CHAPTER ONE

INTRODUCTION

Sustainable capture fisheries and aquaculture play a critical role in food and nutrition security and in providing for the livelihoods of millions of people. Aquaculture accounts for a growing share of the global aquatic food production. The term aquaculture covers all forms of farming of aquatic animals and plants in freshwater, brackish water and saltwater. Aquaculture has the same objective as agriculture – the controlled production of food to improve the supply for our consumption. In the case of aquaculture, the products are aquatic animals and plants that grow in the water. Even in small quantities, fish can have a positive effect on the overall health and nutrition of humans. Fish is an important source of nutrients such as vitamins A, B and D, calcium, iron and iodine. Fish also provides vital amino acids that are often lacking in staple foods such as rice or cassava. It is therefore vital to the food security of many of the world's poor, especially in coastal areas and in Small Island developing States. Through aquaculture, we can produce protein and nutrient-rich food throughout the year.

Low-income farmers who invest in fish farming will be able to generate additional income and food for their family and potentially for the market. To be successful, an aquaculture operation requires much careful planning. The available natural resources, such as water and land, but also the local temperature and other factors influence the choice of the species to be farmed and the production system to be used. Climate does not limit the scale of aquaculture but it can determine the species that can be grown. Aquaculture can be done in a pond, a river, a lake, an estuary or in the sea.

The availability of high-quality water is usually the most crucial resource when making decisions about where, what and how much fish to farm. The most common small-scale aquaculture systems are small- pond fish farming and fish farming in lakes, rivers, dams and reservoirs. Climate change may have various negative impacts on aquaculture, including sea-level and temperature rise, rainfall fluctuations, and natural hazards such as floods and drought (FAO, 2009).

Sea level rise, for example, may threaten inland freshwater aquaculture by causing saltwater and brackish water to move further upstream and into rivers. Existing water bodies may also shrink or their water levels may decrease owing to erosion, drought and increasing temperatures. Increase in temperatures may also lead to lower levels of dissolved oxygen in the water, which would increase mortality of fish.

Through various exercises, such as small group discussions, hands-on tasks and demonstrations, Junior Farmer Field and Life School (JFFLS) participants will learn about the various types of aquaculture that can be used to provide new business opportunities for farmers and fishers.

They will also learn about the importance of preliminary planning and management of daily activities and procedures to ensure a successful aquaculture business.

Madicago sativa Linn, commonly known as ‘Alfalfa’, is a tonic plant rich in proteins, minerals, enzymes and vitamins, Bulk quantity of the whole plant is required in the pharmaceutical industries especially in homoeopathic pharmacies, Hence, there is a great need to cultivate this plant for sustained supply of the drug. Use of good and adequate phosphate containing farm yard manure, timely irrigation and appropriate spacing between plants results in good yield.

Medicago sativa Linn Commonly known as ‘Lucerne’ or Alfalfa’, belongs to the family fabaceae widely occurs in the Caucasian region and in the mountainous regions of Iran, Afghanistan and adjacent localities. The cultivated form probably arose in western Persia, whence it has spread to many countries. It is used as tonic due to presence of high percentage of proteins (60.5%), minerals, enzymes, vitamins etc. Alfalfa is a valuable source of vitamins A and E fresh is rich in Vitamin C (1.78 mg/g) but it loses 80% of vitamins on drying. The enzymes reported in alfalfa are amylase, emulsion, coagulase perxidase, erepsin, lipase, invertage and pectinase (W.I., 1962; Uphof, 1968). It is highly valued as a legume fodder, most important fodder crop in USA where millions of acres are devoted to it. In India it is also valued as green fodder especially for horses and its cultivation is confined to military farms. But is largely used by various pharmaceutical industries especially in homeopathic pharmacy. Updated market survey indicates that there is an increasing demand and use of this drug in preparation of health and vitality tonic i.e. Alfalfa tonic. It favorably influences nutrition, evidenced in ‘tonic up’ the appetite and digestion resulting improved mental and physical vigour with gain in weight (Boericke, 1927). There is a short fall in supply of this important raw drug material and demand is rising day by day due to lack of knowledge of proper cultivation, therefore, it was felt to undertake the experimental cultivation of this important plant for sustained supply by growing the plant for sustained supply by growing the plant in an organized way in our experimental garden we are growing this plant for consecutive six years with success. Cultivation Medicago sativa can be grown on a wide range of soils but does best on rich, friable, well drained loamy soil while it fails to grow in acid soil, It is hardy and drought resistant, can withstand high temperature (40o C to 45o C) and an annual rain fall of 45-50 CM in optimum but can also survive in low rain fall of 35CM.

