): 361 – 372.

Divyagnaneswari, M., Christybapita, D. and Michael, R. D. 2007. Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. Fish Shellfish Immunology **23:** 249–259.

DoFD. 2014. Annual report of Department of Fisheries development, Balaju, Kathmandu.

Doumas, B.T. 1975. Standards for total serum protein assays–a collaborative study. International Journal of Clinical Chemistry **21:** 1159–1166.

Doumas, B.T., Watson, W.A. and Biggs, H.C. 1971. Albumin standards and Management of serum albumin with bromocresol green. International Journal of Clinical Chemistry **31:** 87-96.

Duda-Chodak, A., Tarko, T. and Rus, M. 2011. Antioxidant activity and total polyphenol content of selected herbal medicinal products used in Poland. Herba Prolonica **57**(1): 48-61.

Dugenci, S.K., Nazh, A. and Candan, A. 2003. Some medicinal plants as immunostimulant for fish. Journal of Ethnopharmacology **88:** 99–106.

El-Sayed, A.F.M. 2006. Tilapia Culture. CABI Publishing. Wallingford, GB, 256 p.

El-Sayed, S.A.A., Abdl El-Galil, S.Y. and Rashied, N.A. 2014. Effects of some herbal plants as natural feed additives in comparision with antibiotic on growth performance and immune status of Nile Tilapia (*Oreochromis niloticus*). International Journal of Research in Fisheries and Aquaculture **4**(1): 22-30.

Fagbenro, O. A., Jegede, T. and Fasasi, O. S. 2010. Tilapia aquaculture in Nigeria. Applied Tropical Agriculture **15:** 49-55.

FAO .2015. Fisheries and Aquaculture Development, Food and Agriculture organization, United State.

FAO. 1994. Aquaculture production, FAO, Rome, Italy.

FAO. 2002. Fishery Statistics. Aquaculture production 90(2).

FAO. 2010. A report on "The State of World Fisheries and Aquaculture", Food and Agriculture Organization, United State.

FAO. 2014. A report on "The State of World Fisheries and Aquaculture", Food and Agriculture Organization, United State.

Favias, W.R.L., Roboucas, H.J., Torres,V.M., Rodrigues, J.A.G., Ponles, G.D.C. et al. 2004. Enhancement of growth in Tilapia larvae (*Oreochromis niloticus*) by sulfated D-galactans extracted from the Red marine alga *Botryocladia occidentalis*. Revista Ciencia Agronomica **35:** 189-195.

Feng, J.G., Xu, Y.T. and Chen, Y.T. 1999. Growth performance of eight native broadleave species on hill country in southwestern Zhejiang. Forest Research Beijing **12**(4): 438-441.

Fenichet, R.L. and Chirigos, M.A. 1984. Immune modulation agents and their mechanisms. New York: Marcel Dekker.

Fortes, R. D. 2005. Review of the techniques and practices in controlling Tilapia populations and identification of methods that may have practical applications in Nauru including a National Tilapia plan. Aquaculture Section. Secretariat of the pacific community (SPC), Marine Resources Division, Noumea, New Calednia. p. 1-55.

Francis, G., Harinder, P., Makkar, S. and Becker, K. 2005. *Quillaja saponins*- a natural growth promoter for fish. Animal Feed Science Technology **121**(1-2): 147-157.

Galina, J., Yin, G., Ardo, L. and Jeney, Z. 2009. The use of immunostimulating herbs in fish. An overview of research. Fish physiology and Biochemistry **35**(4): 669-676.

Gammanpila, M., Yakupitiyage, A. and Bart, A.N. 2007. Evaluation of the effects of dietary vitamin C, E and Zinc supplementation on reproductive performance of Nile Tilapia (*Oreochromis niloticus*). SriLanka Journal of Aquatic Science **12:** 39-60.

Gartner, C., Stahl, W. and Sies, H. 1997. Lycopene is more bioavailable from tomato paste than from fresh tomato. American Journal of Clinical Nutrition **66:** 116-122.

