EFFECT OF *Urtica parviflora* Roxb. ON THE GROWTH PERFORMANCE AND HEMATOLOGIC PARAMETERS IN NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758)

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RECOMMENDATIONS

This is to recommend that the thesis entitled "**EFFECT OF HERBAL PLANT *Urtica parviflora* Roxb. ON THE GROWTH PERFORMANCE AND HEMATOLOGIC PARAMETERS IN NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758)"** has been carried out by **Miss Dipa Rai** for the partial fulfillment of Master's degree of Science in Zoology with special paper **Fish Biology and Aquaculture.** This is her original work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisors **"Prof. Dr. Kumar Sapkota** and **Dr. Tirth Raj Ghimire"** this dissertation submitted by **Miss Dipa Rai entitled "EFFECT OF HERBAL PLANT *Urtica parviflora* Roxb. ON THE GROWTH PERFORMANCE AND HEMATOLOGIC PARAMETERS IN NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758)""** is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper **Fish Biology and Aquaculture**.

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This thesis work submitted by **Miss Dipa Rai** entitled **"EFFECT OF HERBAL PLANT *Urtica parviflora* Roxb. ON THE GROWTH PERFORMANCE AND HEMATOLOGIC PARAMETERS IN NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758)"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper **Fish Biology and Aquaculture**.

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

Date..09. Sept. 2019 .....

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is the second most cultured fish in the world after carp. For its culture and growth, several herbs have been used for culture and growth since many years. Interestingly, the stinging nettle (*Urtica parviflora*), due to its accessibility, economically sustainable, and geographic diversity, has been hypothesized to control the growth and immunomodulation in various fish species. Thus, the current study was conducted to determine the role of this herb in the growth and hematologic parameters of Nile tilapia. To address this, a total of 50 fingerling with an average body weight of 12g and 8cm of body length were assigned at random to five treatment diets of *U. parviflora* (0%, 1%, 5%, 10%, 15% of total feed). These diets were fed for 90 days. The results revealed that specific growth rate (SGR), and percentage weight gain (PWG), of fishes fed with *U. parviflora* enriched diet (T_2) was found to be significantly higher ($p < 0.05$) compared to the control group. Similarly, feed conversion ratio (FCR), and economic conversion ratio (ECR) were lower in treatment group in comparison to control group. Other parameters like feed efficiency ratio (FER), survival rate (SR), condition factor (k), and feed intake (FI) were not significantly different in all group of fish. The hematologic parameters results suggest that fish fed with 5% stinging nettle enhanced RBC, platelets, hemoglobin, and monocyte significantly ($p < 0.05$) in comparison to the control group. There was no significant difference for lymphocyte in different treatment groups. In conclusion, this study showed that the addition of stinging nettle powder to the Nile tilapia diets has a potential stimulatory effects on the growth performance and hematologic parameters.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPS

LIST OF APPENDICES

LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
Al	Average Length
ARL	Animal Research Laboratory
Aw	Average Weight
CF	Condition Factor
CDZ	Central Department of Zoology
cm	Centimeter
CO_2	Carbon dioxide
CP	Crude Protein
DLC	Different Leucocyte Count
DO	Dissolve Oxygen
DOFD	Directorate of Fisheries Development
ECR	Economic Conversion Ratio
EDTA	Ethylene diamine tetra acetic acid
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FER	Feed Efficiency Ratio
FI	Feed Intake
FML	Final Mean Length
FMW	Final Mean Weight
FRC	Fishery Research Center
g	Gram
Hb	Hemoglobin

i.e.	That is
IML	Initial Mean Length
IMW	Initial Mean Weight
KI	Potassium Iodide
Min	Minute
mL	Milliliter
mm	Millimeter
N	Normality
NAST	Nepal Academy of Science and Technology
NHPL	National Herbarium and Plant Laboratories
NHRC	Nepal Health Research Council
No.	Number
OIO	Immersion Oil
PH	Hydrogen Ion Concentration
Ppm	Parts per million
RBC	Red Blood Cells
SE	Standard Error
SGR	Specific Growth Rate
SR	Survival Rate
T_0	Control
T_1	Treatment 1
T_2	Treatment 2
T_3	Treatment 3
T_4	Treatment 4
TLC	Total Leucocyte Count

WBC	White Blood Cells
WGP	Weight Gain Percentage
%	Percentage
^{o}C	Degree Centigrade
µL	Microliter
mg/l	Microgram per liter

INTRODUCTION

1.1 Background of the study

Aquaculture is the world's fastest growing food production system, its average growth rate was 3% in between 2003-2016 (FAO 2018). Fishery and aquaculture sector is crucial in meeting FAO's goal of a world without hunger and malnutrition (Silva 2018). Global fish production has peaked at about 171 million tons in 2016 including major fishes carp, tilapia and catfish (FAO 2018). With capture fishery, aquaculture has been responsible for the continuous impressive growth in the supply of fish for human consumption (FAO 2018). Sustainable aquaculture is helping 7.5 billion people by establishing food security, reducing poverty and conserving and using the ocean and other water resources (FAO 2016). In Nepal, fish is helping to reduce food shortage and nutrient crisis and has been one of the best nutrient diet as it contains a high protein source (DOFD, 2015). But, disease outbreaks are increasing at the same time and are being recognized as a potential constraint on aquaculture production, thus, causes massive economic losses to the farmers. Due to increase in population and decrease in water resources, the consumption of aquaculture products is on the increase (Asiedu et al. 2015). To continue its growth in the same rate and reduce fish pathogen, fish feed plays a vital role as it is directly related to fish health. Fish health and human health are interrelated with each other as fish has been consumed as a part of an important diet around the world and healthy fish always determines the status of a healthy human. Thus, the use of quality fish feed has become a concern to increase healthy fish production and to protect human health.

In order to reduce the risk of disease, the level of resistance to infection in the cultured organisms should be increased by the use of better feeds, vaccines, and immunostimulants and by selective breeding for higher disease resistance (Raa et al. 1992). The use of compounds with immunostimulants and/or antioxidant effects as dietary supplement can improve the innate defense on animals providing resistance to pathogens during the periods of high stress (Santose 2009, Han et al. 2012, Faggio et al. 2015). Immunostimulants can enhance the status of hematology (Sakai 1999). Hematology is the study of blood components of the innate immune system such as macrophages, monocytes and granulocytes (Magnadottir 2006). Immunostimulation is considered as a promising alternative therapy to antibiotics because of their broad spectrum nature, cost effectiveness, and eco-friendly disease preventive measure. Using immunostimulants in combination with fish vaccine is an attractive method for increasing the protective capabilities of fish, whose smaller dose of vaccine can boost potency (Jeney and Anderson 1993). Among immunostimulants, depending on their sources, natural ones are preferable because they are biocompatible, biodegradable, cost effective and safe for the environment (Sheikhzadeh, 2013). In recent years, algae, herbs and plant extracts rich in polyphenols, green tea extracts and essential oils have been extensively used in aquaculture for immune enhancement and improved disease resistance (Guardiola et al. 2016).

Hematology deals with the various blood molecules and their physiology determines the systematic relationship among certain species (Pavlidis et al. 2007). Blood parameters are an important auxiliary diagnosis tool when an imbalance on homeostasis of fish is detected. This imbalance can be caused by physiological changes, the occurrence of infectious diseases or parasites that can cause anemia (Tavares-Dias and Moraes 2007, Tavares-Dias et al. 2009). Blood tests are recommended for diagnosis and prognosis of morbid conditions in populations (Shah et al. 2009). The differences in the formation and function of blood cells of animals are due to the different feeding mechanisms and geographical distribution, and the knowledge of the hematology responses to different diets are useful for health status of fish (Yildirim-Aksoy et al. 2007, Lim et al. 2011, Costa et al. 2014).The hematologic profile of a fish population helps to indicate its physiologic status and health. Hematologic values together with other parameters like, Red Blood Cell (RBC), White Blood Cells (WBC), platelets, and hemoglobin could be used to identify and assess the disease and conditions that cause stress in the fish (Tavares-Dias and Moraes, 2007, Pavlidis et al. 2007). For example, RBC can be used for the diagnosis of anemia, while, the leukocytes, the primary line of immunologic defense, provide an important representation of defense cells throughout the body (Tavares-Dias and Moraes, 2007, Affonso et al. 2007, Pavlidis et al. 2007). Similarly, changes in the number or appearance of fish hematology is also one of the most elementary ways to assess the immune system.

However lack of suitable feed choice as well as fish disease is the main problem in aquaculture. Fish feed ingredients play a vital role for growth, health, status and cost product of fish. Hence, selection of right fish feed ingredient is quite challenging, but after analysis of ingredients in detail, it is not a big deal to select an appropriate ingredient. There are many ingredient like plant origin, animal origin, and biowaste origin. But among them, the herbal ingredients are eco-friendly, more economic and easily accessible (Harikrishnan et al. 2011)

It is usually experienced that use of antibiotic and chemotherapeutic agents are practiced to remove pathogen. However, the most important drawback of their application is most of these agents accumulate in fish meat or in environment and they promote the development of resistant pathogen and weaken the immune system of fish (Ardo, et al. 2007). This is even worsening for public health following the transfer of resistance to the environmental and human pathogenic bacteria (Alderman and Hastings 1998, MacMillan, 2001). In addition, antibiotics accumulate in the environment and in the fish from which they cause a potential risk to consumers. Vaccination is regarded as the most effective method in prevention of fish disease, but they are also effective only against one pathogen and their effect is of short term and they do not contribute to the development of immunological memory (Jeney et al. 2009). The negative effects caused by overexposure to synthetic chemicals led public awareness and search for ‘‘green solutions’’, such as synthetic chemical-free food products like organic one. To enable organic fish production, it is essential to develop antibacterial treatments that are based on materials from natural sources (Abutbul et al. 2004). Immune enhancing activity of certain

medicinal herbs has been known for a long time in traditional medicine (Green 1820). Nowadays, it is thus, being applied in fish culture to replace antibiotics and chemotherapeutic agent. Herbs can replace massive use of synthetic antimicrobials for disease control which has been suppressing growth in aquatic animals and can increase eco-friendly product.

Herbal medicines are known to exhibit anti-microbial activity, and disease resistivity to facilitate growth, and maturation of cultured species (Samad et al. 2014). Besides, under intensive farming the anti-stress characteristics, herbs will be of immense benefit without posing any environmental hazard (Harikshnan et. al. 2003). In recent years, herbal plants serve as a new class of growth promoters that provide an alternative feeding strategy to replace antibiotic growth promoters (Hashemi and Davoodi 2011). Administration of herbal extracts or their products at various concentrations through oral (diet) or injection route enhances the growth performance and innate and adaptive immune response of different freshwater fish, marine fish, and shellfish against bacterial, viral, and parasitic diseases (Harikrishnan et al. 2011).

1.2 Urtica parviflora

Urtica parviflora Roxb**.** (Family-*Urticaceae* - Group Angiosperms -flowering plant) is a perennial and monoecious, herb found in moist and partly shady places of evergreen forest (Kumar et al. 2009, Taylor 2009 and Kumar et al. 2014). There are 46 species of flowering plant of the genus *Urtica* **(**The plant list, 2018). The genus *Urtica* is derived from word '*uro*' to burn or '*urere*' denotation to sting (Grieve 1931). It is native to East Asian countries and grows to an altitude of 1,700-2,800 m from sea level (Pandey et al. 2010, Kumar et al. 2014). It is commonly known as Himalayan stinging nettle and locally as Sisnu. The leaves of the plant have stinging hairs which are responsible for the burning sensation and itching sensation following contact with skin surface. It is actually attributed to presence of histamine and 5-hydroxytryptamine (Pandey 2010). The stinging hairs of nettles are used as anti-itch drugs.

Stinging nettle is powerhouse of nutrients. It contains on average 22% protein, 4% fats, 37% non-nitrogen extracts, 9-21% fiber, and 19-29% ash. It is also rich in fatty acids, carotenoid, and phenolic compounds, while its extracts have been reported to improve oxidative stability in brined vegetables (Rutto et al. 2013).The medicinally importance organic compounds found in *U. parviflora* are phytosterols, saponins, flavanoids, tannins, sterols, fatty acids, carotenoids, chlorophylls, proteins, amino acids and vitamins (Vit. C and α-tocopherol) (Kumar et al. 2009, Krystofova et al. 2010, Kataki et al. and Rafajlovska, et al. 2013).

Young leaves of the plant are nutritious and are cooked as food in the Western Himalayan region. This plant is used traditionally to cure various disorders. Young leaves of the plant are used to cure goitre and associated pain and to cure the allergic disorders such as cold and cough. The leaf extract is used in hair wash and to cure baldness. The fresh leaves

and roots of the plant are applied to cure the dislocation of bones (Malaisse, and Mathieu 2008).

Due to the numerous medicinal uses, it is considered as an important medicinal plant (Panday et al. 2010, Kumar et al. 2014). *U. parviflora* contains several chemical compounds like histamine, serotonin (5-hydroxytryptamine), acetylcholine, malic acid, tryptophan, aspartic acid, serine and tyrosine, alkaloids, flavonoids, phenols, polysaccrides, glycosides and tannins (Rafajlovska et al. 2013). Several studies showed beneficial effects of *U. dioica* against different diseases such as rheumatoid arthritis and diabetes (Nazemi et al. 2012).

The *Urtica* spp has been reported to have various pharmacological activities (Shackebaei, et al. 2010, Rafajlovska et al. 2013) such as rheumatoid arthritis (Riehemann et al. 1999), hypotensive (Tahri et al. 2000), anticancer (Koch 2001), immunomodulatory (Akbay et al., 2003), antioxidant (Mavi, et al. 2004), anti‑inflammatory, antiulcer (Gulcin et al. 2004), natriuretic, hepatoprotective (Kanter et al. 2005), hypoglycemic (Golalipour and Khori 2007), insecticide (Zlatko et al. 2007), cardiovascular effects, hypocholesterolemic (Alisi et al. 2008) anti‑colitis, antiviral (Krystofova et al. 2010) antibacterial, antimicrobial, antifungal, analgesic (Gulcin et al. 2004, Hadizadeh et al. 2009, Kukric et al. 2012, Modarresi et al. 2012) and antiandrogenic (Nahata and Dixit, 2014). Similarly, it helps to lessen the swelling of hemorrhoids and stop bleeding from minor skin injuries (Ebrahimzadeh et al. 2015). *U. parviflora* is one of the finest herbs which contains immunostimulants and is believed to improve the status of blood parameters and increase the growth performance of fish.

1.3 Nile tilapia

Tilapia is the second most farmed group of fish after carp and is common name for a large group of fish species within the family Cichlidae. Tilapia is classified into three genera: *Tilapia, Sarotherodon,* and *Oreochromis* based on reproductive behavior (Teichert-Coddington et al. 1997). Tilapia is widely introduced for aquaculture, with many existing strains. *Oreochromis* subspecies are recognized as: *Oreochromis niloticus baringoensis*, *O. niloticus cancellatus*, *O. niloticus eduardianus*, *O. niloticus filoa*, *O. niloticus niloticus*, *O. niloticus sugutae*, *O. niloticus tana* and *O. niloticus vulcani* spp (Froese and Pauly 2019).

Oreochromis niloticus is identified by a large deep-bodied and relatively small head (Genner, et al. 2018) and the presence of regular vertical stripes throughout depth of caudal fin (Eccles 1992, Teugels and Audenaerde 2003). Males are bluish pink, sometimes with a dark throat, belly, and anal and pelvic fins (Genner et al. 2018). However, females are usually brownish, silvery/white beneath with around 10 thin vertical bars (Genner et al. 2018). The body coloration is varies depending on environmental, physiological, and dietary factors (Little and Macingtosh 1995).

Nile tilapia (*O. niloticus*) is mainly the freshwater fish inhabiting shallow streams, ponds, rivers, and lakes. It tolerates brackish water and survives in temperatures between 8 to 42 °C (46 to 108 °F) (Froese and Pouly 2015). It is an omnivore that feeds on both plankton and aquatic plants. They are macrophyte-feeders, feeding on a diverse range of filamentous algae and plankton. Likewise, they are efficient feeders as they can capture and process a wide variety of food items (Loiselle 1994). Nile tilapia is a mouth breeding species. They can be used as biological control for duckweed and mosquito (Cross 2013).