Objective:

1. To evaluate the effect of different alfalfa level supplementation on growth performance of Nile tilapia (*Oreochromis niloticus)*.
2. To determine diets the proximate composition of the studied fish fed with experimental.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture

Global production of fish from aquaculture has grown rapidly over the past four decades, contributing signify cant quantities to the world's supply of fish for human consumption. Aquaculture now accounts for almost half (45%) of the world's food fish (note that 'food fish' or simply 'fish' in this document refers to production of aquatic animals' fish, crustaceans, mollusks, echinoderms, amphibians). Aquatic plants are considered separately. With its continued growth, it is expected that aquaculture will, in the near future, produce more fish for direct human consumption than capture fisheries. Aquaculture, which started as primarily an Asian freshwater food production system, has now spread to all continents, encompassing all aquatic environments and using a range of aquatic species. From an activity that was principally small scale, non-commercial and family based, aquaculture now includes large-scale commercial and industrial production of high-value species that are traded at national, regional and international levels.

largely based on small-scale operations, there is a wide consensus that aquaculture has the potential to meet the growing global demand for nutritious food fish and to contribute to the growth of national economies, while supporting the sustainable livelihoods of many communities (FAO 2006a).

Production trends World aquaculture has grown tremendously over the past 50 years from a production of less than one million tons in the early 1950s to 48.1 million tons in 2005 (all production data provided in this document are based on 2005 production statistics given in FAO Fish Stat+ 2007 database); an average annual growth rate of 8.8% . Current production had a farm-gate value of US$70.3 billion, increasing significantly in value as farmed products move along the market chain to consumers. Of this production, 32.4 million tons (or 67.3%) was produced in the People's Republic of China (hereafter referred to as China) and 22.3% from the rest of the Asia–Pacific region. Western Europe contributed 4.2% with 2.0 million Tonnes (valued at US$6.2 billion), while Central and Eastern Europe contributed 270 000 tonnes or 0.6%. Latin America and the Caribbean and North America contributed 2.9 and 1.3%, respectively. Finally, production from the Near East and North Africa and sub-Saharan Africa accounted for 1.2 and 0.2%, respectively, of the global total for 2005. In addition to fish production, aquaculture activities in 2005 produced 14.8 million tonnes of aquatic plants worth US$7.1 billion. The vast majority of aquatic plant production (99.8%) came from the Asia– Pacific.

2.2 Sudan fisheries

The total annual fin fish production in Sudan is around 140000 tons from fresh water and 8000 tons from marine water (fully future operating estimate), marine resources divided into artisanal fishery (about 3000 tonnes), trawling (about 2000 tonnes), per sine fishery (about 2300 tonnes), shrimp from trawling (about 60 tonnes), shrimp from culture (about 6 tonnes), trochaus (about 724 tonnes), mother of pearl shell (about 12 tonnes) and sea cucumber (about 60 tonnes) Vine et al., (1980). Tuna fishes Not target to fish in Sudanese Red Sea coast, these retaining to limiting in fishing location and gear use by local fishers, most catch taken by hooks and line, seasonally foreign fleets come from Egypt under economic protocol, sign by Sudanese government and Egypt, use to fish in Sudanese trawling area (Southern area), also pure sine use by Egyptian vessels. But since 2010 this fishing activates was a stop.

Since 2010 the only fishing activates running by local fishers with small fibber glass boat and wooding, no regular a statistical data had been taken for fish species or gear types. Only statistical data records taken from the fish market in Port Sudan, all tuna fishes record under name of mackerel fishes. Shark and other marine fish product were recorded also from the fish's local market. No statistical and regular data taken for marine mammal and sea bird.

Sudan fishery in general depending on traditional fishery (Khalid, et al 2008), subjected to use the traditional gear in fishing activates in whole the coast (Reed, 1994). Fishers usually used to fish in limiting areas near their villages that set along the coast, they used a hooks and line for the coral reefs fishes and also they used a gills net, pure sine net for bottom fishes Mishrigi et al., (1993). Mainly their fishing concentrating on coral area especially in the north area where coral reefs and inland distributed there near the coast, in south area they usually used the gill nets for bottom fishes and migration fishes (Fisheries Administration, 2012). Boats and fleets used here are small boats, lengths range between 7 meter to 8 meter for woods boat and fibber glass boats, for bigger one lunches their length range between 9 meter to 13 meter, this usually fish in south area for Trochaus and strombus fisheries. In 2012 the fishing activity can be summarize below, mainly activities in 2012 concentrated in traditional fishing activity, also

Ornamental fishing activity appear, 6 companies starting since 2011 to fish Ornamental fish from the Sudanese coast.