Graftman, L. and Chriswaterguy, B. 1998. Technical Guide for Tilapia Farming. p.51

Hagerman, A.E., Ried, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W. et al. 1998. High molecular weight plant polyphenolics (tannis) as biological antioxidant. Journal of Agricultural and Food Chemistry **46:** 1887-1892.

Hamza, N., Silvestre, F., Mhetli, M., Khemis, I.B., Dieu, M., Raes, M. et al. 2010. Differential protein expression profile in the liver of Pikeperch (*Sander luciopera*) larvae fed with increasing levels of phospholipids. Comparative Biochemistry and Physioogy-Part D **5**(2): 130-137.

Hassan, M. and Javed, M. 2000. Studies on muscle protein profile of fish reared in poultry dropping fertilized ponds. Pakistan Journal of Agricultural Science **37**(1-2): 51-54

Hau, C.H., parrotta, J.A. and Turnbull, J.W. 1997. Tree seed predation on degraded hillsides in Hongkong. Forest Ecology and Management **99**(1/2): 215-221.

Hemapriya, V.S. 1997. Immunostimulatory effect of leaf extracts of few medicinal plants in *Oreochromis mossambicus* (Peters). M.Sc. Thesis. The American College, Madurai, India.

Hepher, B. and Pruginin, Y. 1981. Commercial fish farming with special reference to fish culture in Israel. John Wiley and Sons, New York, USA.

http://www.en.wikipedia.org/wiki/Nile_Tilapia.

Ibrahem, M.D., Fathi, M., Mesalhy, S. and Abd El-Aty, A.M. 2010. Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile Tilapia (Oreochromis niloticus). Fish and shellfish Immunology **29**(2): 241-246.

Jackson, J. K. 1994. Manual of Afforestation in Nepal. 2nd edition. Forest Research and Survey Centre, Kathmandu, Nepal.

Jayaprakash, V. and Euphrasia, J. 1996. Growth performance of *Labeo rohita* (Ham.) to Livol (IHF-1000), an herbal product. Proceeding of the Indian National Science Academy **63**(2): 1-10.

Jayathirtha, M. G. and Mishra, S.H. 2004. Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica*. Phytomedicine **11:** 361-365.

John, G., Salah, M., Rezk, M., El-Naggar, G. and Fathi, M. 2007. Effect of some immunostimulants as feed additives on the survival and growth performance of Nile Tilapia, *Oreochromis niloticus* and their response to artificial infection. Egyptian Journal of Aquatic Biologyand Fisheries **11**(3): 1299 -1308.

Khattab, Y.A., Shalaby, A.M.S., Sharaf, S.M., El-Marakby, H.I. and Rizhalla, E.H. 2004. The physiological changes and growth performance of the Nile Tilapia *Oreochromis niloticus* after feeding with Biogen ® as growth promoters. Egyptian Journal of Aquatic Biological Fish **8:** 145-158.

Kim, D.S., Noah, C.H., Jung, S.W. and Jo, J.Y. 1998. Effect of Obosan supplemented diet on growth, feed conversion ratio and body composition of Nile Tilapia, *Oreochromis niloticus*. Journal of Aquaculture **11:** 83–90.

Kirubakaran, C.J., Alexander, C.P. and Michael, R.D. 2010. Enhancement of non-specific immune responses and disease resistance on oral administration of *Nyctanthes arbortristis* seed extract in *Oreochromis mossambicus* (Peters). Aquaculture Research **41:** 1630-1639.

Labh, S.N. and Chakrabarti, R. 2011. Effects of Different Dietary Supplements of Vitamin C L-Ascorbate-2-Triphosphate Calcium (LATP-Ca) on Growth, Tissue Vitamin C and Liver Ultrastructure of Indian Major Carp Mrigal *Cirrhinus mrigala* (H) During Intensive Aquaculture. Journal of Theoretical and Experimental Biology **7** (4): 195-201.

Labh, S.N. and Shakya, S.R. 2014. Application of immunostimulants as an alternative to vaccines for health management in aquaculture. International Journal of Fisheries and Aquatic Studies **2**(1): 153-156.

Larson, R.A. 1998. The antioxidants of higher plants. Phytochemistry 27: 969-978.