Nile tilapia is native to Africa and has been a major source of protein in many developing countries. It is cosmopolitan due to its aquaculture potential, thus, since 1950s, it has been a choice from many years for including in the tropics and subtropics (Pullin and Maclean 1992). Nile tilapia is exotic in the context of Nepal. At first, it was introduce in 1985 in some government farms of Nepal (Panth 1993). Among four popular culture species like Nile, Blue, Red, and Mozambique, only Nile and Blue Tilapia were introduced in Nepal for the first time from Thailand in 1985 (Shrestha 1994). Though there is general fear of displacement of indigenous fish species. They have played a critical role on food security in Nepal.

Nile tilapia contain low saturated fat, calories, carbohydrates and sodium, and are good source of protein. They also contain the micro nutrients phosphorus, niacin, selenium, Vitamin B_{12}, and potassium. Multiple studies have evaluated the effects of adding flaxseed derivatives (a vegetable source of omega-3 fatty acids) to the feed of farmed tilapia.

O. niloticus is the most important fish species in aquaculture due to their fast growth rate, easy reproduction, resistant to disease, and poor water quality adaptability to a wide range of culture condition, and high consumer acceptability (Little and Macingtosh 1995, Castro et al. 2011, Ferreira et al. 2011). They are considered as aquatic chicken as they contain less Food Conversion Ratio (Pillay 1999 and Fitzsimmon, 2000). But, due to their prolific breeding behavior and lack of monosex production technology, it is being problematic to distribute among farmers in Nepal (Shrestha 2004).

Nile tilapia can be cultured in pens, cage, and earthen ponds. As they feed low on the food chain and accept a wide range of feeds, resistant to poor water quality, disease and handling, good flesh quality and fingerlings easy to produce year round, they are fruitful to cultivate (FAO 2016). The climatic condition of hilly region can control to extend the overbreeding activity of fish as they can't tolerate minimum temperature (8-11ºC). With the help of sustainable culture of Nile tilapia, Nepal could reduce food deficiency and improve the economic condition of farmer, thus Nile tilapia culture is interconnected with public health and economy. Annual world production of tilapia is 5.57 million tons in 2016 (FAO 2016). At 1.3 million tons per annum, China is the largest tilapia producer in the world, followed by Egypt with 0.5 million (FAO 2016).

1.4 Fish feed ingredients

Fish feed ingredients must contain high nutrition. Fish production system essentially depends upon good nutrition for economic production of healthy and high quality product. Fish nutrition has advanced dramatically in recent years with the development of new balanced commercial diets that promote optimal fish growth and health (Craig and Helfrich 2002). To take the advantage of aquaculture fish feed is one of the key factors. In developed country, readymade feed is available for aquaculture sector. But, developing countries like Nepal where aquaculture is in growing phase, there is no readymade feed, and locally available fish are utilized as fish feed. In commercial fish farming, nutrition is crucial factor because it represent 40-59 % of product costs (Craig and Helfrich 2002).

Fishes are species specific; they consume various types of feed with various feeding behavior depending on species. Thus, some are herbivorous, some are carnivorous and some are omnivorous. Their feed could be originate from plant, animal, both or biowaste. In modern aquaculture system, fishes are fed with supplementary feeds along with natural food for maximum yield. The supplementary foods are made according to the species reared. Some of the by-products like poultry, animal husbandry, fish farm, and waste food materials have been utilized as the alternative sources of fish food. Various feed stuffs of plant and animal origin are being used to make supplemental feed for different fish. Fish feed ingredients can be categorized in the following subsections:

1.4.1 Ingredients of plant origin

The type of fish feed are specific according to the species. Various plant origin ingredients have been used in fish feed in order to evaluate their efficiency to promote growth performance, enhance health, and replace animal stuffs such as fodder plants, oil cake and oil meals, aquatic weeds (El – sayed 1999), luecaena leaf (Wee and Wang 1987), legume seed (Keembiyehetty and Gatlin 1993) roof and tubers (Tusan et al. 2013), and cereal and cereal by-products (Santis et al. 2016). Among them, mustard oil cake, soybean, rice barns, maize and wheat flour are mostly used ingredients.

1.4.2 Ingredients of animal origin

Animal origin ingredients are comparatively rich in protein, essential amino acids, especially lysine and methionine that are limiting in plant sources. These include fresh and dry meat of various fish (Casteneres 1990), blood, earthworms (Hilton 1983, Rawling et al. 2012), and insects (Fasakin et al. 2003). But they are expensive in comparison to plant origin feed.

1.4.3 Ingredients in biowaste origin

The biowaste origin of the ingredients include fish silages, milk by products, kitchen by-products, and slaughter house by-products (Cheng et al. 2012) which are regarded as the wastes and are supplemental feeds in aquaculture to reduce the feed expenses. They are beautiful tools of practicing biological waste management.

1.4.4 Miscellaneous feed ingredients

They include cane molasses, brewer's yeast, leaf protein concentrate, and grain distiller by products, algae, yeast, and bacterial protein (Ran and Zhou 2016).

1.5 Objectives

1.5.1 General objective

- To investigate the effects of herbal plant *Urtica parviflora* Roxb**.** on the growth performance and hematologic parameters in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758*).*

1.5.2 Specific objectives

- To determine the Specific growth rate (SGR), Feed conversion ratio (FCR), Survival rate (SR), Feed efficiency ratio (FER), Percentage weight gain (PWG), Condition factor (k), Feed intake (FI) and Economic conversion ratio (ECR) of Nile tilapia fed with different proportions of *U. parviflora* diet.
- To evaluate the Different leucocyte count (DLC), White blood cells (WBCs), Red blood cells (RBCs), hemoglobin and platelets of Nile tilapia fed with different proportions of *U. parviflora* diet.

1.6 Rationale of the study

Recently, a growing interest has emerged in using herbs in animal feeds by both researchers and feed companies as they are eco-friendly, easily accessible and contain economic value (Harikrishnan et al. 2011). Herbs are been used in various countries to control shrimp and fish diseases, and successful results have been reported in India, and Japan (Dey and Chandra 1995, Logambal and Michael 2000). For instance, aqueous extract of *Azadirachta indica* (neem) leaf has shown a positive result when used against *Aeromonas hydrophila* infection in common carp (Harikshnan et. al. 2003). Uses of *U. parviflora* in fish food is consider as one of the best solutions of aquaculture problem. *U. parviflora* has been related as a primary diet of fish but how this plants affects the growth, survival and various hematologic values is not completely known. Thus, this study has been conducted to analyze its effect on growth performance and hematologic parameters.

1.7 Research Hypothesis

The uses of *U. parviflora* on the fish feed, cannot be useful to enhance the growth performance and immune response (Hematologic parameters) of Nile tilapia.

LITERATURE REVIEW

Aquaculture is one of the fastest growing food-producing sectors around the world (Harikrishnan et at. 2011). In fish, nutrients like proteins, essential fatty acids, vitamins C and E, polysaccharides, and some minerals possessed critical role for the activities of effective functions (Barrows 2007). Aquaculture contributes food to world and due to the rapid growth of human population, improvement in this sector is necessary (Born 1999). There has been effect of fish meal quality on growth, feed efficiency, and protein digestibility (Aksnes 1997).

But disease and lack of suitable fish feed are main problem in aquaculture. The use of antibiotics is popular (Grondel et al. 1987*)*. However, there are many problems associated with the use of antibiotics in aquaculture, such as the residues of antibiotics left in the tissue of treated fish, the generation of antibiotic-resistant bacteria and the imbalance of the normal beneficial intestinal flora (Subasinghe 1997).

Indeed food producing animals are one of several potential sources of antibiotic resistant bacteria which may spread from animals to man via the food chain (Harikrishnan et al. 2011). The use of antibiotic therapy is also under criticism due to the potential for enhanced microbial resistance and accumulation of residues in tissues of fishes (Chevassus and Dorson, 1990). Similarly, there are detrimental side-effects associated with the over use of chemicals to treat disease, such as effects on public health associated with residual contaminants in the flesh and environmental hazards associated with waterborne applications (Harper 2002). The pathogens may also transfer their antibiotic-resistance genes into human pathogenic bacteria thus posing a threat to human health and environmental problems (Abutbul et al. 2004, Cabello 2006), leading to failure of antibiotic treatment in some life-threatening conditions which limits the use of traditional therapeutic substances (Miranda and Zimelman 2001, Radu et al. 2003).

To treat disease by supplemented feeding (with herbal extract), rather than painful vaccination and resistant drugs has been one of the best options for farmers of the third world. Most of the herbal plants have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. Herbal medicines are known to exhibit anti-microbial activity, anti-stress activity, facilitate growth, and maturation of cultured species without including environmental hazard (Harikshnan et al. 2003). Administration of herbal extracts or their products at various concentrations through oral (diet) or injection route enhance the innate and adaptive immune response of different freshwater fish, marine fish, and shellfish against bacterial, viral, and parasitic diseases (Harikrishnan et al. 2011). The accumulation of chemicals in the environment and in the fish have led to the imposition of stringent regulations that limit the use of antibiotics and a number of chemicals (Alderman and Hastings 1998, Treves-Braun 2000) that are harmful to the environment as well as consumers (Smith et al. 1994). The massive use of

synthetic antimicrobials for disease control has been suppressing growth in aquatic animals.

2.1 Effect of Supplementary Feeds and Herbal Plants on Growth Performance and Hematologic Parameters

Legume seeds could replace about 33% of fish meal on trial of sixteen isocaloric diets containing one of the three protein levels i.e. 20, 25 and 30% made by partial and total replacement of fish meal with legume seeds Vigna catiang (cowpea) and Phaseolus mungo (black gram) on Nile tilapia fingerlings without any adverse effect on growth rate (De- silva and Keembiyehety 1993).

Shalaby et al. (2006) investigated that Garlic (*Alliumsativum*) and chloramphenicol diet can significantly increase SGR, PER, and decreased FCR on Nile tilapia. Similarly, can significantly increase blood parameters like, RBC, hemoglobin content where there was no difference in mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of Nile tilapia.

Chinese herbs like Astragalus has ability to enhance the immune system. Analysis shows that *A. radix* contains polysaccharides, monosaccharides, flavonoid and alkaloid, together with choline, betaine, folic acid, various amino acids, mucoitin, gum, cellulose, and 14 trace minerals, including selenium, zinc, and iron, which are essential micronutrients for man and animals(Wang and Bourne S 1999). Yin et al. (2006) performed experiments with tilapia showed that *A. radix* had a positive influence on the immune system by acting as a booster.

Nile tilapia fed with dehulled solvent extract of soybean meal and expeller pressed soybean meal as alternative of fishmeal showed no significance difference in final mean weight, survival rate and feed conversion ratio (Nguyen and Allendavis 2009).

Immanuel et al. (2009) reported medicinal plants *Cynodon dactylon*, *Aegle marmelos, Withania somnifera,* and *Zingiber officinale* can increase growth performance, protein, albumin, globulin, cholesterol, glucose, triglyceride levels, Leucocrit value, phagocytic index and lysozyme activity in *O. mossambicus*.

Keri et al. (2011) describes the Length -Weight relationship (LWR), relative condition factor (k) and dietary maltose of the Nile tilapia fingerlings (*O. niloticus*) to determine the growth pattern. There was significance correlation between length and weight, while condition factor was computed near by 1.62 to 1.79 which indicated good health condition during the experiment and it is indicating an isometric growth, which is the desirable for fish of fish farm.

Ginseng is an immunomodulator, containing herb that can produced several cytokines (TNF-α, IL1β, IL2, IL6, IFNγ, GMCSF) and stimulate lymphoid cells to proliferate (Tan and Vanitha 2004) and enhance both B and T-cell mediated immune responses (Yun et al.

1993). *Echinacea* is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses (Meshaly et al. 2008). Sabry et al. (2014) reported that diet of *Ginseng and Echinacea* extract growth performance, IgM, globulin, total leukocyte count, NBT, and lysozyme in *Oreochromis niloticus.*

Verbascum, plant of the family *Scrophulariaceae*, is one of plant about 323 species globally (Akdemir et al. 2011). The species are also used to treat haemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea, and have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B (Akdemir et al. 2011). Measuring the immunological and biochemical parameters in fish species treated with herbal derivatives may be a good method to evaluate the possible effects of an herbal drug on fish health (Faggio et al. 2014). Katayoon Nofouzi et al. (2016) investigated *Verbascum speciosum* supplementation significantly enhanced the growth performance, Serum complement, total antibody, haemagglutination titer, and lysozyme activities.

Imanpoor and Roohi (2016) reported that Sangrovit (0.05%) increase in final weight, weight gain, and specific growth rate, and total protein, and decreased food conversion ratio, blood glucose and cholesterol levels.

Dietary supplementation with essential oil of clove, basil, and ginger was useful for improving growth, immune response and disease resistance with significant difference in thrombocytes, total leucocytes, lymphocytes and neutrophils (Brum and Martins 2017).

Diet containing different levels of supplemental glutamine and/or arginine fed to juvenile Nile tilapia showed significant effects on weight gain, feed intake, feed efficiency ratio and protein efficiency ratio. Diet with combine supplement of glutamine and arginine at 1% gave more improved growth performance than diet supplemented individually with glutamine and argenine (Pereira and Gatlin 2017).

Inosine monophosphate (IMP) enhance cellular muscle growth and up regulate growth-related gene expression in juvenile Nile tilapia having potential feed additive and supplementation of $\geq$ 0.2% IMP for better growth enhancement. It showed IMP supplementation enhances muscle growth by promoting hyperplasia and hypertrophy, it up regulate the growth related gene expression in Nile tilapia Juvenile (Asaduzzaman and Kedar 2017).

Dietary spray-dried plasma supplementation mainly at the level of 51.83% g/kg enhances growth performance, intestinal health, and hematologic profile and could induced stress resistance in Nile tilapia. It affects leucocytes, lymphocytes and neutrophil counts (Araujo and Barros 2017).

Leya et al. (2017) investigated curcumin diet can increase percentage weight gain, feed efficiency ratio, feed conversion ratio, specific growth rate and protein efficiency ratio in *Cirrhinus mrigala* and showed significantly higher total immunoglobulin, serum

protein, serum albumin, Immunoglobulin, respiratory burst activity, and myeloperoxidase activity.

Different oil sources with 32% CP diet and isoproteic isoenergetic 3,300 kcal DE kg-1 each implemented with a different oil source T1: linseed oil (OL), T2: soy oil (SO), T3: fish oil (OF) and T4: corn oil (CO) used to feed breeding males of Nile tilapia (*Oreochromis niloticus*) shown that, no significant difference for the percentage of red cells, mean corpuscular volume, hematocrit, and mean leukocyte percentage. However, for the mean corpuscular hemoglobin concentration (CHCM) and hemoglobin, fish fed soybean oil presented significantly higher values than other treatments. The soybean oil, characterized by iron with a high bioavailability, led to higher levels of hemoglobin and CHCM in Nile tilapia, which possibly can reflect a greater oxygenation in fish. However, the fish oil originated greater values of both components of the blood (hematocrit, red cells) and immune (thrombocytes, monocytes, neutrophils and lymphocytes) system (Navarro et al. 2018).

2.2 Significance of *Urtica* Spp in Health Status

The hydromethanolic extract of *U. parviflora* protect the myocardium by decreasing the elevated level of malondialdeyde, elevating the diminished levels of glutathione, superoxide dismutase, catalase, and high density lipoprotein, with a concomitant decrease in the elevated levels of low density lipoprotein, and Triglyceride (Hooker Dalton 1890). It protect the cells from injuries caused by reactive oxygen species (Pandey et al. 2010). Similarly, hydroethanolic extract of *U. parviflora* significantly reduced the increased activities of aspartate aminotransferase, alanine transaminase, alkaline phosphatase, creatine phosphokinase and lactate dehydrogenase (LDH) (Hooker Dalton 1890).

The leaves and roots of plant are used internally as a blood purifier, emmenagogue, diuretic nasal and menstrual haemorrhage, rheumatism, eczema, anaemia, nephritis, haematuria, jaundice, menorrhagiaand diarrhea (Wetherilt 1992, Tucakov 1997, Khare 2007).

The orally administered extract of *U. parviflora* could reduce elevated levels of aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total biliruvin, and serum protein in rat (Bomber 2009).

Soner et al. (2016) identified methanolic extract of *Pleurotus ostreatus* and *U. dioica* increase growth rate, feed conversion ratio, phagocytic activity, lysozyme activity, and Nitroblue tetrazolium (NBT) activity in *Oncorhynchus mykiss.*

Sara et al. (2018) reported, *U. dioica* decreased CD4- CD8- lymphocytes and increased CD4+ CD8+ lymphocytes in the thymus, while in the blood, it increased CD4+ cells, monocytes and total T lymphocytes in malnutrition rat.