2.3 Tilapia species

2.3.1-Biology

Tilapia is one of the most widely cultured fish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2009), and this contribution has been exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia.

Tilapias are easily cultured and highly adaptable to a wide range of environmental conditions. Tilapia feed on a wide variety of dietary sources, including phytoplankton, periphyton, zooplanktons, larval fish, and detritus.

Adult tilapias are principally herbivorous but readily adapt to complete commercial diets based on plant and animal protein sources. In the United States, the most commonly farmed tilapia species are, in order, Nile(*Oreochromis niloticus*), Mozambique (*O.mossambicus*), blue (*O. aureus*), and hybrids (Green, 2006). This publication provides a brief overview of environmental and nutritional requirements of tilapia.

Tilapia can tolerate a wider range of environmental conditions including factors such as salinity, dissolved oxygen, temperature, pH, and ammonia levels than most cultured freshwater fishes can. In general, most tilapias are highly tolerant of saline waters, although salinity tolerance differs among species. Nile tilapia is thought to be the least adaptable to marked changes (direct transfer, 18 parts per thousand in salinity); Mozambique, blue, and red belly (*T. zilli*) are the most salt tolerant (El-Sayed 2006).

With the exception of Nile tilapia, other tilapia species can grow and reproduce at salinity concentrations of up to 36 parts per thousand, but optimal performance measures (reproduction and growth) are attained at salinities up to 19 parts per thousand (El-Sayed 2006). Tilapia are, in general, highly tolerant of low dissolved oxygen concentration, even down to 0.1 mg/L (Magid and Babiker, 1975), but optimum growth is obtained at concentrations greater than 3 mg/L (Ross, 2000). Temperature is a major metabolic modifier in these fish. Optimal growing temperatures are typically between 22° C (72° F) and 29° C (84° F); spawning normally occurs at temperatures greater than 22° C (72° F). Most tilapia species are unable to survive at temperatures below 10° C (50° F), and growth is poor below 20° C (68° F). Blue tilapia are the most cold tolerant, surviving at temperatures as low as 8° C (46° F), while other species can tolerat temperatures as high as 42° C (108° F; (Sarig, 1969; Morgan, 1972; Caulton, 1982; Mires, 1995).

Other water quality characteristics relevant to tilapia culture are pH and ammonia. In general, tilapia can tolerate a pH range of 3.7 to 11, but best growth rates are achieved between pH 7 to 9 (Ross, 2000). Ammonia is toxic to tilapia at concentrations of 2.5 and 7.1 mg/L as unionized ammonia, respectively, for blue and Nile tilapia (Redner and Stickney, 1979; El-Sherif et al., 2008) and depresses feed intake and growth at concentrations as low as 0.1 mg/L (ElSherif *et al.*, 2008). Optimum concentrations are estimated to be below 0.05 mg/L (El-Sherif *et al.*, 2008).

Plant protein and chemical composition of alfalfa meal

The use of leaf protein concentrate has great potential in animal nutrition. However, the use of alfalfa protein concentrates as a protein source in diets of Nile Tilapia (Oreochromis niloticus) was investigated by Oliver – Novoa *et. al.*, (1990). This concentrate is produced by grinding the plant followed by separating the juice by pressing the juice then coagulated by heating. Leaves of plants are considered as sources of protein, minerals and carotene. This review will focus on leaves used in fish nutrition. With a little will be mentioned about their use in the nutrition of monogastric animals. Alfalfa means the best fodder. It is a legume and its leaves and stems were exploited commercially a principles ingredients in compose feed manufacture.

Alfalfa does not appear to contain the nutritional factors that limit its use in composed feeds for this it is safe to be incorporated into composed fish feed. Leaves of Alfalfa contain 25% protein or higher and it's a good source for vitamins E, K and carotene (Patrik and Schoible 1980). The use of alfalfa leaf protein concentrate as a protein source in -45- diets of Nile Tilapia (Oreochromis Niloticus) was investigated by Olvera-Novoa (1990). In contrast to the above finding, Omar, *et. al*., (1994) showed that fish meal protein can substituted at 5, 10, 20 and 30% by alfalfa leaf meal protein.

2.4 Effect of alfalfa meal on body weight and gain of Nile tilapia

Little information is available on the incision of alfalfa meal in diets of Nile tilapia where leaves of paints are considered as sources of protein, minerals and carotene. This review will focus on leaves used in fish nutrition with a little will be mentioned about their use in the nutrition of monogastric animals. Studies on green plant leaves as dietary sources for fish have focused on the use of leaf protein concentrates such as Rye grass and alfalfa leaf protein concentrate (Ogino et. al., 1978 and Olvera –Novoa et. al., 1990).