Lee, K.J. and Dabrowski, K. 2003. Interaction between vitamins C and E affects their tissue concentrations, growth, lipid oxidation and deficiency symptoms in Yellow Perch (*Perca flavescens)*. British Journal of Nutrition **89:** 589-596.

Lee, S. H., Oe, T. and Blair, I. A. 2001. Vitamin C induced decomposition of lipid hydroperoxides to endogenous genotoxins. Science **292:** 2083-2086.

Lim, C. and Lovell, R.T. 1978. Pathology of vitamin C deficiency syndrome in channel Catfish. Journal of Nutrition **108:** 1137-1141.

Lin, J.G., Chen, C.L., Xu, C.J. and Zhang, S.H. 2000. Comparision of the physical and mechanical properties of three species of ring porous woods from plantations and natural forests. Journal of Fujian College of Forestry **20**(1): 59-61.

Logambal, S.M. and Michael, R.D. 2000. Immunostimulatory effect of azadirachitin in *Oreochromis mossambicus* (Peters). Indian Journal of Experimental Biology **38:** 1092–1096.

Logambal, S.M. and Michael, R.D. 2001. Azadirachtinan- immunostimulant for *Oreochromis mossambicus* (Peters). Journal of Aquaculture in the Tropics **16:** 339–347.

Lovell, R.T. 1991. Nutrition of aquaculture species. Journal of Animal Science **69:** 4193-4200.

Madhuri, S., Sahni, Y.P. and Pandey, G. 2012. Herbal feed supplements as drugs and growth promoter to fishes. International Research Journal of Pharmacy **3**(9): 30-33.

Mair, G. 2001. Genetics in Tilapia aquaculture. Tilapia production in the Americas 136 - 148.

Martin, S.A.M., Vilhelmsson, O., Medale, F., Watt, P., Kaushik, S., Houlihan, D.F. 2003. Proteomic sensitivity to dietary manipulations in Rainbow Trout. Biochimica et Biophysica Acta **1651**(1-2): 17-29.

Mastan, S.A. 2015. Use of Immunostimulants in aquaculture disease management. International Journal of Fisheries and Aquatic Studies **2**(4): 277-280.

Masumoto, T., Sokawe, H. and Shimeno, S. 1991. Ascorbic acids role in Aquaculture nutrition. Preceedings of the Aquaculture Feed Processing and Nutrition Workshop, 19 – 25 September 1991. American Soybean Association, Republic of Singapore: 42-48.

Megbowon, I., Adejonwo, O.A., Adeyemi, Y.B., Kolade, O.Y., Adetoye, A.A.A., Edah, C.A. et al. 2013. Effect of Garlic on growth performance, nutrient utilization and survival of Ecotype Cichlid, 'Wesafu'. ISOR Journal of Agriculture and Veterinary Science **6**(3): 10-13.

Merchie, G., Lavens, P. and Sorgeloos, P. 1997. Optimization of dietary vitamin C in fish and crustacean larvae: a review. Aquaculture **155:** 165-181.

Mesalhy, S.A., Fathi, M.M. and john, G. 2008. *Echinacea* as immunostimulatory agent in Nile Tilapia (*Oreochromis noloticus*) via. earthern pond experiment.8th International Symponium on Tilapia in Aquaculture.

Metwally, M. 2009. Effects of Garlic *Allium sativum* on some antioxidant activities in Nile Tilapia *Oreochromis niloticus*. World Journal of Fish and Marine Science **1**(1): 56-64.

Minomol, M. 2005. Culture of Gold fish *Carassius auratus* using medicinal plants having immunostimulant characteristics. M.Phil. Thesis. MS University, India.

Mishra, R.N. 2002. African catfish *Clarias gariepinus* as a predator for population control in mixed-sex Nile Tilapia *Oreochromis niloticus*. M.Sc. Thesis. Institute of Agriculture and Animal Sciences, Tribhuvan university, Rampur, Chitwan, Nepal.

Ndong, D. and Fall, J. 2011. The effect of Garlic (*Allium sativum*) on growth and immune responses of hybrid Tilapia (*Oreochromis niloticus x Oreochromis aureus*). Journal of Clinical Immnunology and Immunopathology Research **3**(1): 1-9.