It was also reported that *Urtica spp* prevents the damage of liver tissue structure in rats (Turkdogan et al. 2003). Similarly, *U. dioica* has been shown to have a protective effect

against hepatic ischemia-reperfusion (Kandis et al. 2010), hyperglycemia (Otles and Yalcin, 2012), and hyper-cholesterolemia (Nassiri- asl et al. 2009). Supplementation of *U. dioica* leaves beverage have a significant protective effect against TCA-induced liver injury (Celik and Tuluce 2007).

Although, *Urtica Spp* is used for some *in vivo* and *in vitro* experiments, there is little evidence for the effect of *U. parviflora* on fish diet and health, and there is not any report about the effect of *U. parviflora* on growth performance and hematologic parameters in *O. niloticus*. Similarly, though there are many other plant and animal originate fish feed they are not easily accessible and neither economic. Therefore, the aim of the present study was to investigate the effects of *U. prviflora* on growth performance and hematologic parameters in *O. niloticus.*

MATERIALS AND METHODS

3.1 Experimental Site

The study was carried out in Central Department of Zoology (CDZ), TU, Kirtipur Nepal, and Nepal Academy of Science and Technology (NAST), Khumaltar, Kathmandu, Nepal.

3.2 Materials

3.2.1 Experimental setup apparatus

a) Air-pump
b) Aluminum foils
c) Aquarium (60cm×45cm×38cm)
d) Aquarium Filter
e) Aquarium Heater
f) Aquarium thermometer
g) Buckets
h) Extension cord
i) Filter tubes
j) Fish handling net
k) Ingredients (fish feed)
l) Methylene blue
m) Oxygen plastic bag
n) Polyester threads
o) Siphoning tube
p) Water supply pipes

3.2.2 Analytic apparatus

a) Ammonium, nitrate and nitrite kit
b) Beakers
c) BOD bottles (300mL)
d) Burette
e) Conical flask (100mL)
f) Container for anesthetic bath
g) Droppers
h) Eppendorf tubes
i) Ethylene Diamine Tetra Acetic Acid (EDTA) vials
j) Gloves
k) Ice cubes
l) Manual counter
m) Measuring cylinder

n) Pipettes (5mL, 1000µl, 100µl)
o) RBC pipette (BLAUBRANO, GERMANY)
p) Slides and its coverslip
q) Slide box
r) Soft towels
s) Stand
t) Sterile cotton swab
u) Syringe (1ml)
v) WBC pipette (BLAUBRANO, GERMANY)

3.2.3 Instrumental apparatus

a) Ammonia, Nitrate and Nitrite kit
b) Blood transportation box
c) Compound Microscope (OPTIKA MICROSCOPE ITALY)
d) Hemocytometer with Neubauer grid (MARIENFELD, GERMANY, 0.0025mm^2)
e) Hemoglobin tube
f) Hydrogen ion concentration (pH) meter
g) Microscopic Camera (OPTIKA HDMI easy camera 1280×960)
h) Refrigerator
i) Sahli's hemometer
j) Steel scale (30cm)
k) Thermometer
l) Weighting balance

3.2.4 Chemicals for water quality test

a) Concentrated Sulfuric acid (H_2SO_4)
b) Erichrome black-T indicator
c) Hardness buffer
d) Hydrochloric acid (HCl 0.1N)
e) Manganese sulphate ($MnSO_4$. $4H_2O$)
f) Methyl orange
g) Patassium Iodide (KI)
h) Phenolphthalein
i) Sodium carbonate (Na2CO3, 0.1N)
j) Sodium EDTA
k) Sodium hydroxide (NaOH, 0.05N)
l) Sodium sulphite
m) Sodium Thiosulphate ($Na_2S_2O_3.5H_2O$)
n) Starch (Indicator)

3.2.5 Chemicals for Hematology test

a) Ammonium oxalate reagent
b) Distil water
c) HCl (0.1N)
d) Immersion oil
e) Leishman's stain
f) RBC diluting fluid (STANBIO REAGENTS (P) LTD)
g) WBC diluting solution (STANBIO REAGENTS (P) LTD)

3.3 Experimental Setup and Fish Collection

This study was assigned to evaluate the effects of *Urtica parviflora* formulated feed on growth performance, feed utilization efficiency, and hematological parameters in Nile tilapia (*Oreochromis niloticus)* in CDZ and NAST, Kathmandu, Nepal.

A total of 150 fingerlings of *O. niloticus* were collected from Agricultural (Fisheries) research Centre, Begnas Lake, Pokhara on 23rd October 2018. The stock were transported to the CDZ laboratory in oxygenated plastic bags. Upon arrival, they were kept in 2% potassium permanganate solution for three to 5 minutes as prophylactic measures. Fishes were stocked in 3 tanks (60cm×45cm×38cm=98-100L water), 40 in each. All tanks were maintained at 22^{0}C by using aquarium heater. Fishes were fed twice a day with commercial feed containing 35% CP. After acclimatization of 40 days (2nd December) fishes were transferred to experimental tank.

Experiment was conducted for 90 (2nd December-5th March) days in five different tank with size of (60cm×45cm×38cm). A total of 50 fingerling with an average body weight of 12g and 8cm of body length were assigned at randomly to five treatment diets with, 10 fingerlings in each tank. 45 fishes were tagged by using polyester thread in their caudal fin for individual identification. During experiment, 3% of body wt. of fishes was feed twice every day. The control group was fed with diet T_o (without nettle powder) and the experiment groups T_1, T_2, T_3, and T_4 were fed with diet containing nettle powder 1%, 5%, 10%, and 15% respectively. To optimize water quality, removal of waste and uneaten food were siphoned out and about one third water was changed daily.

3.4 Ingredients Collection, Analysis and Control Feed Composition

Ingredients (fish meal, soybean meal, mustard oil cake, wheat, and rice bran) were collected from fish food factory, Balaju. Crude protein, moisture, fat, ash, and fiber of ingredients were analyzed by using feed analyzer- ANTARIS II DR (FT-NIR Analyzer) India. Four different experimental diet containing 35% CP were prepared by using Pearson's square Method. [At first, ingredients were divided into two types according to their crude protein like a) Basal feeds CP <20 (rice bran and wheat) and b) Protein Supplement CP >20 (fish meal, mustard oil cake, and soybean meal). Then, fish feed was decided to prepare of 35% CP (according to FAO 26-36% feed is suitable for tilapia

fingerlings). Crude protein content of formulated feeds were analyzed by ANTARI II DR (FT-NIR Analyzer), India.

Table 1. Proximate composition of different feed ingredients:

Ingredients	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)
Fish meal	45.6	12.3	15.50	3.81	1.79
Mustard oil cake	34.41	8.29	11.83	13.36	1.11
Soybean meal	34.11	10.69	12.32	7.17	1.06
Wheat flour	10.04	12.36	6.51	7.24	1.65
Rice bran	13.3	9.96	6.52	16.13	2.23
Stinging nettle	22. 3	17.58	15.41	-5.27	1.77

Table 2. Diet composition for control feed:

Ingredients	**Percentage (%)**	**Crude Protein (%)**
Rice bran	6	0.798
Fish meal	28	13.68
Soybean meal	40	13.64
Mustard oil cake	20	6.89
Wheat flour	6	0.6
Total	100	35

Finally, the above composition (without mixing nettle powder) was consider as control diet for fish group T_0 and stinging nettle powder(1% for T_1, 5% for T_2, 10% for T_3 and 15% for T_4) was added on this composition and each composition was fed to experimental fish group like T_1, T_2, T_3 and T_4 respectively.

3.5 Collection and Identification of Herb and Proximate Feed Preparation:

U. parviflora was collected from Shantiban (27º 36´ 52" N, and 85º 21´ 56" E) Kathmandu. The spp was authenticated by Ganga Datta Bhatta, Department of plant Resources and National Herbarium and Plant Laboratories (NHPL/ KATH), Nepal**.** A voucher specimen was deposited in the Department of plant Resources and National Herbarium and Plant Laboratories (Accession No: 137909). The tender tops were harvested with gloves and scissor. The collected tender tops were then shade dried, on plastic sheet in room temperature. After the twigs get dried it was grinded (150 µm mixture) to fine powder. The final powder was stored in plastic bag, air tied, and was stored in refrigerator. Stored powders were added to the different treatment groups like 1%, 5%, 10%, and 15% concentrations. The proximate compositions of each of the feed were carried out in accordance with Pearson Square Method.

Table 3. Composition of different treatment feed fed to different experimental tilapia fingerlings:

Ingredients	Control(T_0)	Treatment 1 (T_1)	Treatment 2 (T_2)	Treatment 3 (T_3)	Treatment 4 (T_4)
Rice bran (%)	6	6	6	6	6
Fish meal (%)	28	28	28	28	28
Soybean meal (%)	40	40	40	40	40
Mustard oil cake (%)	20	20	20	20	20
Wheat flour (%)	6	6	6	6	6
Stinging nettle powder (%)	0	1	5	10	15

3.6 Feeding Experiment

The experimental fish were fed at 3% of their body weight per day for three months continuously. Pallet was prepared before feeding (twice every day). Feed for next period was adjusted according to 15 days interval weight sample. Feeding was done twice, in the

morning at 11.00 am and in the evening at 04.00 pm. To optimize water quality, removal of waste and uneaten food were siphoned out and 1/3rd water was changed daily.

3.7 Water Quality Parameters and Analysis

During experimental period, water parameters were tried to maintain as suitable for *O. niloticus*. All tanks were maintained at 22° C by using aquarium heater. Aeration and filtration was supplied by sponged filter. To optimized water quality, removal of waste and uneaten food was siphoned out and 1/3rd of water was changed daily. The water quality variables: DO, CO_2, alkalinity, acidity, hardness, ammonia, nitrate, nitrite and pH were recorded

3.7.1 Temperature

Temperature was measured by using aquarium thermometer and mercury thermometer.

3.7.2 Dissolved Oxygen (DO)

To determine the dissolved oxygen of water, 300 mL of sample water was filled in Biological Oxygen Demand (BOD) bottle. Immediately, 2 ml of manganese sulfate was added. Again 2 ml of alkali-iodide-azide was added on the same manner and sample was mixed by inverting BOD bottle the several times and was let it for settle. Then, 2 ml of sulfuric acid was added in the same sample. After that, 100 ml of water sample was transformed into the conical flask and 2 ml of starch solution was added to form blue color. Finally, sample was titrated against sodium thiosulfate, till colorless and total consumed sodium thiosulfate was noted. The value obtained by this method was calculated by as follows using follows:

$$Dissolved\ Oxygen\ (DO) = \frac{V \times N\ of\ titrant \times 8 \times 1000}{v2(\frac{v1-v}{v1})}$$

Where, V= volume of $MnSO_4$ + KI

V_1= volume of BOD bottle

V_2= volume of sample taken

3.7.3 Free- carbon Dioxide (CO_2)

To determine the free CO_2, 100 ml of sample was taken in a conical flask and few drops of phenolphthalein indicator was added. This sample was titrated against 0.05N of NaOH until the slight pink end point was recorded. Calculation was done by using the following equation:

$$Free\ (CO2) = \frac{(V \times Normality\ of\ NaOH \times 1000 \times 44}{V}$$

Where, V = Volume of water sample taken

3.7.4 Hardness

The total hardness of water was estimated by titrating the water sample against EDTA using Eriochrome Black -T (EBT) indicator. 50 ml of sample was taken in a conical flask and 2 ml of buffer solution was added in it. The solution was shaken till the color change wine red and then titrated with EDTA until the color of the solution changed to blue. The volume of EDTA consumed was recorded. Total hardness was calculated by using following equation:

$$Total\ hardness\left(\frac{mg}{l}\right) = \frac{EDTA\ used\,(mL)\times 1000}{sample\ used\,(mL)}$$

3.7.5 Acidity

Acidity was determined by titration method. A total of 100 mL of sample was taken in a conical flask and 1 ml of methyl orange indicator was added on it. Then yellow color was appeared which means that the absent of methyl orange acidity. Then, a few drop of phenolphthalein was added in the solution. This solution was titrated against 0.05 N of NaOH until the slight pink end point was detected. Calculation was done by using the following equation:

$$Acidity = \frac{(V\times Normality\ of\ NaOH\times 1000\times 50)}{sample\ taken\ (mL)}$$

3.7.6 Alkalinity

To determine the free Alklinity, a total of 100 ml of sample was taken in a conical flask and a few drops of phenolphthalein indicator was added. There was not formed any color which indicate that absence of phenolphthalein alkalinity. Then, few drops of methyl orange were added on the same sample. This sample was titrated against 0.1 N of HCl until the orange color end point was recorded. The following equation was used to calculate alkalinity:

$$Alkalinity = \frac{(V\times Normality\ of\ HCl\times 1000\times 50)}{sample\ taken(mL)}$$

3.7.7 Hydrogen ion concentration (pH)

The pH was measured by using a calibrated pH meter (HI 98107, HANNA Instrument).

3.7.8 Ammonia, Nitrate and Nitrite

Ammonia, Nitrate, and Nitrite were measured by using API FRESHWATER MASTER TEST KIT.

3.8 Fish Sampling Procedure and Analysis Growth Parameters

Length, weight, and water quality parameters were measured at an interval of 15 days of the experimental period. Prior to the experiment, all the 50 experimental fishes from different treatment group were captured with scoop net and their individual length and weight were recorded to the closest cm and closest gram. A steel measuring scale and sensitive electronic balance was used for measuring the lengths and total body weight of individual fish. This experiment was conducted at the Central Department of Zoology. Experimental data collected during the trial was used to determine the SGR, FCR, SR, FER, PER, WGP, FI, k, and ECR by using various formulas:

1. $\text{Specific Growth Rate(SGR)} = \frac{\text{In (FMW)} - \text{In(IMW)}}{\text{Culture period (days)}} \times 100$

   Where, FMW= Final mean weight of fish

   IMW= Initial mean weight of fish

2. $\text{Feed Conversion Ratio(FCR)} = \frac{\text{Quantity of feed intake (g)}}{\text{Net weight gain(g)}}$

3. $Survival\ Rate\ (SR\ \%) = \frac{Total\ harvested\ number}{Total\ stocked\ num\ ber} \times 100$

4. $Feed\ Efficiency\ Ratio(FER) = \frac{1}{FCR}$

5. $\text{Weight Gain Percentage(WGP)} = \frac{\text{Final weight gain(g)} - \text{initial weight gain (g)}}{\text{Initial weight gain (g)}} \times 100$

6. $\text{Fulton's Condition factor (k)} = \frac{\text{Body weight (g)}}{\text{(Total length) 3}} \times 100$

7. $\text{Feed Intake(FI)} = \frac{\text{Total weight diet offered offer in a given period}}{\text{No.of survival fish}} \times 100$

8. ECR = FCR × feed cost

9. Length-weight relationship

The length-weight relationship can be used as an indicator of fish condition (Froese 2006). To study the length weight relationship, the standard length (L) and total weight (W) were observed to find out the variations from the expected weight of an individual fish with a specific length in order to ascertain the pattern of growth and standard wellbeing, of the fish. The data of length weight relationship was analyzed by the following the Le cren's (1951) method.

$W = a\ L$

$\text{Log } W = \log a + b \log L$

Where, W = total weight

L = total length

a = coefficient related to body form

b = exponent indicating isometric growth

$a = \bar{y} - b\,\bar{x}$

$$b = \frac{\Sigma xy - \frac{[(\Sigma x)-(\Sigma y)]}{n}}{\Sigma X2 - \frac{(\Sigma x)2}{n}}$$

When b=3 isometric or allometric growth,

b < 3 negative allomeric growth and when b >3, positive allomeric growth.

3.9 Blood Collection and Analysis

Blood samples were obtained from all the 50 experimental fishes from different treatment group fish at the end of experiment (after 90 days). Fish were anesthetized dipping them in ice cubes box container, for collection of blood samples for further hematological studies, around 500µL blood samples were drawn from the cardiac vein puncture of each fish using 1 mL insulin syringe, and was collected in EDTA containing vials. Blood samples were stored in the refrigerator at 4º C in NAST ARL for hematologic parameter analysis.

Hematologic values were determined by various methods. Blood sample was transferred to the ARL in EDTA vials in a blood transportation box. Differential Leukocyte Count (DLC) analysis was done using Leishmann staining technique as described by Cheesbrough (2000) with slight modification. Total Leukocytic Count (TLC) was performed using the improved Neubaur chamber, Natt and Herrick's solution as diluting fluid and 1:100 diluted blood according to the Oliveira et al. (2008). Determination of Red Blood Cells count (RBC) and platelets was performed using Neubauer hemocytometer, as described by Oliveira et al. (2008). Hemoglobin was measured using Sahli's Hemoglobinometer.