Shireman *et al*., (1978) found that supplementation of alfalfa meal in diets of grass carp improved growth rate. Also Pantastico *et. al* (1986) illustrated that supplementation of Azolla plant in diets enhanced growth of Nile Tilapia (Oreochromis Niloticus) incages. Wee, K.L.X. and S.S. Wang (1987) reported that growth performance of Nile Tilapia (Oreochromis Niloticus) reduced by increase of Leucaena leaf in the diets. While, Omar et al., (1994) showed that control group of Nile Tilapia (Oreochromis Niloticus) had significantly (P<0.05) better -47- weight gain as compared with groups of fishes fed diets containing 9,10,20 and 30% levels protein from alfalfa meal. Also, he added that increasing plant leave meal decreased growth rate of blue Tilapia (*Oreochromis oureus*) fish. However, Miguel, et. al., (1990) indicated that diets containing alfalfa leaf protein gave the best results with growth rates higher than those obtained with a fish meal based diets when the plant protein replaced up to 35% of the fish meal protein in the diet, but the increasing level depressed growth rate. On the other hand Emil (2001) reported that body weight of Nile Tilapia decreased as the level of Azolla meal incased. The same author showed that body weight was numerically higher for fish fed 5% Berseem leaf meal followed by group fed combination of 5% Berseem leaf meal and 5% Mung been Seeds. Also, feeding of Berssem leaf meal (5and 10%) and combination of 5% Berssem and 5% Mung been seeds induced insignificantly higher body weight gain as compared to the control group. Ali et al., (2003) reported that growth performance of Nile Tilapia was reduced when the inclusion level of alfalfa meal was over 5% in the as compared with the control diet. Furthermore, Miguel et al., (2003) indicated that growth rate of Nile tilapia (Oreochromis Niloticus) reduced when levels of alfalfa meal -48- increased in the diet (15, 25, 35, 45 and, 55%). The same author added that the leaf protein could be included at levels of up to 35% of the dietary protein in feeds of Tilapia.

Wee, K.L.X. and S.S. Wang (1987) reported that feeding Nile Tilapia on *Azolla pinnata*, Medic Sativa and *penniseetum purpureum* had significant effect on final body weight. He added that daily gain was between 0.86 to1.13 g. Also, Pullin et al,. (1987) indicated that adding Azolla in diets of

Nile Tilapia (Oreochromis Niloticus) increased growth rate. Soliman (1994) indicted that Nile Tilapia (*Oreochromis Niloticus*) fish fed diets containing 1,2,3,5 and 8% plant leaves had significantly the best final body weight gain as compared with control group. On the other hand, Olvera-Novoa *et. al.*, (1990) indicated that the use of purified alfalfa leaf protein in diets of *O.mosambicus* reduced performance at higher inclusion level. Hassan et. al., (1997) indicated that the supplementation of different plant protein sources in diet of carp fishes resulted insignificant ($P<0.05$) variation growth. The results of Miguel, et. al., (1997) indicated that the highest growth rate was observed in Nile tilapia (Oreochromis Niloticus) when the diets contained 20-30% of Cowpea protein concentrate in replacement with Fish meal. Omar *et.al*.,(1994) indicated that when fish Tilapia meal protein as -49- substituted at 5, 10, 20, and 30% level by alfalfa leaf meal body weight gain decreased significantly with increasing the substitution level. Olvera-Novoa (1990) showed that feeding Nile Tilapia on diets containing 15 and 35% alfalfa meal exhibited the highest growth response but the lowest response was observed for group fed 55% substitution level.

2.4.1 Specific growth rate (SGR)

Soliman (1994) indicated that fish fed diets containing different levels of plant leaf (1, 2, 3, 5 and 8%) had significantly the best specific growth rate. Al-Asgah, *et. al*., (2003) found that no significant differences were observed in specific growth rate of Nile Tilapia when fed on diets containing 5% Alfalfa meal. Miguel, *et. al*., (1997) reported that specific growth rate of Nile Tilapia was significantly improved when fed on diets containing Azolla pinnata, Medicago *penniseetum purpureum*. Sadek and Moreau (1998) found that specific growth rate of Nile Tilapia was 1.8 when cultured in ponds.

Cruz and Laudencia (1978) obtained a 2.3% increase in SGR when copra meal supplied 5% of the protein in the diet of Nile tilapia. Omar et. al., (1994) reported that SGR of Nile tilapias fish was significantly decreased when fish meal in its diets was substituted by alfalfa meal at 5, 10, 20 and 30 % -50- levels and the decrease was more pronounced at the higher substitution levels.