Nguyen, D.D., Nguyen, N.H., Nguyen, T.T., Phan, T.S., Nguyen, V.D., Grabe, M. Et al. 1996. The use of a water extract from the bark of *Choerospondias axillaris* in the treatment of second degree burns. Scandinavian Journal of Plastic and reconstructive surgery and hand surgery **30**(2): 139-144.

Nya, E.J. and Austin, B. 2009. Use of Garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in Rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases **32:** 971–977.

Omoregie, E., Igoche, L., Ojobe, T.O., Absalom, K.V. and Onusiriuka, K.V. 2009. Effect of varying levels of sweet Potato (*Ipomea batatas*) peels on growth, feed utilization and some biochemical responses of the cichlid (*Oreochromis niloticus*). African Journal of Food, Agriculture, Nutrition and Development **9:** 700-712.

Pantha, M.B. 1993. Aquafeeds and feeding strategy in Nepal. Proceedings of the FAO/AADCP Regional Expert Consolation on Farm-made Aquafeeds, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand.

Paudel, K. C. and Parajuli, D.P. 1999. Domestication and Commercialisation of Lapsi tree: A potential income source through agroforestry in the middle hills of Nepal. In: Ministry of Science and Technology, Scientific World, Vol. 1, No. 1, Kathmandu, Nepal, 116–120.

Paudel, K.C. 2001. Participatory domestication of lapsi tree (*Choerospondias axillaris*. Roxb.) in Nepal, Part I- occurrence and distribution in Nepal, Part II- softwood cutting propagation. A report submitted to the ICIMOD, Kathmandu.

Paudel, K.C. 2003. Domesticating Lapsi *Choerospondias axillaris* Roxb. (B.L. Burtt and A.W. Hill) for fruit production in the middle mountain agroforestry systems in Nepal. Himalayan Journal of Sciences **1**(1): 55-58.

Paudel, K.C., Pieber, K. And Klumpp, R and Laimer, M. 2003. Evaluation of Lapsi tree *Choerospondias axillaris* for fruit production in Nepal. Die Bodenkultur **54**(1): 3-9.

Paudel, K.C., Pieber, K., Klumpp, R. and Laimerda da Camara Machado, M. 2000. Promotion of Lapsi tree (*Choerospondias axillaris*) for fruit production in Nepal. International Conference Workshop Food Security Urban and Peri urban system in developing countries. ISSU, University of Bodenkultur Nov.15-18: 15.

Penaflorida, V.D. 1995. Effect of Papaya leaf meal on the Penaeus monodon postlarvae. Israeli Journal of Bamidgeh **47**(11): 23-25.

Prasad, S. and Padhyoy, V.K.B. 1993. Chemical investigation of some commonly used species. Aryavaidyan **6:** 262-267.

Priyadarshini, M., Manissery, J.K., Mohan, C.V. and Keshavnath, P. 2012. Effect of ImmuPlus on growth and inflammatory response to Fruend's. Complete adjuvant in common carp *Cyprinus carpio* (L.). Turkish Journal of Fisheries and Aquatic Sciences **12:** 291-299.

R Core Team 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rai, S., Shrestha, M.K., Jha, D.K. and Acharya, D. 2007. Growth performance of Sahar and mixed Nile Tilapia in monoculture and co-culture system in Chitwan. IAAS research Advances **1:** 187-193.

Rao, V. and Chakrabarti, Y.R. 2005. Stimulation of immunity in Indian major carp (*Catla catla*) with herbal feed ingredients. Fish & Shellfish Immunology **18:** 327-334.

Rao, Y.V. and Chakrabarti, R. 2005. Stimulation of immunity in Indian major carp *Catla catla* with herbal feed ingredients. Fish Shellfish Immunology **18:** 327-334.

Rombout, J.H., Huttenhuis, H.B.T., Picchietti, S. and Scapigliati, S. 2005. Phylogeny and ontogeny of fish leucocites. Fish and Shellfish Immunology **19:** 441-455.

Roxburg, W. 1832. Flora Indica 2nd ed. Mission Press, India, 453 p.