3.9.1 DLC Method

DLC is important for the diagnosis of various blood related disorders. Generally, it is performed to check the normal number or distribution of different leucocytes. It also gives the morphology of different cells. There are three major steps involved in differential cell count:

a) Preparation of blood smear

Clean grease free slide was taken and about 5 µL of blood from EDTA vial was obtained and placed it on a corner of a slide. With the help of spreader, a very thin smear was prepared on the slide. Blood film was about 1 cm from the edge of slide and 5 mm in width. The angle between spreader and slide was about 45°.

b) Staining of Smear

The dry blood film was then covered with Leishman's stain drop by drop. The stain was eventually distributed over the entire smear, and left for 1 - 2 min (fixing). Then, distill water was added drop by drop over dry smear and was well mixed. After 10 min, slide was dried (air dried) and observed under 100× microscope using OIO.

c) Microscopic Examination:

First stained blood smear were examined under, low power objective. The background color and distribution of cells were noted. In an ideal staining smear, three zones can be identified: hick area or head of smear, the central area is body and at the end of smear is tail region.

The portion of the smear in the body region, slightly before the tail end was chosen. The slide was observed under oil immersion objective (×100) by putting a drop of oil over the slide.

Each type of white cell observed was counted. The observations was counted on a piece tabular form. The cell counter has different keys for different types of WBC. A total of 100 cells were counted with Leishman stain, the cells were observed as:

1. Lymphocyte

Dark blue nucleus with light blue cytoplasm was observed as lymphocyte.

$$Lymphocyte(\%) = \frac{Total\ No.of\ lymphocyte\ Observed}{Total\ no.of\ cell\ counted} \times 100$$

2. Neutrophil:

Purple colored nuclei with pink cytoplasm was detected as neutrophils.

$$Neutrophil(\%) = \frac{Total\ No.of\ Neutriphils\ Observed}{Total\ no.of\ cell\ counted} \times 100$$

3. Monocytes:

Monocyte were the pink cytoplasm with purple color nucleus.

$$Monocyte(\%) = \frac{Total\ No.of\ Monocyte\ Observed}{Total\ no.of\ cell\ counted} \times 100$$

3.9.2 WBC Counting Method

WBC are the effector cells of the immune system and circulate throughout the bloodstream and lymphatic system. An infection or a physical injury results in either increase or decrease of WBC depending on etiology of infection. Thus, WBC count is a valuable metric for diagnosis and prognosis of several diseases.

The coverslip was put on the top of grid area in the Neubauer Chamber. Around 5 µl blood sample was taken in Eppendorf with the help of micropipette and was diluted with around 100 µl WBC diluting solution, was left for 5 min. The sample was load into the loading area in the chamber. The cells were counted in the 4 large squares under objective (40×) microscope. While counting cells, 90° lines was chosen. The cells of those lines (eg: L-shape) were counted only in all squares for maximum accuracy.

$$Total\ WBC\ count\left(\frac{Cell}{\mu L}\right) = \frac{No.of\ cells\ in\ 1\ large\ square\ counted \times dillution\ factor \times}{Volume\ factor(0.1)}$$

Dilution factor= reciprocal of dilution (20)

Volume factor = (width x length x height) = 0.1 (Neubauer's chamber)

3.9.3 RBC Counting Method

First, the glass slide was cleaned with cotton and alcohol. It was allowed to air dry, and the cover slip was cleaned. RBC dilution fluid was taken immediately. This was shaken vigorously for 2 min. Initial two drops of this dilution fluid was discarded. The drop of sample was taken on the Neubauer Hemocytometer. Then, it was covered with coverslip and mounted it on the stage. First, it was observed under the low power (10×) and adjusted it and then, switched to high power (40×). The number of RBC was calculated only in 5 small boxes and summed up those number. Hence, the total number of RBC in 80 squares was calculated. The total number of Red blood cells present in a unit blood by given formula:

$$Total\ RBC\ count\left(\frac{Cell}{\mu L}\right) = \frac{cell\ counted \times dillution\ factor \times 106}{0.2 \times 0.2 \times 0.1 \times 10}$$

3.9.4 Platelets Counting Method

A total of 380 µl filter ammonium oxalate reagent was measured and dispensed into a small tube. 20 µl of blood in tube was added and mixed properly. A coverslip was put on counting area of Neubaur chamber and the mixed solution was added in chamber and avoid over filling. The chamber was left for 20 minutes and kept in petri-dish and it was closed to avoid drying and contamination. After 20 minutes the chamber was kept under microscope. Platelets was observed at objectives (40×) microscope. The platelets was counted in 5 small squares and calculated as follows:

$$Platelet\ count\ \left(\frac{cell}{\mu L}\right) = \frac{cell\ counted \times 20 \times 106}{0.2 \times 0.1}$$

20=1:20 dilution

0.2=mm area counted

0.1= mm depth of chamber

3.9.5 Hemoglobin (Hb) counting Method (Sahli's Hemoglobinometer)

To count Hb, 0.1 N HCl was prepared and filled into Sahli Haemometer tube up to 2 % mark. The pipette was placed in Sahli Haemometer at a given place (in between two windows). The blood was filled into Hemometer pipette up to 20 microliter marks. Then, the blood sample was transferred into Haemometer tube (containing 0.1 N HCl). After 10 minutes, distilled water was added slowly-slowly drop wise in inside tube and it was shaken well with the help of glass rod. Distilled water was added till the color of blood was matched with the side window. The reading was observed and noted down. That was the percentage of hemoglobin.

3.10 Statistical Analysis

Statistical differences between diets were evaluated by t-test, Kinetics of length and weight with respect to days and diet were analyzed by using liner regression, length-weight relationship was represent using Pearson correlation coefficient and relation among length, weight and hematologic values were established by Corplot using R Software of version R-5.3.2. Experimental data were evaluated as mean ± Standard Error (SE).

RESULTS

4.1 Water Quality Parameters of Experimental Tank

Mean value of water quality parameters (Table 4) were recorded during the current experiment using titration method, thermometer and pH meter. The value of water quality parameters were not significantly different among different treatments (p>0.05).

Table 4: water quality parameters of different tanks where different proportions of *U. parviflora* diet were fed to *O. niloticus* (Mean ± SE).

Parameters	**Control (T_0)**	**Treatment 1 (T_1)**	**Treatment 2 (T_2)**	**Treatment 3 (T_3)**	**Treatment 4 (T_4)**
Temperature (ºC)	22 ± 0.00	22 ± 0.00	22 ± 0.00	22 ± 0.00	22 ± 0.00
DO (mg/l)	4.23 ± 0.4	4.55 ± 0.24	4.26 ± 0.37	4.22 ± 0.23	4.36 ± 0.2
CO_2 (mg/l)	11.94 ± 0.65	11.62 ± 0.8	11.34 ± 0.91	12.25 ± 1.16	12.25 ± 0.8
Hardness (mg/l)	84.57 ± 5.06	84.43 ± 4.26	85.43 ± 9.04	81.42± 7.04	80 ± 4.9
Alkalinity (mg/l)	392.85±7.14	405.71± 6.67	410 ± 6.64	407.14±7.14	396.4 ± 6.5
Acidity (mg/l)	24.3 ± 2.3	23.92 ± 2.3	24.64 ± 2.8	24.28 ± 2.3	24.64 ± 2.4
Ammonia (ppm)	3 ± 0.2	2.57 ± 0.3	2.85 ± 0.26	2.85 ± 0.26	3 ± 0.2
Nitrite (ppm)	1.14 ± 0.21	0.92 ± 0.20	0.92 ± 0.17	1.36 ± 0.26	1.35 ± 0.17
Nitrate (ppm)	92.85 ± 5.65	91.42 ± 4.04	92. 85± 5.21	92.85 ± 5.65	91.42 ± 3.4
pH	7.7 ± 0.11	7.8 ± 0.07	7.8 ± 0.13	7.8 ± 0.08	7.7 ± 0.11

4.2 Growth Performance

At the end of the experiment period, the highest SGR was recorded in treatment 2 (0.8 ± 0.11) followed by treatment 3 and the lowest was in control and treatment 4 (0.59 ± 0.06). Similarly, highest PWG was recorded in the treatment T2 (12.8 ± 1.90) and lowest was in the

treatment 4. FCR was lower in the treatment 2 (4.42 ± 1.25) than control group while FER (0.28 ± 0.04) and ECR (171.6) were higher in treatment 2 group compared to control. Statistical tests showed that growth parameters like PWG, and ECR are highly significant (p <0.05) in treatment 2 group in comparison to control where others parameters are not highly significant (p >0.05) (Table 5).

Table 5: Effect of stinging nettle fed diet on the growth performance of *O. niloticus*
Values are presented as mean ± SE (n = 50). IML = Initial mean length, FML = Final mean length, IMW = Initial mean weight, and FMW = Final mean weight. Values in the same column with different superscripts letters are significantly different (p< 0.05).

Parameters	Control 0 (T_0)	Treatment 1 (T_1)	Treatment 2 (T_2)	Treatment 3 (T_3)	Treatment 4 (T_4)
IMW(g)	12.68 ± 2.35	12.23± 3.36	11.13± 3.36	11.43 ± 2.21	11.53 ± 1.75
FMW(g)	22.21 ± 5.46	22.23 ± 4.57	22.86 ± 3.17	21.28 ± 3.15	19.59± 1.75
IML(cm)	8.74 ± 0.67	8.7 ± 0.62	8.09 ± 0.58	8.29 ± 0.76	8.39 ± 0.68
FML(cm)	10.41 ± 0.77	10.37 ± 0.87	10.44 ± 0.57	10.38 ± 0.63	9.96 ± 0.90
SGR (%)	0.59± 0.06 ^{b}	0.6 ± 0.08^{ab}	0.8 ± 0.11 ^{a}	0.69 ± 0.09^{ab}	0.59± 0.06 ^{b}
FCR (%)	5.42 ± 1.42 ^{a}	5.11 ± 1.27 ^{ab}	4.42±1.25 ^{b}	4.95±1.31 ^{ab}	5.48±1.04 ^{a}
SR (%)	100.00 ± 0.0 ^{a}	100.00 ± 0.0 ^{a}	100.00 ± 0.0 ^{a}	100.00 ± 0.0 ^{a}	100.00 ± 0.0 ^{a}
FER (%)	0.24± 0.03 ^{ab}	0.23 ±0.03 ^{ab}	0.28 ± 0.04 ^{a}	0.24 ± 0.03 ^{ab}	0.20 ± 0.02 ^{b}
PWG (%)	9.68± 1.48 ^{bc}	10.42 ± 1.37 ^{ab}	12.8 ± 1.90 ^{a}	10.96 ± 1.51 ^{ab}	8.93± 1.03 ^{c}
CF(K)	1.84± 0.03 ^{b}	1.93± 0.03 ^{ab}	1.93 ± 0.03 ^{ab}	1.90± 0.02 ^{ab}	1.94± 0.03^{a}
FI	6.98±0.50 ^{a}	6.94± 0.54 ^{a}	6.63 ± 0.65 ^{ab}	6.55 ± 0.54 ^{ab}	6.36 ± 0.44 ^{b}
ECR(NRs.)	234.09 ^{a}	217.1 ^{b}	171.6 ^{c}	178.9 ^{bc}	181^{bc}

4.2.1 Specific Growth Rate (SGR)

The highest SGR was recorded in treatment 2 (0.8 ± 0.11) and lowest was in control and treatment 4 (0.59 ± 0.06). There was no any significance difference between control and treatment 4.

From initial day to day 45 the SGR pattern was in ascending order in all treatments T_0, T_1, T_2, T_3 and T_4. Among all treatment, treatment 2 has increases rapidly. After day 45, SRG had been quite declined and from day 60 to day 90 the value of SRG was almost closed. Beyond them, treatment 2 has the highest SRG and control and treatment 4 had the lowest value of SRG (Figure 1).

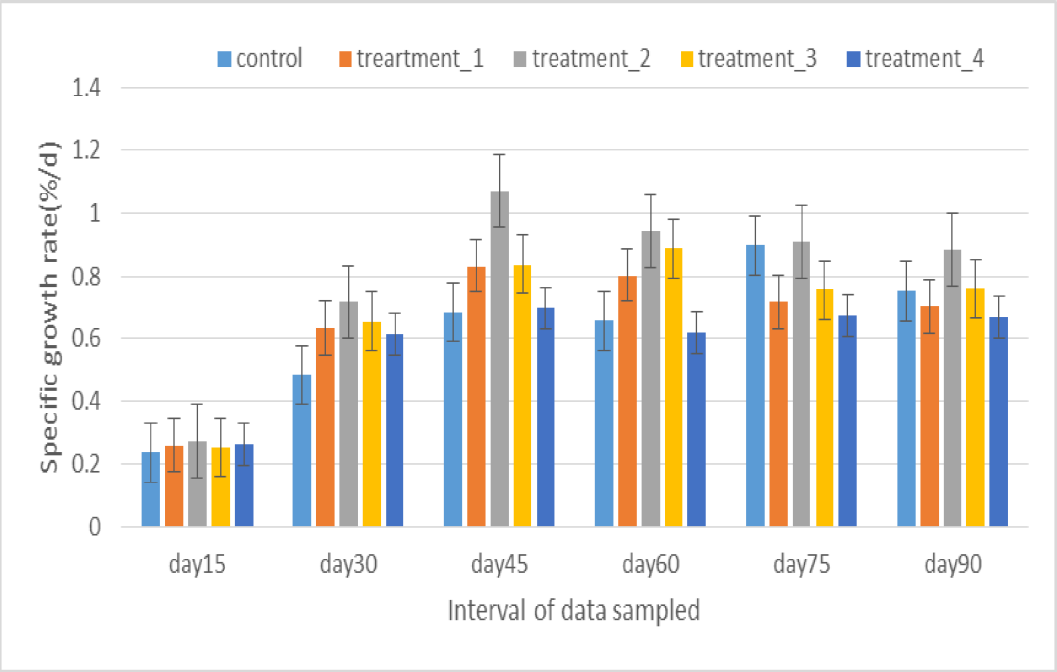


Figure 1: SGR under different treatments fed with different proportion of *U. parviflora* in *O. niloticus* during different sampling days. Values are expressed as mean ± SE.

4.2.2 Food Conversion Ratio

The FCR of *O. niloticus* kept in different tanks and fed on five different types of feed have been calculated in every 15, 30, 45, 60, 75, and 90 days. The highest FCR (5.42 ± 1.42 %) was found in the control (T_0), while the lowest FCR (4.42±1.25%) was measured in treatment 2 (T_2). In initial 15 days FCR was very highest it might be because of stress (while tagging).

The lower the value of FCR the better the food conversion to fish flesh. Thus, treatment 2 gives the best result in comparison with control and treatment 4. Finding FCR in the experiment is ranged from 4.42-5.48 (Figure 2).

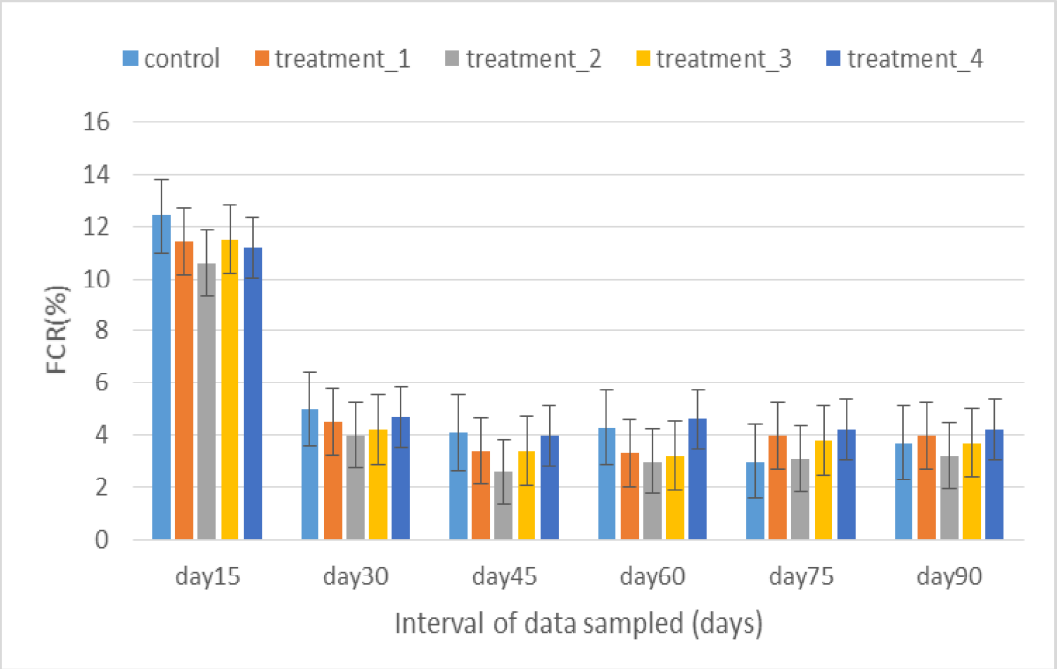


Figure 2: FCR under different treatment fed with different proportion of *U. parviflora* powder in *O. niloticus*. Data are represented by mean ±SE**.**

4.2.3 Survival Rate (SR)

The experimental fish is highly tolerated fish, and thus the SR of this fish was higher. During experiment, the SR was 100 % in all group of treatment.