Olvera-Novoa (1990) indicated that SGR of Nile tilapia was best when fed on diets containing 35% of alfalfa meal.

Abdel-Hamid et al.,(2000) indicated that SGR of Carps was greater (0.98 %/day) when fed on M.Sativa diets. Moustafa (1993) indicated that for Nile tilapia the SGR was 0.75; 0.944; 0.991 and 1.061 %/day for 20, 24, 28 and 32 % protein level in diets when fish were stoked at a rate of 120 fish/m3 in Cages.

2.4.2 Feed conversion ratio (FCR)

Pullin, *et. al.,* (1987) showed that adding Azolla meal in diets of Nile Tilapia (Oreochromis Niloticus) increased feed conversion ratio .Also, Soliman (1994) indicated that fish fed diets containing different levels of plant leaf 1,2,3,5 and 8% had significantly the best food conversion ratio. Al-Asgha, *et.al.* (2003) reported that no significant differences were observed in feed conversion ratio of diets containing 15% of alfalfa meal. Furthermore, Omar, et. al., (1994) found that feed conversion ratio were higher in Nile Tilapia fish fed control diets as compared to fish fed on diets containing 5,10,20,and 30% protein from Alfalfa meal. Ogino, et. al., (1978) indicated that alfalfa meal could replace up to 43-40% -51- of total protein in diets for fishes without any loss in feed conversion ratio when compared with casein control ration.

Olvera, *et. al*. and Chaves (1988) they reported that feed efficiency utilization decreased of Tilapia fed diet containing 15 % of dietary inclusion of raw *Sesbania grandifflora* seed meal. However, Emil (2001) showed that feed conversion ratio of Nile tilapia decreased when fed on diets containing Azolla meal at 5 %. Also, he indicated that FCR decreased significantly in group fed on diets containing berssem leaf meal (5 and 10%), mung been seed (10 and 15%) and combination of 5% of both as compared to the control group, while FCR was numerically increased in groups fed 15% berseem leaf meal and 5% mung been seeds as compared with control group. Cruz and Laudencia (1978) obtained an l.53% increase in FCR when copra meal supplied 5% of the protein in the diet.

Also, Chou, *et. al*., (2004) reported that existing significant differences in FCR of fish when the replacement fish meal with soybean meal in diets up 40%. Hassan, *et. al*., (1997) indicated that the supplementation of different plant protein sources in diet of carp fishes had no significant ($P<0.05$) variation in FCR. Roy (1994) showed that fish fed the water hyacinth leaf meal free diet exhibited the best FCR followed by the group fed 25% and 50% water hyacinth containing diets.

Labib, *et. al*., (1994) illustrated -52- that no significant differences in FCR of fishes fed diet containing 0, 5, 15 and 25% Egyptian mallow leaf meal and control group. Olvera-Novoa (1990) reported that FCR for Nile Tilapia fed 35% substitution of alfalfa meal was best, while FCR depressed with increases in the substitution level in the diet.

2.4.3 Feed utilization and feed intake

Pantha (1982) showed that diet utilization did not affected when diet containing 40.1% plant protein. Wee, K.L. and S.S. Wang (1987) showed that feed utilization of Nile Tilapia decreased with increasing Leucaena leaf meal levels in the diets.

Nour, *et. al*., (1989) indicated that feed utilization of Nile Tilapia decreased by feeding diet containing 10, 20, and 30% water hyacinth leaf meal.

Olvera –Novoa e*t. al*., (1990) indicated that the use of purified alfalfa leaf protein in diets of O.mosambicus reduced feed utilization at higher inclusion level. Abdel-fattah and Saida (1990) showed that feed utilization efficiency of Nile tilapia was not affected by feeding on diet containing Azolla meal at a level of 25 %. Moreover, Omar *et. al*., (1994) indicated feed utilization reduced of Nile Tilapia fed different levels of Alfalfa meal (5, 10, 20 and 30%).

Omar *et. al*., (1994) indicated that feed utilization was poorer when Nile Tilapia fed diets containing leave plant -53- protein. Hassan, *et. al*., (1997) indicated that the supplementation of different plant protein sources in diet of carp fishes had no significant (P<0.05) effects on fed utilization. Soliman (1994) showed that nutrient utilization of Nile Tilapia (Oreochromis Niloticus) improved when fed on diets containing 1,2,3,5 and 8% plant protein sources as compared with control group.

Al-Asgah, *et. al*., (2003) showed that nutrient utilization reduced of Nile Tilapia when fed diets containing 5% Alfalfa meal, as compared with control group.

The efficiency of fed utilization decreased linearly when the level of Alfalfa meal in diets increased over 5%. Hossain, *et. al*., (2001) indicated that feeding fishes in diets containing Sesbania meal had no affect on feed Utilization.