Sahoo, P.K. and Mukherjee, S.C. 1999. Influence of the immunostimulant, chitosan on immune responses of healthy and cortisol-treated Rohu (*Labeo rohita*). Journal of Aquaculture in the Tropics **14**(3): 209-215.

Salah, M.A., Nashwa, M.A.A. and Mohamed, F.M. 2001. Effect of Garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*. Proceedings of 8th International Symposium on Tilapia in Aquaculture, 12-14, October 2008. Cairo, Egypt: 277-296.

Sambhu, C. and Jayaprakash, V. 2000. Livol (IHF-1000), a new herbal growth promoter in white Prawn *Penaeus indicus* (crustacean). Indian Journal of Marine Sciences **30:** 38-43.

Schuchardt, D., Vergara, J.M., Palaciso, H.F., Kalinowski, C.T., Cruz, C.M.H., Izquierdo, M.S., et al. 2008. Effects of different dietary protein and lipid levels on growth, feed utilization and body composition of red porgy (*Pagruspagrus*) fingerlings. Aquaculture nutrition **14**(1): 1-9.

Shadakshari, G.S. 1993. Effect of bioboost forte, Livol and Amchemin AQ on growth and body composition of common carp *Cyprinus carpio* (Linn.). M.F.Sc. Thesis. University of Agriculture Sciences, Bangalore, India.

Shah, D.J. 1978. Ascorbic acid (Vitamin C) content of Lapsi-pulp and peel at different stage of maturation.Research Bulletin 2035 B.S. Food Research Section, HMGN, Department of Food and Agricultural Marketing Services, Kathmandu.

Shakya, S.R. and Labh, S.N. 2014. Medicinal use of Garlic *Allium sativum* improves fish health and acts as an immunostimulant in aquaculture. European Journal of Biotechnology and Bioscience **2**(4): 44-47.

Shalaby, A.M., Khattab, Y.M. and Abdel Rahman, A.M. 2006. Effect of Garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia (*Oreochromis niloticus*). Journal of Venomous Animals and Toxins Including Tropical Diseases **12:** 172-201.

Shang, Y.C. 1996. The Role of Aquaculture in World Fisheries. Proceeding of the world Fisheries Congress, Theme 6 (T.G. Heggberget Ed.). Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, India : 24-42.

Shelton, W.L. 2002. Tilapia culture in the 21st century. In Proceedings of the International Forum on Tilapia Farming in the 21st Century, 2002. Philippne Fisheries Association Incorporation, Los, Banos, Laguna, Philippines 2002.

Shi, S., Li, Z. X., Tian, F. J., Bai, Y. F., Tian, L., Yang, Y. M., et al. 1985. Effect of flavanoid from *Choerospondias axillaries* fruit on left ventricle function and hemodynamics of anaesthesia dog. Inner Mongolia Pharmaceutical Journal 2**:** 14-15.

Shiau, S.Y. and Hsu, T.S. 1995. L- Ascorbyl-2-Sulfate has equal antiscorbutic activity as L-ascorbyl-2-monophosphate for Tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquaculture **133:** 147-157.

Shivaprasad, H.N., Kharya, M.D., Rana, A.C. & Mohan, S. (2006). Preliminary immunomodulatory activities of the aqueous extract of *Terminalia chebula*. Pharmaceutical Biology **44**(1): 32-34.

Shrestha, J. 1994. Fishes, Fishing Implements and Methods of Nepal. Craftsman Press, Bangkok, 102 p.

Shrestha, M.K. and Bhujel, R.C. 1999. A preliminary study on Nile Tilapia *Oreochromis niloticus* polyculture with common carp *Cyprinus carpio* fed with duckweed *Spirodela* in Nepal. Asian Fisheries Science **12:** 83-89.

Shrestha, M.K. and Pandit, N.P. 2012. A Text book of Principle of Aquaculture 2^{nd} ed. Graphic Design and Printing Support, Nepal, 7 p.