4.2.4 Feed Efficiency Ratio (FER)

The values of FER have been calculated at the end of 15, 30, 45, 60, 75 and 90 days experiment. Usually, no significant difference of feed efficiency found in control, treatment 3. The FER was higher (0.28 ± 0.04 %) in treatment 2 and lower (0.20 ± 0.02) in treatment 4 (Figure 3).

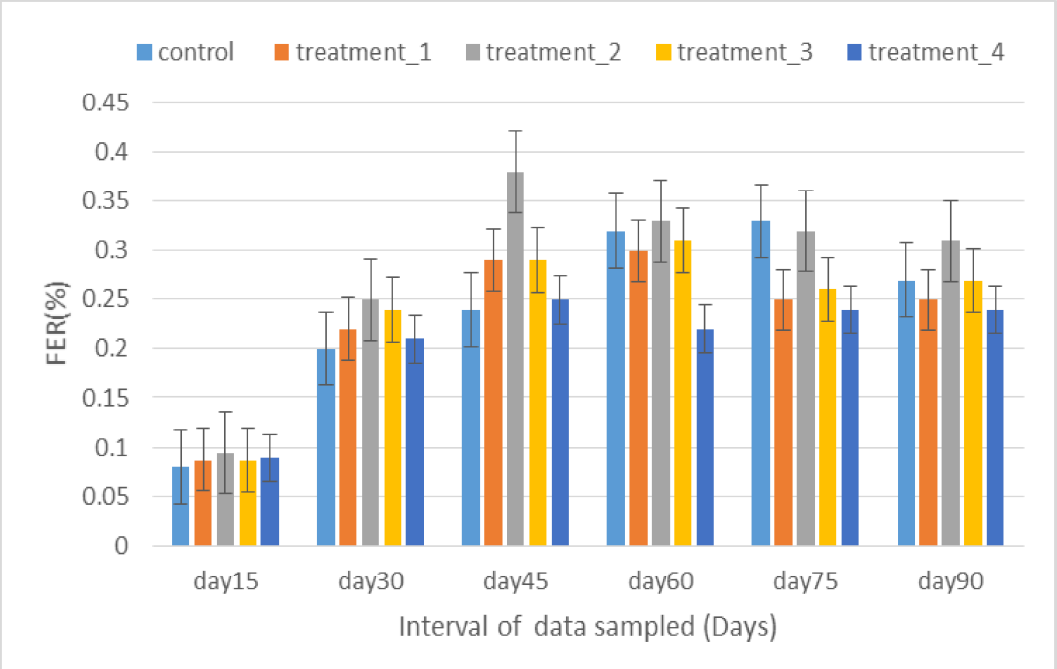


Figure 3: FER of different treatment group of *O. niloticus* fed with different proportion of *U. parviflora* powder diet. Values are expressed as Mean ±SE.

4.2.5 Weight Gain Percentage (WGP)

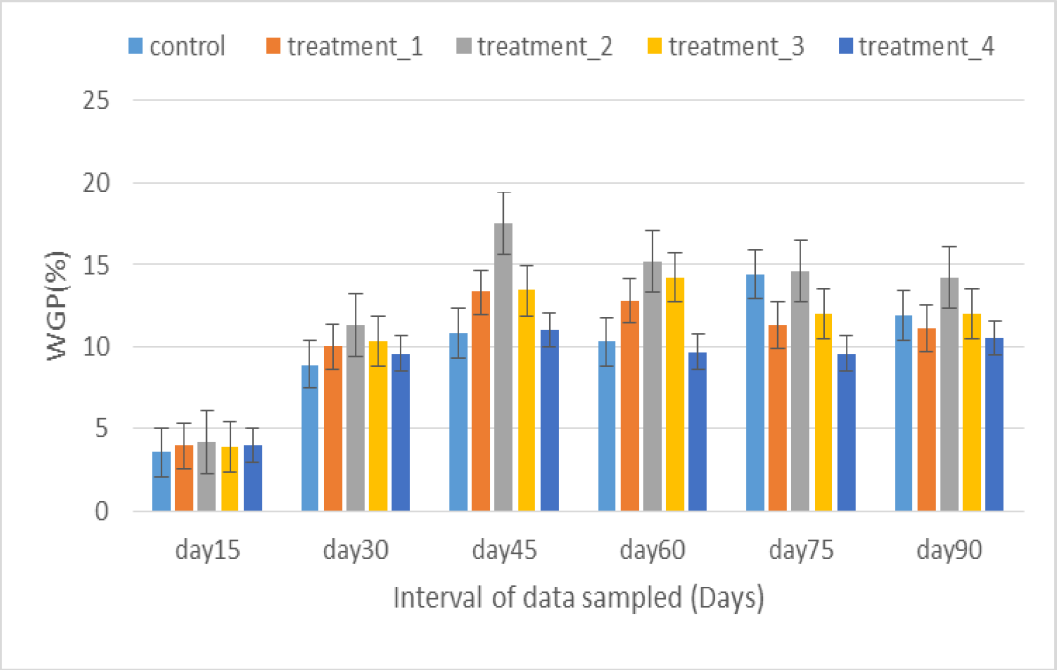


Figure 4: WGP under different treatments fed with different percentage of *U. parviflora* in *O. niloticus* during different sampling days. Values are evaluated as mean ± SE.

The highest WGP (12.8 ± 1.90) was recorded in treatment 2 and lowest WGP (8.93± 1.03) was in treatment 4. There was no any significance difference between control and treatment 4 (Figure 4).

4.2.6 Condition Factor (k)

The values of condition factor (k) were calculated during the experiment periods at the end of 15, 30, 45, 75 and 90 days. The k was highest in treatment 4 (1.94 ± 0.03 %) and lowest in control 1.84 ± 0.03). However, k (1.93 ± 0.03 %) in the treatment 1 and 2 were more or less similar with treatment 3 (1.90 ± 0.02 %). The values of k of the fish ranged from 1.84 - 1.94 (Figure 5).

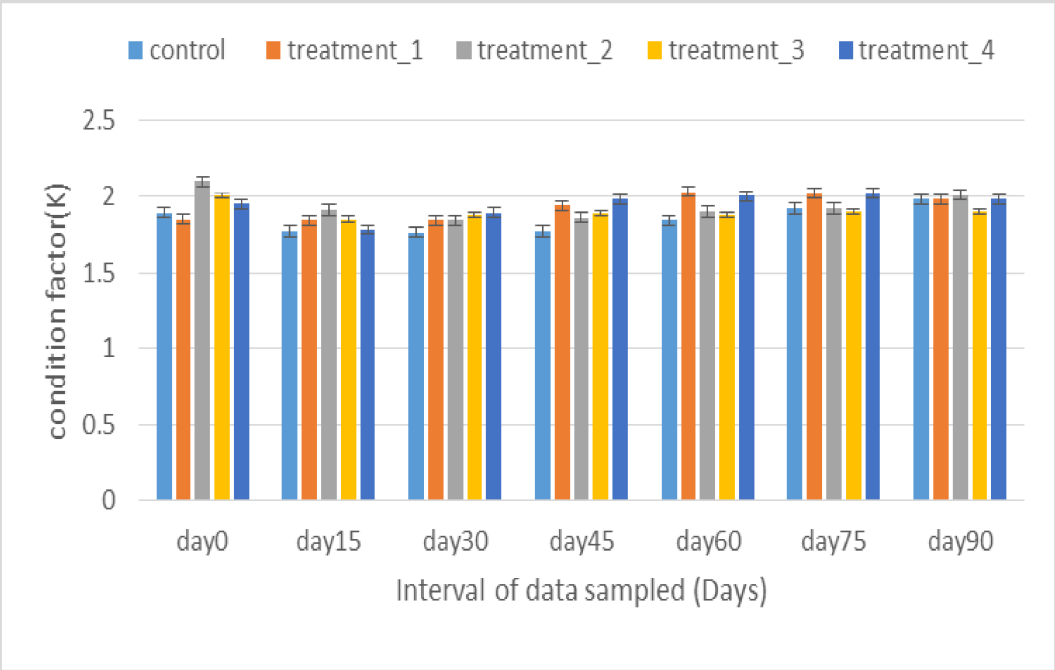


Figure 5: Condition factor (K) under different treatments fed with different percentages of *U. parviflora* in *O. niloticus* during different sampling days. Values are presented as mean ± SE.

4.2.7 Feed Intake (FI)

The highest FI (6.98 ± 0.50) was recorded in control group and the lowest FI (6.36 ± 0.44) was in treatment 4. Feed intake was increased from day initial days to end of experiment as their weight was increased (Figure 6).

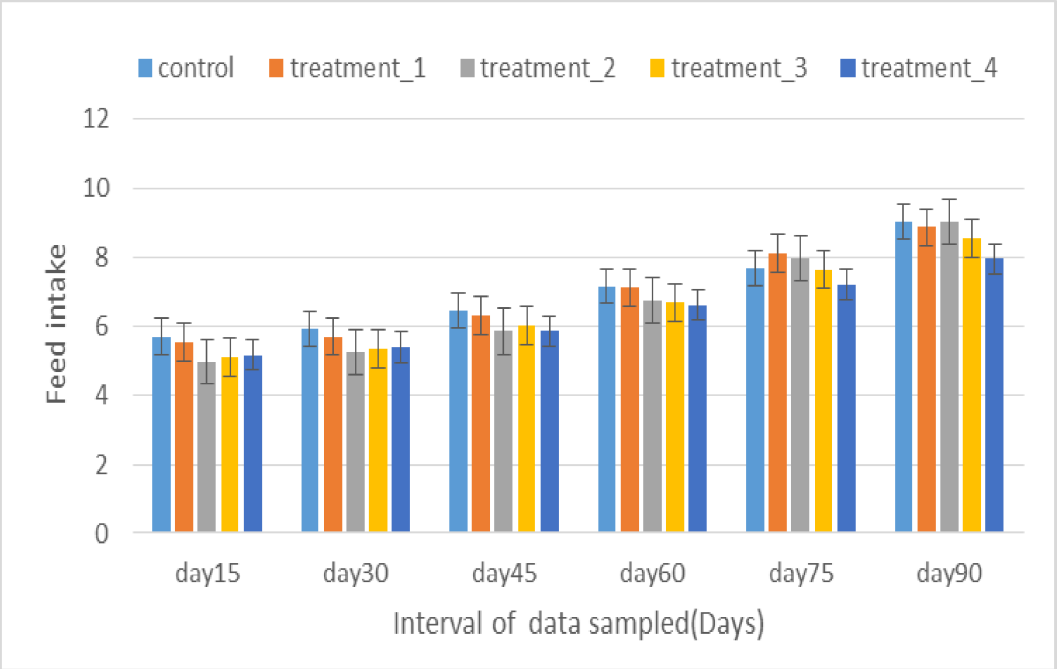


Figure 6: Feed Intake (FI) under different treatments fed with different percentages of *U. parviflora* in *O. niloticus* during different sampling days. Values are expressed as mean ± SE.

4.2.8 Economic Conversion Ratio (ECR)

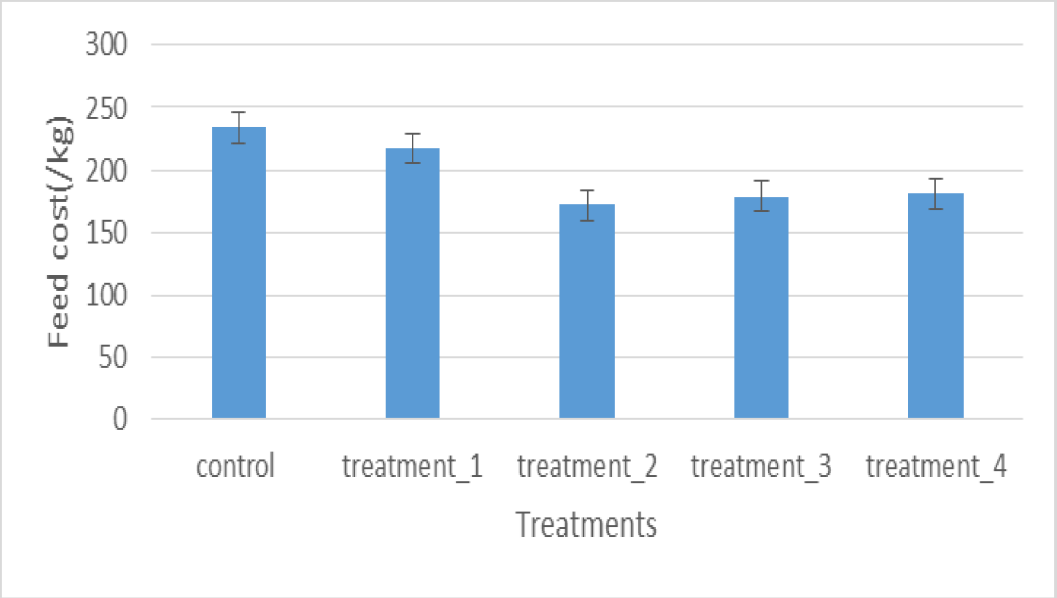


Figure 7: ECR under different treatments fed with different proportions of *U. parviflora* in *O. niloticus* during different sampling days. Values are presented as mean ± SE.

The cost of each experimental feed was determined on the basis of the cost of the ingredients used for its manufacturing. The ECR was 234.09 Rs. for control feed and

171.6 Rs. Treatment 2 feed (Figure 7). Higher the ECR, feed cost is more and vice versa. Therefore, here treatment 2 feed cost low and control feed cost more.

4.2.9 Kinetics of Weight gain with respect to diet and days

After feeding formulated diet, the fish weight was increased significantly higher with interval of time. At first 15 day, there was no significant increased (p<0.05). But, after 15 days, weight of fish was increased significantly. For example, at 30 days, weight of fish was increased highly significant (p<0.0012) in comparison to initial day. Similarly, at 45 days, weight of fish was increased highly significantly (p < 4.2e-09), at 60 days (p< 4.1e-15), at 75 days (p<2.22e-16), at 90 days (p < 2.22e-16) in comparison to initial day (Figure 8).