Al-Ashgh, *et. al*., (2003) illustrated that feed intake reduced in Nile Tilapia fed diets containing Alfalfa meal as compared with control group.

2.2.6. Protein efficiency ratio (PER).

Miguel, et al., (1990) indicated that protein efficiency ratio was higher in fishes fed control diet, 15, and 35 alfalfa meals as compared with other treatments group Soliman (1994) indicated that Nile Tilapia fish fed diets containing 1,2,3,5, and 8% plant leaves had significantly the best protein efficiency ratio as compared with control group. Also, AlAsgha, *et. al*., (2003) reported that protein efficiency ratio in -54-Nile Tilapia was not affected significantly when fed diets containing 10 or15% Alfalfa meal. While, Emil (2001) reported that protein efficiency ratio of Nile Tilapia were worst when fed Azolla meal at 15% level as compared with other group The same author reported that the best PER was recorded for fish fed 10% berseem meal followed by the group fed combination of 5% berseem meal and 5% mung been seeds.

Mohanty and Dash (1995) showed that use of a diet containing 60 % of dry Azolla improved the PPE to 1.77 Chou (2004) reported that significant differences existed in PER of fish when the replacement fish meal with soybean meal in the diet reached 40%. Miguel, *et. al*.,(1997) indicated that the PER of Tilapia was the best when cowpea protein concentrate replaced 40% of Fish meal in the diet. Stone, *et. al*., (1999) indicated that PER value was best for fish fed on silage.

Omar, *et. al*., (1994) indicated that when fish Tilapia fed meal protein as substituted at 5, 10, 20, and 30 % by alfalfa leaf meal PER decreased significantly with increasing the substitution. The same author showed that adding of 15% Leucaena leaf meal to diets of Tilapia resulted in higher PER.

Labib, et. al., (1994) illustrated that PER was similar for fishes fed diet containing 0, 5, 15 and 25% Egyptian mallow leaf meal to the control group.

Goel, *et. al*., (1977) indicated that PER was improved by addition of the cauliflower leaf -55- protein concentrate to diets of fish containing the wheat flour.

2.4.4 Protein and energy utilization

Miguel, *et. al*., (1990) indicated that carcass nitrogen deposition were best for fishes fed 15,35,25 and 15% alfalfa meal as compared with other dietary treatment or control group. But the highest value for nitrogen utilization for fished fed diet containing 15% Azolla meal. Also, the highest energy utilization was recorded for the group fed 10 % Berseem meal, while the lowest were records for the group fed 5% Berssem meal and 5% and 10% mung bean seeds. Chou (2004) reported that replacement of Fish meal with soybean meal up to 40 % in diets of tilapia resulted in significant differences in NPU.

Omar, *et.al.* (1994) showed that replacing Fish meal protein with alfalfa leaf meal at 5, 10, 20 and 30 % levels in Tilapia diets decreased the apparent net protein utilization with each increases in the level of alfalfa leaf protein. However, Roy (1994) showed that fish fed the water hyacinth leaf meal free diet exhibited the best protein utilization followed by the group fed 25% and 50% levels respectively.

Labib, *et. al* (1994) illustrated that net protein utilization was similar for fishes fed 0,5,15 and 25% Egyptian meal and control group.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area-Experimental site

The study was conduct in fish hatcheries at Department of fisheries Science and Wildlife, Collage of Animal Production Science and Technology, Sudan University of science and technology. The study started from April and lasted twin teen of July 2016 for 8 weeks.

3.2 Experimental design

Oreochromis niloticus fry with an average initial weight “0.01-0.015” were collected from the fresh water aquaculture center,Hulua kuku acclimatized to experiment conditions for a period 10 days in 1000 liters capacity experiment tal tank, before the start of actual experiment during this period they were kept on the same standard diet as feed previously at the hatchery .

Forty randomly captured fished (divided into four replicates of 30 fish in each). The fish were kept in plastic tank (34*22*18)filled with dechlorinated and well –aerated –tap water and fitted with waste filtration facility, Compressed air was used maintain the oxygen supply .

Fish were fed a mixture of the all tested experiment diets in order to habituate them to locally formulated feed after acclimatization, regular four isoenitrogeous and isonergetic diets containing different levels of alfalfa meal (0 %,5% ,10% and 15 %). The formulated diet containing Control(0%), T1(alfalfa 5%),T2 (alfalfa 10%) ,T3(alfalfa 15%)

Alfalfa leaves exposure to the sun to become dry then grinding the leaves in powder from adding for the dry ingredient nutrient supplemental and water we got the paste cutting for small pieces and dried in air for 1 day. The dried material was grinded in electric mill then stored for starting nutrition.