Sivagurunathan, A., Xavier, B.I., Saraswathi, G.S. and Mariappan, A. 2012. Immunostimulatory potential of dietary Amala (*Phyllanthus emblica*) in growth and haematology of *Tilapia mossambicus* with *Pseudomanas aeruginosa*. International Research Journal of Pharmacy **3**(7): 165-168.

Sivaram, V., Babu, M.M., Citarasu, T., Immanuel, G., Murugadass, S. and Marian, M.P. 2004. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture **237:** 9-20.

Sivasankar, P., Santhiya, A.V.A. and Kangana, V. 2015. A review of plants and herbal extracts against viral diseases in aquaculture. Journal of Medicinal plants studies **3**(2): 75-79.

Siwicki, A.K., Morand, M., Terech-Majewska, E., Niemczuk, W., Kazuń, K. and Glabski, E. 1998. Influence of immunostimulants on the effectiveness of vaccines in fish: in vitro and in vivo study. Journal of Applied Ichthyology **14:** 225-227.

Soliman, A.K., Jauncey, K. and Roberts, R.J. 1986a. The effect of varying forms of dietary AA on the nutrition of juvenile Tilapia (*Oreochromis niloticus*). Aquaculture **52:** 1-10.

Srivastav, P.K. and Chakrabarti, R. 2010. Effect of Dietary Supplementation of *Achyranthes aspera* Seed on the Immune System of *Labeo rohita* fry. Israeli Journal of Aquaculture **64:** 786-795.

Stahl, W. and Sies, H. 1992. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. Journal of Nutrition **122:** 2161-2166.

Subasinghe, R. P. 2005. Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Preventive veterinary Medicine **67:** 117-24.

Tewary, A. and Patra, B.C. 2008. Use of vitamin C as an immunostimulant. Effect on growth, nutritional quality and immune response of *Labeo rohita* (Ham). Fish Physiology and Biochemistry **34:** 251-259.

Turan, F. 2006. Improvement of growth performance in Tilapia (*Oreochromis niloticus* Linnaeus) by supplementation of Red Clover (*Trifolium pretense*) indiets. Israeli Journal of Aquaculture **58:** 34-38.

Unnikrishnan, G. 1995. Effect of Livol on growth, food utilization and body composition of the Indian major carp *Catla catla* (Ham.). M.Sc. Thesis. University of Kerala, India.

Van Hai, N. 2015. The use of medicinal plants as immunostimulants in aquaculture: a review. Aquaculture **446:** 88-96.

Veliogu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural Food Chemistry **46:** 4113-4117.

Verreth, J. 1999. Growth and feeding metabolism in fish larvae 1st international course on fish larvae nutrition. M.Sc. Thesis. Wageningen Agricultural University, Wageninge. The Netherland.

Wang, H., Xiang, G.D., Zhou, G.C., Li, C. and Yao, W.B. 2008. In vitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food chemistry **106:** 888-895.

Wattes, M., Munday, B.L. and Burke, C.M. 2001. Immune responses of teleost fish. Australian Veterinary Journal **79:** 570-574.

Yadav, C.N.R. 2006. Tilapia- An Introduction and Prospects of its culture in Nepal. Our Nature **4:** 107-110.

Yakubu, A.F., Obi, A., Okonji, V.A., Ajiboye, O.O., Adams, T.F., Olaji, E.D. et al. 2012. Growth performance of Nile Tilapia *Oreochromis niloticus* as affected by stocking density and feed types in water flow through system. World Journal of Fish and Marine Sciences **4**(3): 320-324.

Yoshida, T., Sakai, M., Kitao, T., Khlil, S.M. and Araki, S. et al. 1993. Immunomodulatory effects of the fermented products of chicken egg, EF203, on Rainbow trout, *Oncorhynchus mykiss*. Aquaculture **109** (3-4): 207-214.

Zhou Y. 2003. 35 treatment cases of cerebral arteriosclerosis by chuanxiong tianma decoction. Henan Traditional Chinese Medicine **1**(4): 22–23.

Zhou, D.M., Zhu, G.Q., Wu, S.Y., Wu, Y.F., Hu, H.J. nad Ye, C.W. 1997. Preliminary report on tree species selection for the cultivation of *Lentinus edodes.* Journal of Zhejiang Forestry Science and Technology **17**(1): 18-23.