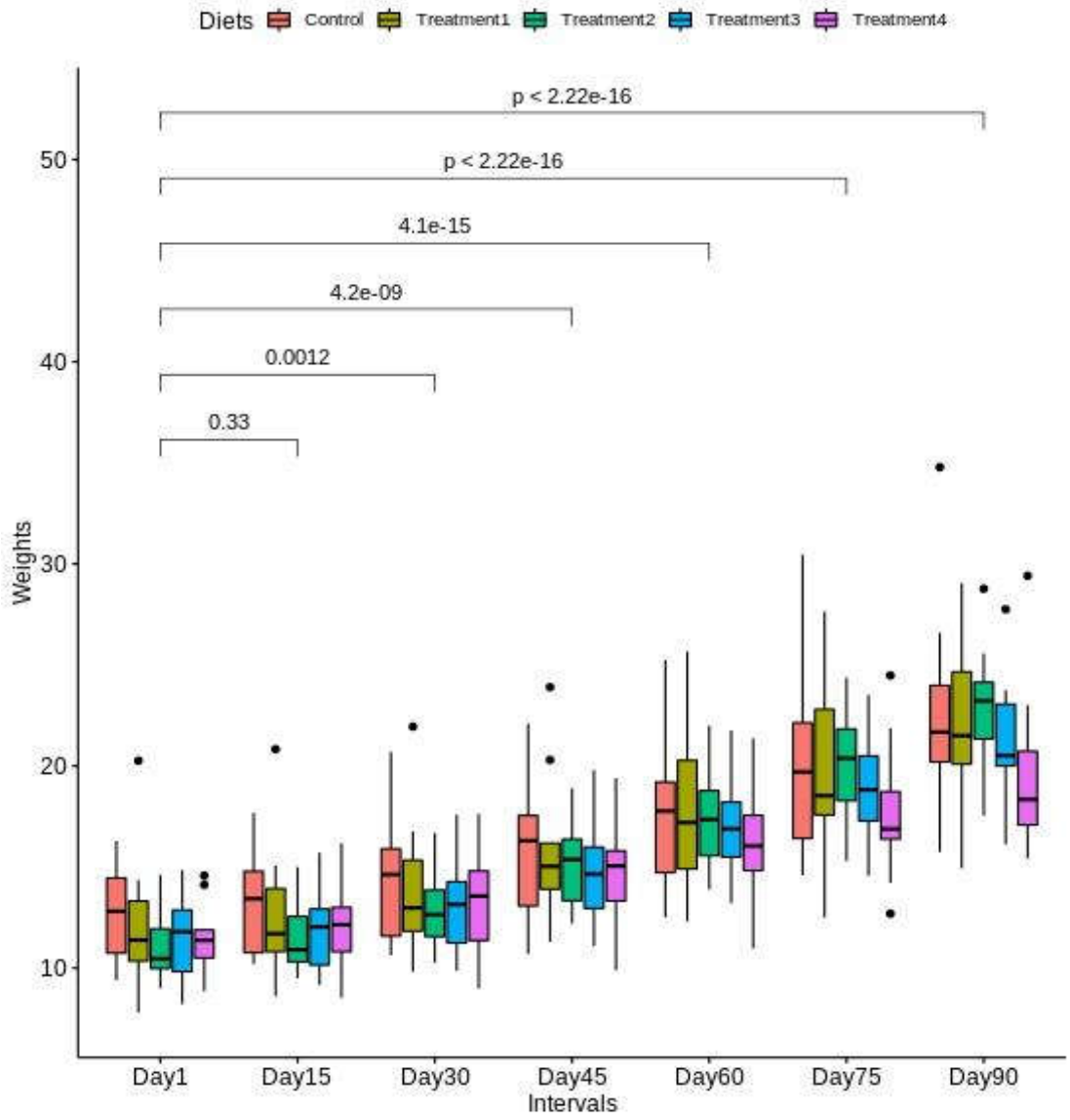


Figure 8: kinetics of weight gain with respect to diet and days. Data were expressed as Mean ± SE

4.2.10 Kinetics of Length with different diets and days

After feeding formulated diet, the length of fish increased with respect to time. At day 15, there was no highly significant increased (p <0.05) on length. At day 30, length of fish was increased significantly (p < 6.1e-05) in comparison to day 1. Similarly, at day 45, length of fish was increased significantly (p <1e-08), at day 60 (p <3.7e-13), at day 75 (p< 2.22e-16), and at day 90 (p < 2.22e-16) in comparison to day 1 (Figure 9).

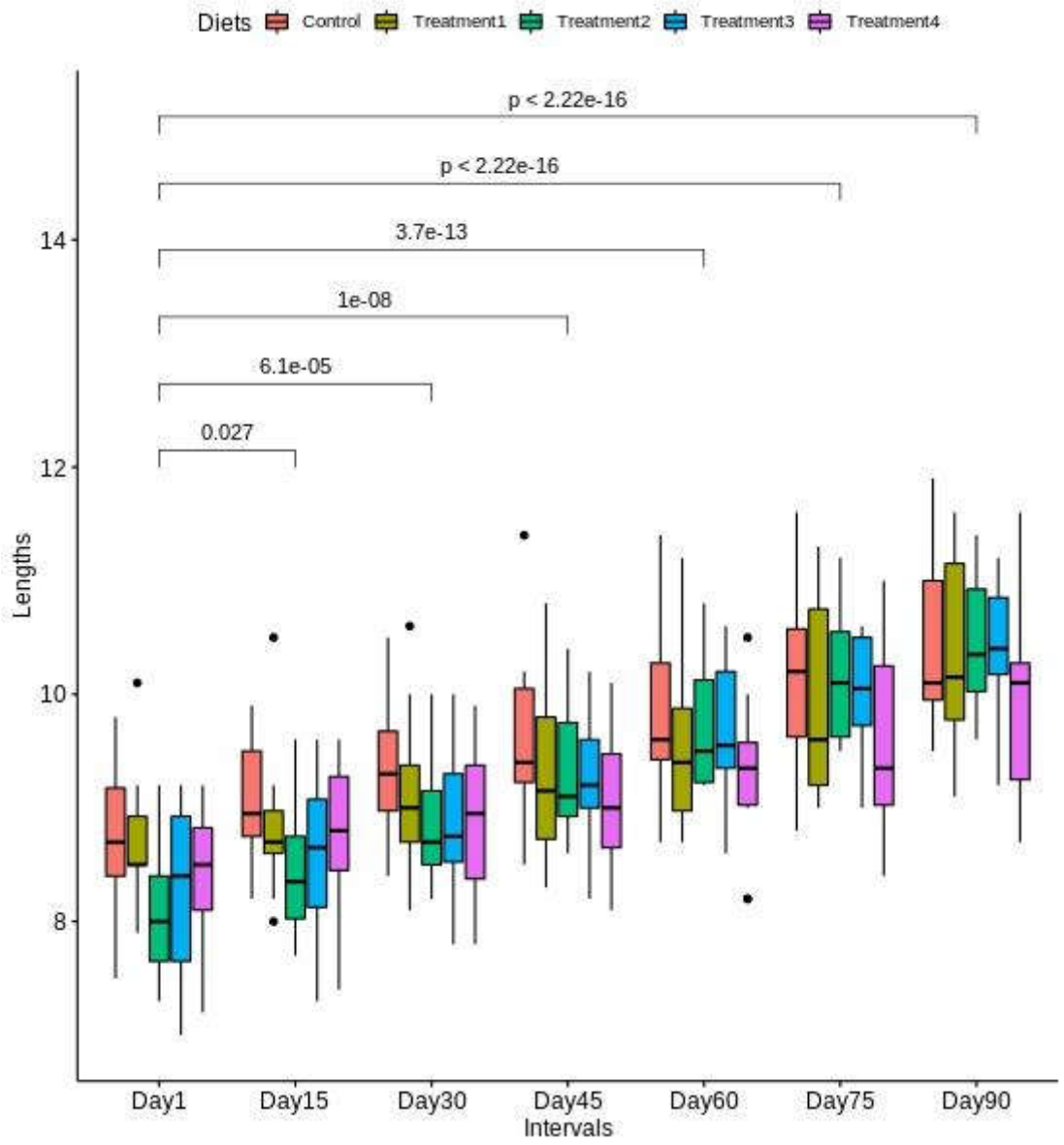


Figure 9: Kinetics of length with different diets and day Data were expressed as Mean ± SE.

4.2.11 Length-weight relationship

A total of 50 individuals of *O. niloticus* weight ranged from 11.13 to 22.86 g. Similarly, the length ranged from 8.09 to 10.44 cm. The length-weight relationship of *O. niloticus* was highly correlated [Correlation coefficient (r^2) = 0.8648 (p<2e-16)] (Figure 10).

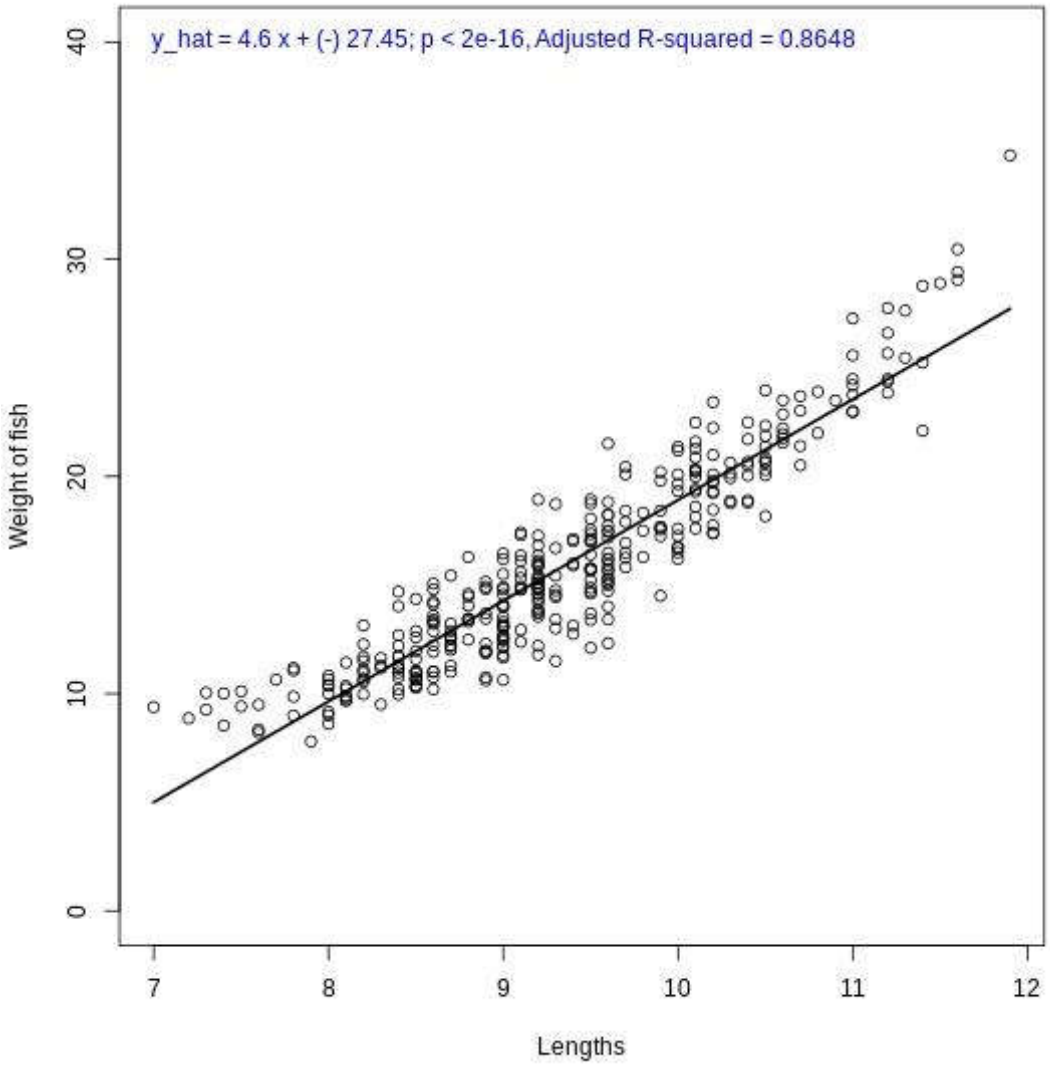


Figure 10: Length-weight relationship of *O. niloticus* fed with different proportions of *U. parviflora* diet. Data were presented as Length-weight correlation coefficient**.**

4.3 Hematologic parameters

The effect of *U. parviflora* fed diets to the fish at different levels on the mean final hematologic parameters such as Lymphocytes, Monocytes, Neutrophils, WBCs, RBCs, Platelets and Hemoglobin were recorded at the end of experiment. Lymphocytes were the most common leucocyte cell type. There wasn't highly significant difference between lymphocytes. The value of Lymphocyte percent was higher (93.86 ± 0.51) in treatment 3 and lower (91.94 ± 0.69) in treatment 2 group. Similarly Monocyte percentage was higher

(5.54 ± 0.40) in treatment 2 group and lower (4.39 ± 0.38) in treatment 3 group followed by control group. And Neutrophil percentage was higher (2.81 ± 0.68) in control group and lower (1.77 ± 0.23) in treatment 3 group. Eosinophil and Basophils were absent. All treated groups showed a significant increase in total leukocyte count (TLC or WBC) except treatment 1 group that showed a slight increase in comparison with control (p<0.05). Total WBC varied from 57,060 to 66,190 µL and RBC from 1,340,000 to 1,790,000 µL. RBC (×10^6/ µl) was increased in all treatment group and was significantly higher (1.79 ± 0.13) in treatment 2 groups except (1.34 ± 0.05) treatment 4 group that showed significantly lower in comparison with (1.5 ± 0.07) control group. Platelets were significantly increased in all treated group in comparison to control group. Treatment 2 showed higher Platelets (10^3/µl) value (108.9 ± 7.87) and control group showed lower Platelets (10^3/µl) value (95.5 ± 10.43). The value of Hemoglobin (g/dl) was significantly higher in all treated group except treatment 4 group. Hemoglobin range was in between 7.39 - 5.54 g/dl in treatment group (Table 6).

Table 6. Hematologic parameters in *O. niloticus* fed with different proportion of *U. parviflora.* Values are presented as Mean ± SE. (N=50)

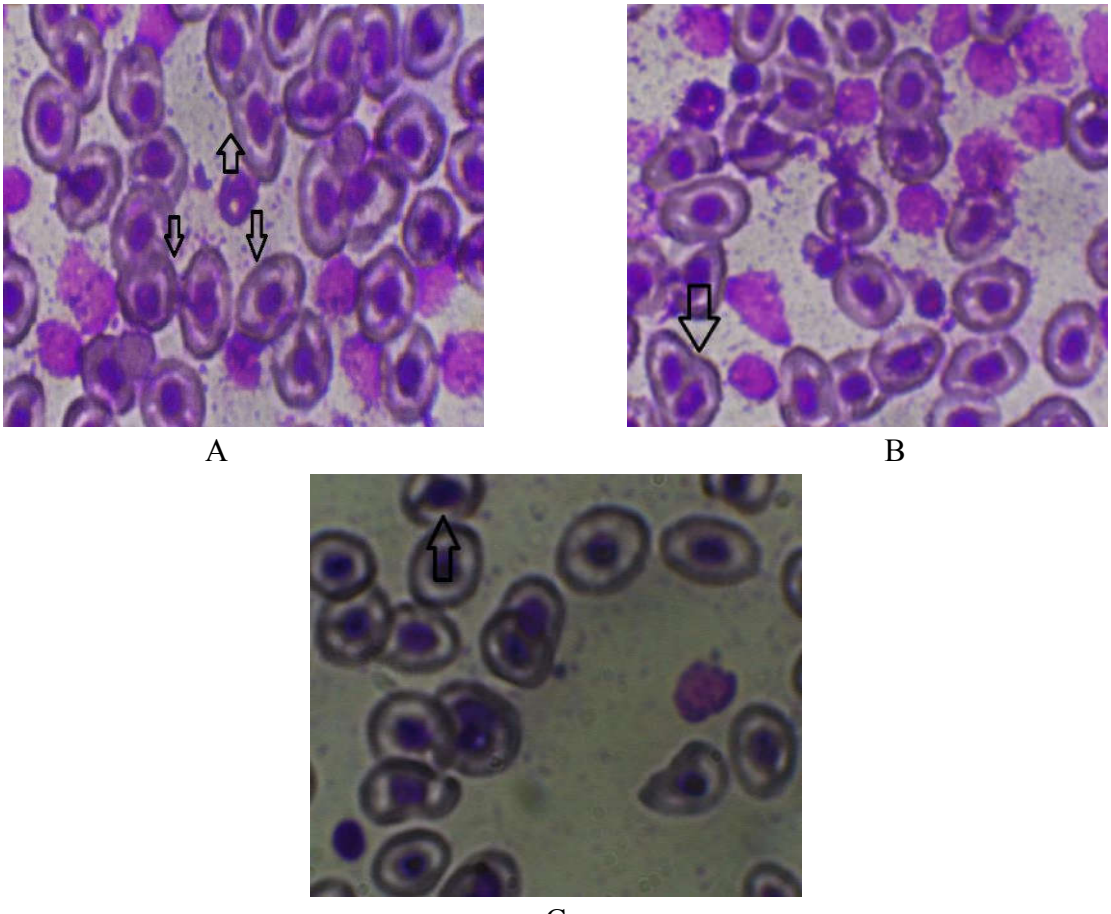
Parameters	**Control**	**Treatment 1 (T_1)**	**Treatment 2 (T_2)**	**Treatment 3 (T_3)**	**Treatment 4 (T_4)**
Lymphocytes (%)	92.5 ± 1.04^{a}	92.65 ± 0.62^{a}	91.94 ± 0.69^{a}	93.86 ± 0.51^{a}	92.76 ± 0.63^{a}
Monocytes (%)	4.82 ± 0.55^{b}	5.37 ± 0.51 ^{ab}	5.54 ± 0.40^{a}	4.39 ± 0.38^{bc}	5.11 ± 0.44 ^{ab}
Neutrophils (%)	2.81 ± 0.68^{a}	1.84 ± 0.52^{b}	2.55 ± 0.32^{ab}	1.77 ± 0.23^{b}	2.34 ± 0.20^{ab}
WBC (×10^3/ µL ^{3})	57.06 ± 6.2 ^{b}	57.93 ± 2.15^{b}	61.97 ± 4.62^{ab}	60.28 ± 5.82^{ab}	66.19 ± 2.34^{a}
RBC (×10^6/ µL)	1.5 ± 0.07^{bc}	1.71 ± 0.09 ^{ab}	1.79 ± 0.13^{a}	1.64 ± 0.3^{b}	1.34 ± 0.05^{c}
Platelets (10^3/µL)	95.5±10.43^{b}	106.6 ± 9.64^{a}	108.9 ± 7.87^{a}	102.7 ± 8.8 ^{ab}	99.4 ± 0.01^{ab}
Hemoglobin (g/dL)	5.54± 0.24^{bc}	6.23 ± 0.33^{ab}	7.39 ± 0.41^{a}	6.2 ± 0.25 ^{ab}	5.75 ± 0. 30^{b}

4.3.1 Distinguishing Characters of Different Leukocytes:

According to form, shape and color, neutrophils, lymphocytes, and monocytes could be identified. Lymphocyte (figure: 11a) contains round cells with large round nucleus surrounded by a thin rim of cytoplasm which stained a dense deep red/violet color; the cytoplasm was either a dark blue ring or not visible. The range of diameter was in between (4.8 - 7) µm.