In all experimental feeding regimes feeding was done three times daily at 08:30, 11:30 and 01:30 h.

Table 3.1-Containing different levels of Alfalfa.(On dry mater basis).

Ingredient	**0% Control**	T1 **5%**	**T2 10%**	**T3 15%**
Fish meal	400	375	350	300
Grand not cake	150	125	100	100
Surgeon meal	100	100	100	100
Wheat been meal	150	120	150	150
Broad flower	50	50	50	50
Starch	100	100	100	100
Mineral mix	10	10	10	10
Vitamin mix	9	9	9	9
Sun flower oil	31	31	31	31
Alfalfa	**-**	50	100	150
Total	1.000	2.000	1.000	1.000

Table 3.2: Proximate chemical composition of diet with different levels of alfalfa meal(on dry basis)

Parameters Treatments	M	D.M	C.P	E.E	Ash	C.F	N.F.E
T1 Control	5.50±0.70[a]	94.50±0.70 [c]	30.00±0.56[b]	4.05±0.07[a]	12.50±0.70[c]	2.98±0.02[a]	44.96±0 .61[b]
T2	4.50±0.70[b]	95.50±0.70[b]	30.10±0.14[b]	3.95±0.7[b]	12.50±0.70[c]	2.94±0.05[a]	46.01±0.15[a]
T3	3.50±0.70[c]	96.50±0.50[a]	31.00±0.28[a]	4.25±0.07[a]	13.50±0.70[b]	2.87±0.02[a]	44.87±1.22[b]
T4	5.50±0.70[a]	94.50±0.70[c]	31.35±0.49[a]	4.25±0.07[a]	14.50±0.70[a]	2.77±0.02[a]	41.56±2.05[c]

[a,b,c,d] Means in the same column with superscripts are significant different at level ($p<0.05$).
whereas:
D.M= Dry matter, C.P= Crude protein, E.E= ether extract, C.F= crude fiber , N.F.E= nitrogen free extract.

3.3 Management of the tanks and fry

For maintenance of good health and growth of fry frequent cleaning of tanks and feeding of fry were performed .All the tanks were scrabble daily for removing accumulated inside surface of rearing tanks ,feces ,waste and particles of food and dead bodies of fish were siphoned at systematic period. During the experiment time water was exchanged with new water at a rate of 20 % of water column daily to keep the water quality suitable for te experimental fish, water was empty out completely every 15 days and all tanks were cleaned and fish wastes were removed, after that filled with new water .

Water quality parameter including water temperature (C), Dissolved Oxygen (DO) concentration (mg/l), pH (degrees) ,ammonia (NH_3) (mg/l), nitrate and nitrite were measured by aqua-zoal water kits .Water quality parameter were measured every 15 days before sampling was performed. All tested water quality parameters were within optimal levels required for growth and evolution of Nile tilapia.

3.4 Growth performance measures

Body weight gain (WG) = final body weight –Initial body weight

Specific growth rate (SGR) = loge final weight- loge initial weight/Time (days)

Percentage weigh gain (%) = final weigh -initial weight/initial weight × 100

Survival rate (%) = initial number of fish stock-mortality/initial fish stock

Feed exploitation

Food conversion ratio (FCR) = Feed fed/fish weight gain

Protein efficiency ratio (PER) = mean weight gain (g)/protein intake

3.5 Proximate composition determination

The proximate composition for experimental diets and fish carcass were measured according to AOAC (1990). As follows:

3.5.1 Moisture Content Determination:

The samples were first weight (Initial weight) then dried in an electric oven at 1050C for 24-30 hours to obtain a constant weight. The moisture content was calculated as follows:-

Moisture content (%) = $$\frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

3.5.2 Crude Protein Determination:

The Kjeldal method for estimation of nitrogen was applied. Nitrogen content was converted to protein percentage by multiplying by 6.25 as follows:

$$\textbf{Protein \%} = \frac{(Va - Vb) \times N \times 14 \times 6.25}{1000 \times Wt} \times 100$$

Whereas:

Va = volume of HCL used in titration

Vb = volume of sodium hydroxide of known normality used in back titration

14 = conversion factor of ammonium sulfate to nitrogen

6.25= conversion factor of nitrogen to protein

Wt= weight of sample

N= normality of NaoH

3.5.3 Crude Fat Determination:

Fat content of each sample was determined according to Soxhlet method by ether extract using 2 gm of fish samples. Extraction continued for 5 hours at 100 0C before finding the weight of the extract fat. Fat percentage was then calculated as follows:

$$\textbf{Fat \%} = \frac{\text{Extracted fat weight} \times 100}{\text{Sample weight}}$$

3.5.4 Ash Content Determination:

Ash was determined by heating 1 gm at 5500C in muffle furnace until a constant weight was obtained. Ash content percentage was given by the following formula: **Ash % =** Ash weight x 100

Sample weight

3.6 Statistical Analysis

Results were expressed as means ± standard deviation (SD). Data were statistically analyzed using ANOVA one-way analysis of variance. Comparisons among means were made by (LSD) when significant F- values were observed ($P < 0.05$), using SPSS version (16).