Zhou, D.M., Zhu, G.Q., Zhu, J.G., Jiang, G.H., Wu, S.Y., Wu, R.F. et al. 1999. Studies on artificial breeding techniques for short-rotation term mushroom forests. Journal of Zhejiang Forestry Science and Technology **19**(6): 1-10.

APPENDIX I

S. N.	Parameters	T1	T2	T3	T4
		Mn±SE			
1	Survival (%)	84.12±6.54	92.06±5.89	93.65±3.27	85.71±5.66
2	Initial Length (cm)	2.72±0.17	2.72±0.17	2.72±0.17	2.72±0.17
3	Final Length (cm)	4.21±0.19	4.53±0.06	5.09±0.07	4.75±0.18
4	Length Gain (%)	54.84±6.93	66.60±2,37	87.23±2.43	74.84±6.46
5	Initial Weight (cm)	0.284±0.37	0.284±0.37	0.284±0.37	0.284±0.37
6	Final weight (cm)	0.89±0.14	1.14±0.06	1.75±0.08	1.42±0.18
7	Weight gain (%)	214.21±47.87	301.78±20.78	517.07±26.79	403.37±61.93
8	Specific Growth Rate	2.0±0.16	2.29±0.06	2.80±0.05	2.54±0.16
9	Feed Conversion Ratio	14.67±0.91	11.07±0.58	7.18±0.31	7.85±0.15
10	Feed Efficiency Ratio	7.09±0.09	9.07±0.47	13.96±0.61	12.72±0.17
11	Total Protein	8.33±0.3	10.66±0.9	15.33±0.3	17.66±0.9
12	Albumin	6.66±0.3	8.66±0.3	12.33±0.7	15.33±0.3
13	Globulin	1.66±0.3	2.0±0.6	3.0±0.6	2.33±0.9
14	A/G Ratio	4.5±1.3	5.16±1.5	4.52±1.1	8.91±3.4

ESSENTIAL PHOTOPLATES

Photo 1. Electric weighing machine

Photo 2. Measuring length of fish

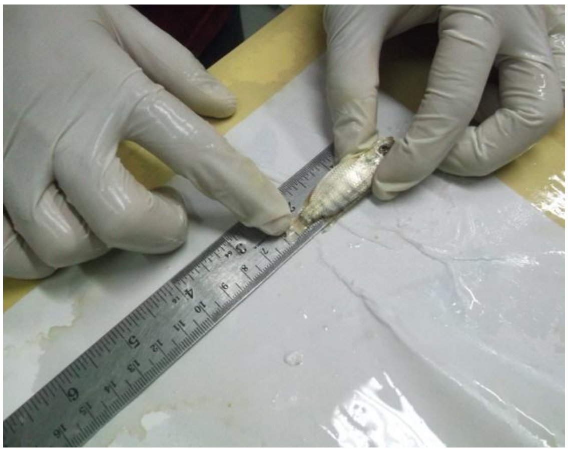

Photo 3. Measuring length of fish

Photo 4. Measuring weight of fish

Photo . culture condition

Photo . Changing water

Photo . Feeding diet to fish

Photo Weighing feed

Photo Dissection of fish

Photo Weighing liver of fish

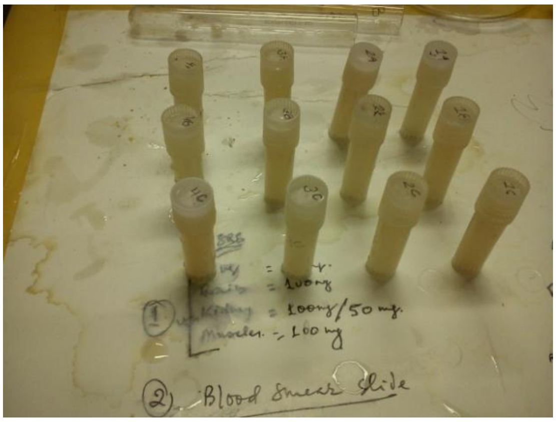

Photo Sample for homogenization

Photo . Centrifuging machine