Monocytes (figure: 11b) were the largest and the scarcest of the cells identified. The nucleus stained dark blue/purple, with a pale blue cytoplasm that occasionally displayed extended processes and gave the cells an asymmetrical outline. The range of diameter was in between (8.8 - 10.9) µm.

Neutrophils (figure:11c) had round/oval cells stained similarly to the lymphocytes but were easily distinguishable by the significant difference in size, their granulated cytoplasm and eccentric nuclei which often appeared either two- or three-lobed or shaped like the human kidney. The diameter of the neutrophil cells was in between (9.6 - 12.8) µm.



A B C

Figure 11: Microscopic examination of blood smear of *Oreochromis niloticus* shown the presence of A) Lymphocyte B) Monocyte C) Neutrophil.

4.4 Relation among length, weight and hematologic values

RBC was positively correlated with weight (+0.8) and length (+0.82) of fish. Similarly, platelet was positively correlated with both length (+0.3) and weight (+0.38) of fish, hemoglobin was positively correlated with weight (+0.7) and length (+0.6) of fish. Neutrophil was positively correlated with weight (0.29) of fish, whereas there was no any relation between neutrophil and length. Monocyte was positively correlated with weight (+0.25) and there was not any relation between monocyte and length. Lymphocytes was negatively correlated with weight (-0.5) and no relation (r^2=0) between lymphocytes and length. Total leucocyte was negatively correlated with both weight (-0.6) and length (-0.7) of fish (Figure 12).

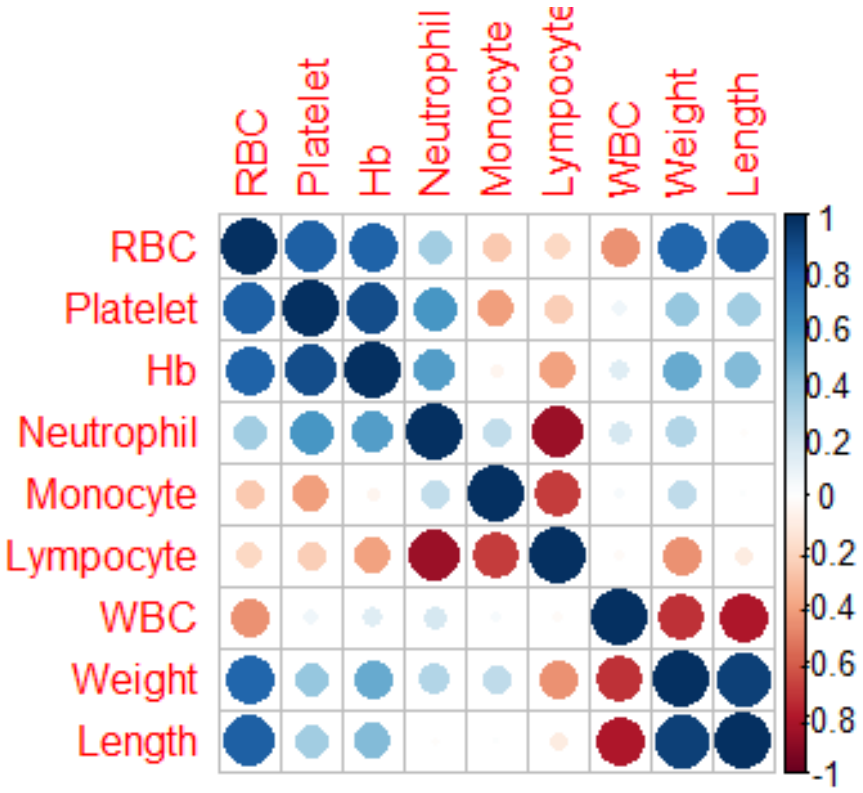


Figure 12: Relation between length, weight, and hematologic value.

DISCUSSION

The current study was carried out to assess the effect of herbal plant *U. parvilora* on growth performance and hematologic parameters in Nile tilapia. Results demonstrate that powder of *U. parviflora* induced high growth performance and hematologic parameters. Herbs reported to improve animal growth performance by stimulating action on gut microflora and furthermore the herbals active principles in the diets induce the secretion of the digestive enzyme and the growth promoter in herbs induced high protein synthesis (Citarasu 2010, Lee et al. 2012).

The specific growth rate of all treated groups in this study was higher than control group except treatment with 15% *U. parviflora*. These results correspond with (Sabry et al. 2014) who examined the effect of feeding *Echinacea* extract and oxytetracycline on the growth of tilapia for two months they found the specific growth rate showed higher value in comparison with other groups. These results agree with (Immanuel et al. 2009) who found the enhanced SGR of *O. mossambicus* fed the four diets (*Cynodon dactylon, Aegle marmelos, Withania somnifera* and *Zingiber officinale,*) was significantly greater than control diet-fed fish. (Alam 2009) reported that dietary vitamin level help to increase the level of SGR. (Abarra and Ragaza 2017) who replaced fish meal with Knife fish (*Apteronotus albifrons*) and fed to Nile tilapia higher the level of SGR. Though the level of protein is lower (22%) *U. parvifora* could give best result for SGR.

The best FCR (lower value) was for groups feed with 5% of stinging nettle powder followed by groups fed with 1%, 10%, and 15% nettle powders then control. Similarly, FI was almost similar. These findings are consistent with Sabry et al. (2014) who reported lower FCR in groups received *Echinacea or Ginseng* extracts or both with oxytetracycline. Similarly, results agree with Immanuel et al. (2009), Alam M.J (2009), Abarra and Ragaza (2017). *U. parviflora* helps to reduce FCR and increases feed utilization rate in comparison to untreated group.

The survivability of all groups showed no significant change in comparison with control group. There was no mortality in experimental fish because feed and all water quality parameters were monitored and maintained as Nile tilapia needed.

The feed efficiency was higher in fish group diet containing 5% *U. parvifora*, followed by control, 1%, 10% and lower in 15%. Feed efficiency ratio is related with FCR so *U. praviflora* also help to increase feed efficiency ratio. These findings were agree with Alam M.J (2009), Abarra and Ragaza (2017), but, do conflict with Immanuel et al. (2009) who reported that there was no significance difference of FER in Nile tilapia while feeding herbal extract. *U. parvifora* contain 10 % fiber which help to increased feed efficiency in Nile tilapia.

The percentage weight gain of group that feed 5% *U. parvifora* powder was significantly higher than others groups. Fish fed with 1%, and 10% also increased but 15 % reduced

weight. This might be because the herb has that chemical which can enhance weight or any chemical that enhance feeding uptake and increase weight. These findings was consistent with Immanuel et al. (2009), Alam M.J (2009), Sabry et al. (2014) and Abarra and Ragaza (2017).

Regarding the condition factor (k), all groups were significantly higher. K reveals in between biotic and abiotic factor in the physiological condition of the fish which reflect the overall welfare of the animal (Hopkins 1992). Generally, its study is done in natural condition. But, in control experiment, it's mandatory to know the k of fish to verify the wellbeing of fish heath. Current study contradicts with Sabry et al. (2014) who recorded no significant change in the k of Nile tilapia fed *Echinacea*.

The ECR was higher for control feed and lower for fish fed with 5% *U. parvifora*. Higher the ECR, feed cost is more and vice versa. Most of the food supplementary research are being held in aquaculture to reduce the cost effect. Aquaculture focused on eco-friendly, economic, and easily accessible feeds. Economic value is directly related with lifestyle of farmer, thus, while selecting fish feed, choosing of those economic feed is very critical in aquaculture.

Every animal in its life exhibits growth both in length and in weight and relationship between these two has both applied and basic importance. The length-weight relationship is one of the standard methods that yield authentic biologic information and is of great importance in fishery assessments. This is particularly useful for computing the biomass of a sample of fish from the length frequency. The correlation between length and weight varies between -1 to 1. In this study, length-weight relationship was highly correlated with correlation coefficient (r^2) = 0.8648 (p<2e-16) suggesting the current feed formulation is one of the best option in Nile tilapia culture.

The ability of herbal plant *U. prviflora* to improve the growth performance of Nile tilapia was varied and might be attributed to the possible mode of action of them as growth promoters as mentioned by few researchers (Sabry et al. 2014). To develop alternative method for growth promotion and disease management in aquaculture, attention has been focused in identifying novel drugs, focused on plant sources. These drugs may be delivered to the cultivable fishes either through feed supplementation or oral delivery through natural diets. Several herbs have been tested for their growth promoting activity in different fishes (Citarasu et al. 2002, Sivaram et al. 2004, Vasudeva Rao et al. 2006, Immanuel et al. 2009).

Hematologic parameters are greatly influenced by environmental conditions, such as temperature, pH, dissolved oxygen and seasonality. Similarly, gender, age, origin, breeding system, feeding and lineage, nutritional and health status, and stress which may also interfere with the results obtained in tests. (Teixeira et al. 2000, Tavares-Dias and Moraes 2004). Similarly, it is known that exogenous agents can change hematologic parameters and that these provided valuable information for fishery biologists in the assessment of fish health (Banaee et al. 2008).

There was no any significance difference between numbers of lymphocytes (91.94 - 93.86 %) in all feeding groups. This finding was consistent with (Navarro et al. 2018) who reported that there was no significant difference in average percentages of lymphocytes after feeding different oils.

Monocytes was highest in fish fed with 5% herb followed by 1% and 15% herb. Monocytes were found in between 5.54 - 4.82 %. These findings correspond with (Navarro et al. 2018) who found no significant difference to average percentages of monocytes after feeding different oils.

Neutrophils was the lowest in fish fed with 10% and 1% followed fish fed with 15% and 5% and highest in control group. Neutrophil range was in between 1.77 - 2.81%. These finding was consistent with (Navarro et al. 2018) there was no significant difference to average percentages of Neutrophils after feeding different oils.

Eosinophil and Basophils were absent in this experiment. These findings were consistent with (Nilza et al. 2003). Basophils and Eosinophil are generally lacking in fish blood, but studies by Pitombeira and Martins (1970), Ezzat et al. (1974) indicated that they are present in at least some fish species. In studies by Ranzani-Paiva et al. (2000), the highest percentage of Eosinophil was found in non-parasitized *S. Borelli.*

Similarly, the total leukocyte count of all experimental groups were significantly increased in comparison with *U. parviflora* unfed group. These findings correspond with (Sabry et al. 2014). WBC range was in between 66.19 – 57.06 × 10^3 /µL. However, it conflict with (Navarro et al. 2018) who found that there was no significant difference in average percentages of leukocytes after feeding with different oils.

Erythrocytes of treated group were significantly increased in comparison with control group. There range was in between 1.34 - 1.71 × 10^6 /µl, which was within the range described for *Schizodon borellii*, 2.09 x 10^6/mm^3 and *Prochilodus lineatus*, 2.33 x 10^6/mm3 (Ranzani-Paiva et al. 2000). Navarro et al. (2018) reported that *O. mosambicus* treated with fish oil presented higher RBC value. On the other hand, it is significantly lower than those described for *O. niloticus* 6.93 x 10^6/mm^3 (Nilza et al. 2003) in semi intensive farm.

Fish thrombocytes represent a link between innate and adaptive immunity (Passantino et al. 2005) and express surface and intracellular molecules that are involved in the immune function (Kollner 2004). It is already agreed that the fish thrombocytes are blood phagocytes that form one of the protective barriers (Tavares-Dias and Moraes 2004, Prasad and Charles 2010, Prasad and Priyanka 2011). In current study number of platelets was found significantly increased in all treated group in comparison to control group. Higher was in fish group fed with *U. prviflora* 5%. Their range was in between 108.9 - 95.5 × 10^3 /µL. However, this has conflicts with a research (Navarro et al. 2018) because there was no significant difference in average number of platelets after feeding different oils.

Hemoglobin (Hb), the major component of mature erythrocytes, is responsible for transporting oxygen to the several tissues (Tavares-Dias and Morase 2004). Fish treated with 5% provided higher values for Hb followed by 1% and 10% herbs. All groups contained higher Hb than control group. Its range was in between 7.39 - 5.54 g/dl in Nile tilapia, and was similar to those reported for Florida red tilapia (7.3g/dl) and *Schizodon borellii* (7.7g/dl) (Ranzani-Paiva et al. 2000), but was lower than those for "Nile tilapia" 10.52 g/dl (Nilza et al. 2003). Araujo et al. (2011), observed a reduction in the hemoglobin rate in tilapia fed with linseed oil after cold stimulation. Another researcher Salvador et al. (2013) observed an increase in hematocrit and hemoglobin rates in Nile tilapia fed with fatty acid and vaccine supplementation. The dietary iron can be absorbed by the gastrointestinal tract, and subsequently stored and incorporated to the hem group of hemoglobin (Bianchi, Silva and Oliveira, 1992). Hence, either presence of high iron or other compound found in *U. parviflora* could be the factor and further studies should be elaborated.

A correlation matrix was established to compare the degree of association among the biometric data and hematologic parameters and among each of their water parameters. The weight and length of fish were positively correlated with DO, hardness and pH and negatively correlated with alkalinity, ammonia and nitrite. Similarly, water quality parameter with hematologic parameters and hematologic parameter with length weight were correlated with each other. Hence there was all type (positive and negative) relation in between hematologic, water parameters and length and weight data so they relate with each other.

Traditionally, different forms of plant sources are globally used to treat different disease (Patel and Udayabanu 2013). Toldy et al. (2005), (Coskun et al. 2005) and Behazadi (2016) showed that *Urtica spp* decreased Reactive Oxygen Species (ROS), increasing the antioxidant enzyme activity of superoxide dismutase (SOD). Phytochemical analyses of hydro-alcoholic extract of *Urtica spp* showed that the polyphenols that are found in *Urtica* spp included tannin, anthocyanin, chlorogenic acid, cafe oil malic, syringic, myricetin, quercetin, kaempferol, rutin, ellagic, isorhamnetin, p-coumaric, ferulic, naringin, fumaric, and vanillic (Otles and Yalcin 2012). Phenolic compounds and especially a group of flavonoids which are present in *Urtica* spp seem to be responsible for the antioxidant activity (Golalipour et al. 2011, Joshi et al., 2014, Khare et al. 2012) and play an important role in stabilizing lipid peroxidation (Bahmani et al. 2014), which is very important for aquatic organism like fish. Jeong et al. (2012) reported that consumption of quercetin can decrease plasma glucose levels and total cholesterol and increase HDL-cholesterol, hepatic GSH-Px activity and plasma adiponectin which is a hormone produced from adipose tissue and reduces insulin resistance. Most of the studies are carried out in *U. dioica* rather than *U. parviflora.* Most of the experiment are carried out in rat and some are in human, hence, *Urtica* spp is still an unexplored natural herb in sector of aquaculture. Further detailed studies involving the extract of *urtica* at different concentrations and kinetics in vitro and in vivo will give us better understandings of its effects on growth performance and hematologic parameters.

CONCLUSIONS

The current experiment, effect of herbal plant *U. parviflora* on growth performance and hematologic parameters in Nile tilapia showed that, *U. parviflora* can increased SGR, WGP, FER, FI, k, length-weight ratio and decreased FCR and ECR. Similarly, feeding diet contains *U. parviflora* helps to increased Monocytes and decrease Neutrophils, there was no significant difference in Lymphocytes and Esinophils and Basophils were absent. It also help to increase WBC, RBC, platelets and Hb in comparison to control group. Growth performance, feed utilization efficiency and hematologic parameters of the fish fed with stinging nettle powder 5% (T_2) was the best among newly formulated five dietary compositions.

Inclusion of *U. parviflora* in fish diet increase specific growth rate, feed efficiency ratio, hemoglobin level, RBC number, platelets and monocytes. Similarly it help to reduced feed cost and Feed conversion ratio. Hence, *U. parviflora* could be one of the best herbal plant to use as fish food.

In conclusion, the current study has revealed that long term dietary administration of *U. parviflora* diet considerably help to improve growth performance and hematological parameters of fingerlings of Nile tilapia.

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APPENDIX I: Permission Letter from Ethical Review Board

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 2584

Date: 25 March 2019

Ms. Dipa Rai
Principal Investigator
Tribhuvan University
Kathmandu

Ref: **Approval of thesis proposal** entitled **Effect of Herbal Plant Urtica parviflora Roxb. on the Growth Performance and Immune Modulation in Nile Tilapia (Oreochromis niloticus Linnaeus, 1758)**

Dear Ms. Rai,

It is my pleasure to inform you that the above-mentioned proposal submitted on **12 February 2019** (**Reg. no. 109/2019**) has been approved by Nepal Health Research Council (NHRC) National Ethical Guidelines for Health Research in Nepal, Standard Operating Procedures Section 'C' point no. 6.3 through Expedited Review Procedures.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **October 2019.**

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and **submit progress report in between and full or summary report upon completion.**

As per your thesis proposal, the total research budget is **Self-Funded** and accordingly the processing fee amounts to **Rs 1,000.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

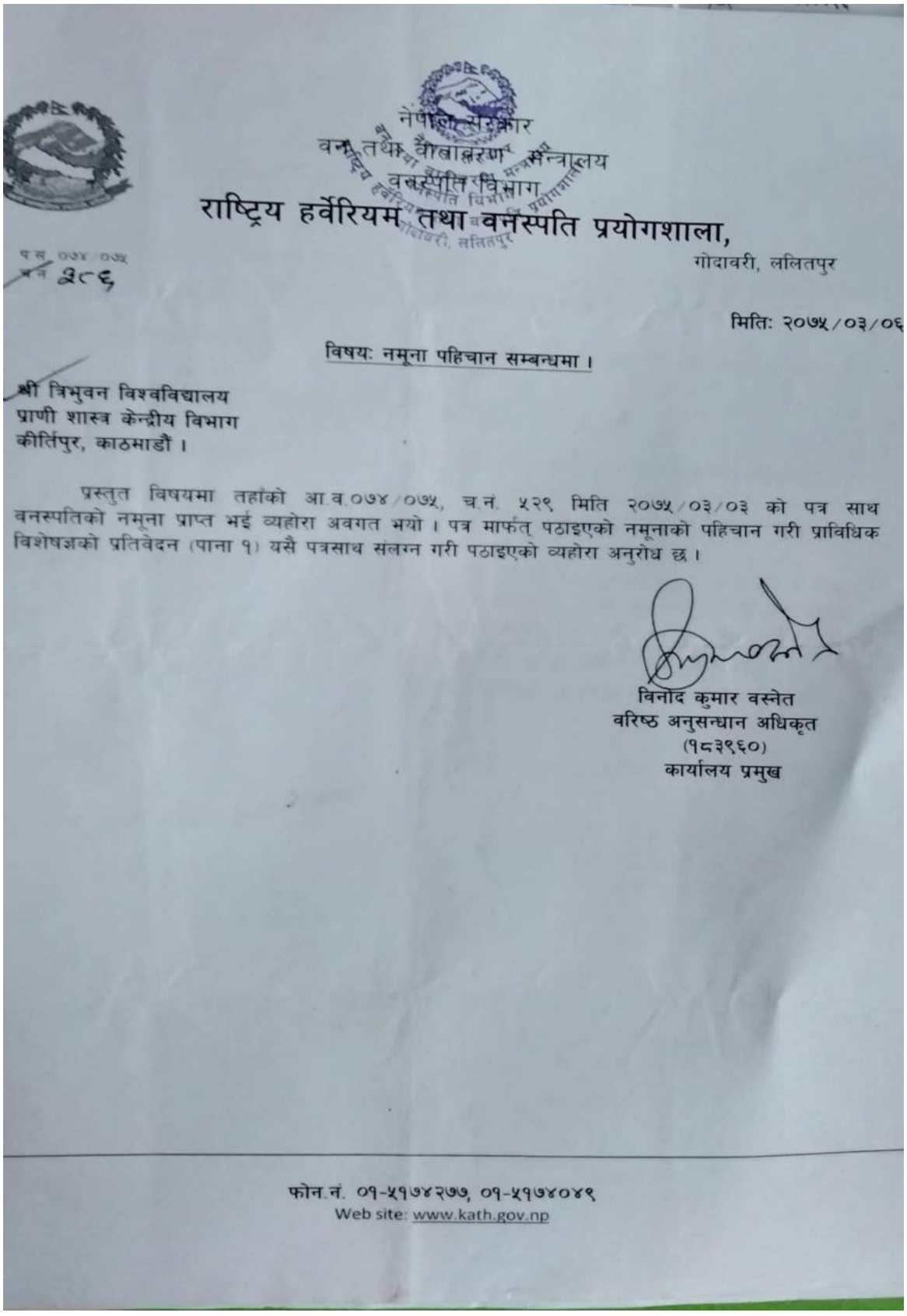
If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Nirbhay Kumar Sharma
Deputy Chief Administrative Officer

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.org.np, E-mail: nhrc@nhrc.org.np

APPENDIX II: Herbal Identification Report



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वनस्पति विभाग

राष्ट्रिय हर्वेरियम तथा वनस्पति प्रयोगशाला,

गोदावरी, ललितपुर

प.सं. ०७४/०७५
च.नं. ३८६

मितिः २०७५/०३/०६

विषयः नमूना पहिचान सम्बन्धमा ।

श्री त्रिभुवन विश्वविद्यालय
प्राणी शास्त्र केन्द्रीय विभाग
कीर्तिपुर, काठमाडौं ।

प्रस्तुत विषयमा तहाँको आ.व.०७४/०७५, च.नं. ५२९ मिति २०७५/०३/०३ को पत्र साथ वनस्पतिको नमूना प्राप्त भई व्यहोरा अवगत भयो । पत्र मार्फत् पठाइएको नमूनाको पहिचान गरी प्राविधिक विशेषज्ञको प्रतिवेदन (पाना १) यसै पत्रसाथ संलग्न गरी पठाइएको व्यहोरा अनुरोध छ ।

विनोद कुमार वस्नेत
वरिष्ठ अनुसन्धान अधिकृत
(१८३९६०)
कार्यालय प्रमुख

फोन.नं. ०१-५१७४२७७, ०१-५१७४०४९
Web site: www.kath.gov.np

प्राविधिक विशेषज्ञको प्रतिवेदन

१. नमूना परीक्षण गर्न पठाउने निकाय/व्यक्ति :- श्री त्रिभुवन विश्वविद्यालय, प्राणी शास्त्र केन्द्रीय विभ
कीर्तिपुर, काठमाडौं । (दिपा राई)

२. प्राप्त नमूनाको विवरण :- सिस्नो भनिएको वनस्पतिको नमूना १ (एक) था

३. यस कार्यालयमा प्राप्त मिति :- २०७५/३/०५

५. परीक्षणका आधारहरु:-
(क) हर्वेरियममा भएको नमूनाहरु संगको तुलनात्मक अध्ययन ।
(ख) सन्दर्भ सामग्रीहरुको अध्ययन ।

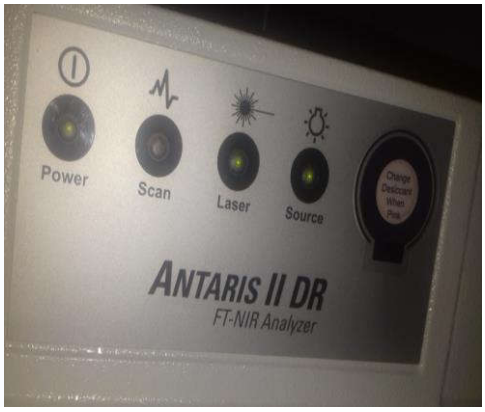
६.पहिचान प्रतिवेदन :- प्राप्त नमूनाहरुको Morphological अध्ययन र यस राष्ट्रिय हर्वेरियम तथा वनस्पति प्रयोगशालाको हर्वेरियममा राखिएका नमूनाहरु संगको तुलनात्मक अध्ययन गर्दा उक्त नमूना **Urticaceae** वनस्पति परिवारको ***Urtica parviflora*** Roxb. भएको प्रमाणित हुन गएको ।

७. परीक्षण गर्ने अधिकारी :-

गंगा दत्त भट्ट
सहायक अनुसन्धान अधिकृत
(१३७९०९)

APPENDIX III: PHOTO PLATES

1. Fishery Research Station, Begnas



2. Feed Analyzer

3. Researcher Collecting *U. parviflora*

4. Powder form of *U. parviflora*

5. Ingredients about to grind

6. Experimental Setup

7. Researcher & Co- Supervisor in CDZ Lab

8. Fish Tagging with polyester thread

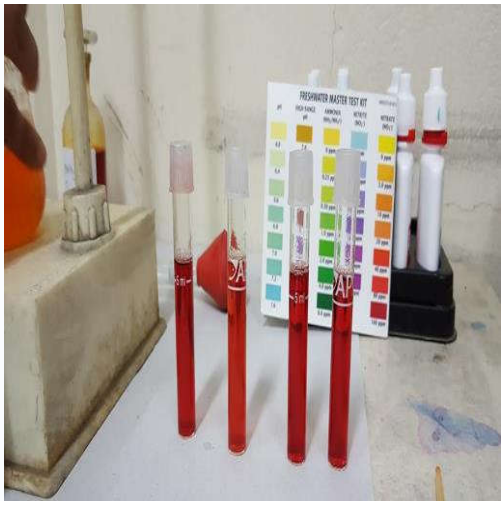
9. Fish Weighting

10. Length Measurement

11. Researcher Testing Water Quality

12. DO Test

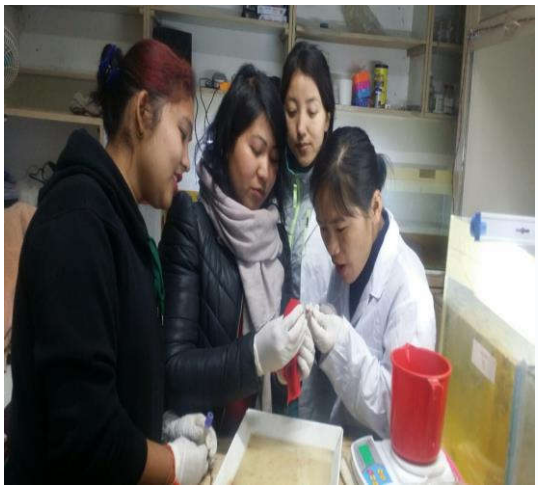
13. Alkalinity Test



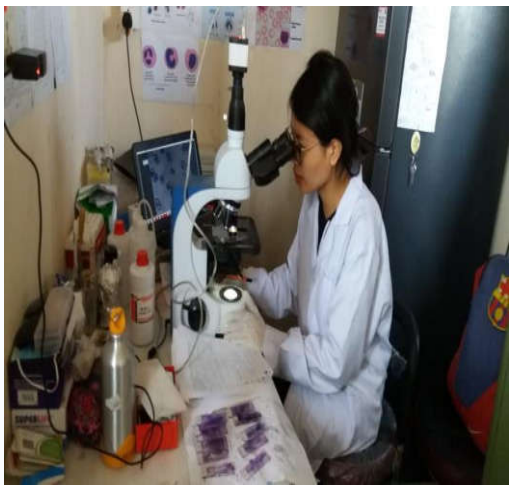
14. Ammonia Test

15. Blood Drawn From Cardiac Puncture

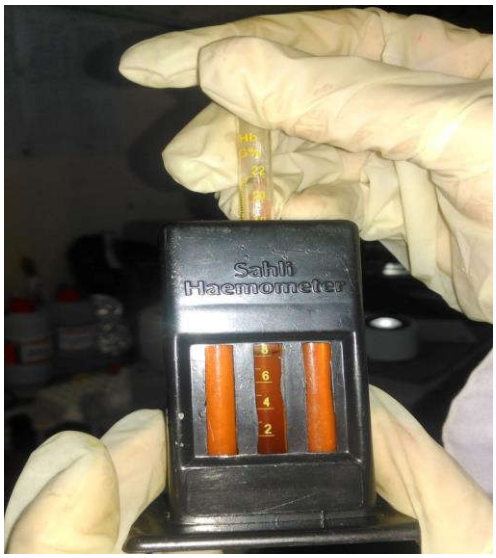
16. Researcher Collecting Blood Sample



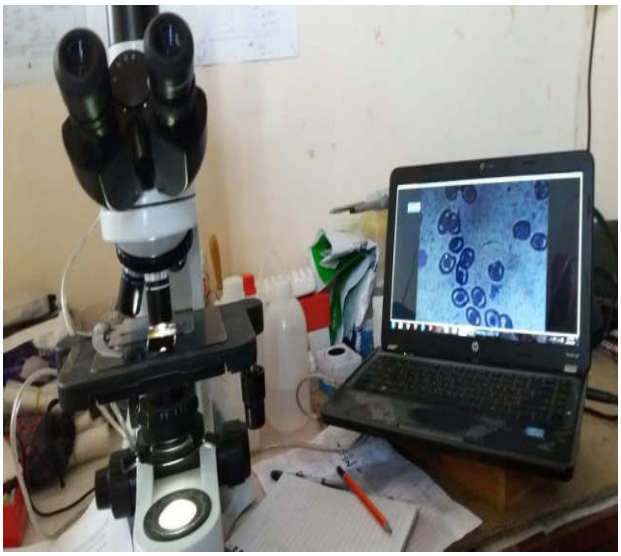
17. Collecting Blood Samples



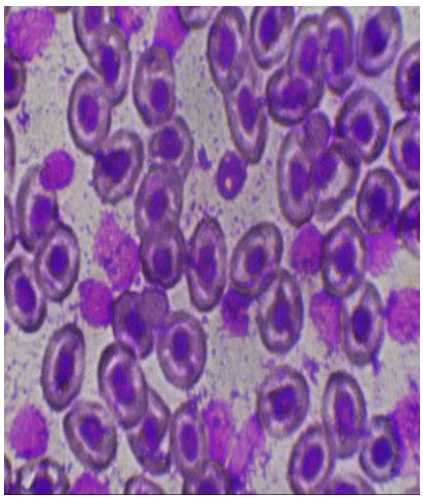
18. Researcher Analyzing DLC



19. Hemoglobin Test

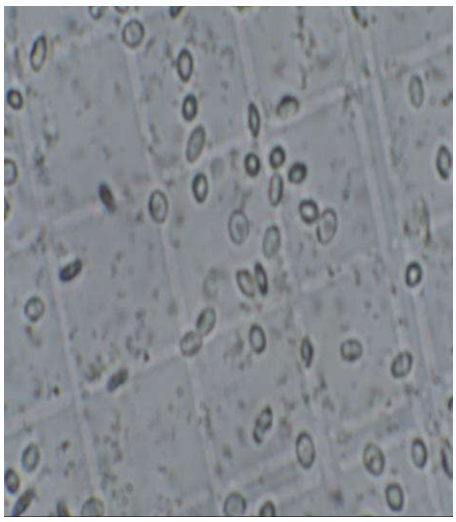


20. DLC Counting

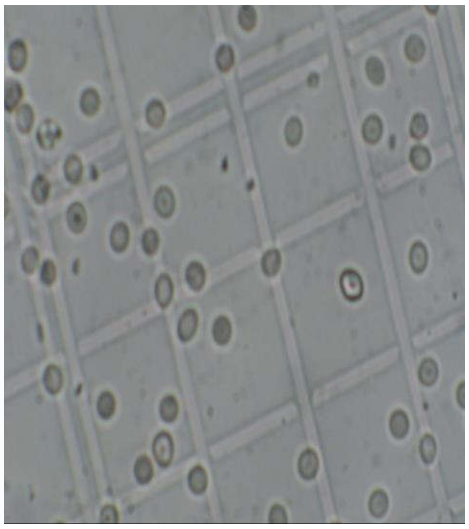


21. DLC (100×)

22. WBC (40×)



23. RBC (40×)



24. Platelet (40×)