CHAPTER FOUR

RESULTS

Data of growth performance of *Oreochromis Niloticus* fed different levels of alfalfa meal is presented in table 4.1 significant (P <0.05) differences were observed in body weight gain of fish fed different levels of alfalfa meal.

Proximate analysis studied fish showed some degree of variation in the protein, fat, ether extract, ash and nitrogen free extract for fish fed (5%, 10%, and 15%) of alfalfa meal (Table 4.2).

Table 4.1: illustrate growth performance of *Oreochromis niloticus* feed with different level of alfalfa meal.

Parameters Treatment	WG	SGR	PER	FCR	PI
T1 control	18.15 ± 3.21^{b}	1.29 ± 0.07^{a}	3.90 ± 0.69^{b}	1.51 ± 0.26^{a}	4.65 ± 0.0^{a}
T2	11.25 ± 10.0^{d}	0.85 ± 0.71^{b}	2.54 ± 2.14^{c}	1.89 ± 0.52^{a}	4.50 ± 0.0^{a}
T3	15.83 ± 1.45^{c}	1.24 ± 0.03^{a}	3.53 ± 0.31^{b}	1.70 ± 0.15^{a}	4.51 ± 0.0^{a}
T4	19.49 ± 1.88^{a}	1.32 ± 0.04^{a}	4.14 ± 0.40^{a}	1.39 ± 0.12^{a}	4.70 ± 0.0^{a}

^{a,b,c,d} Means in the same column with superscripts are significant different at level ($p<0.05$).

Whereas:

WG= weight gain, SGR= specific growth rate, PER= protein efficiency rate, FCR=food conversion ratio,

PI= Protein intake.

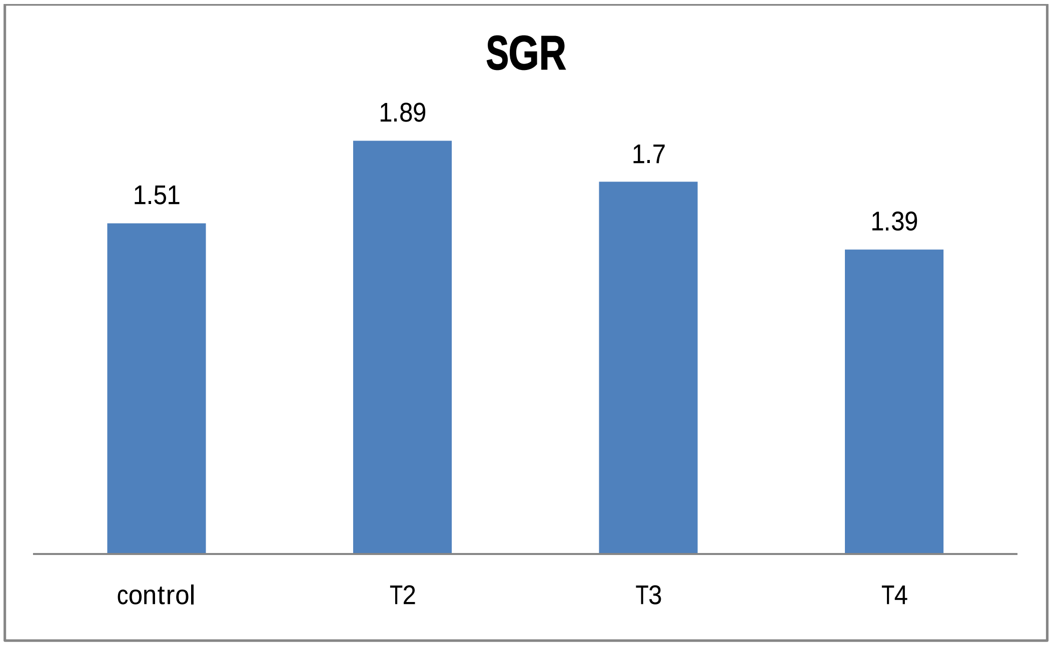

Figure (1): Food conversion ratio (**SGR**) of studied fish fed with difference diets containing different alfalfa meal level.

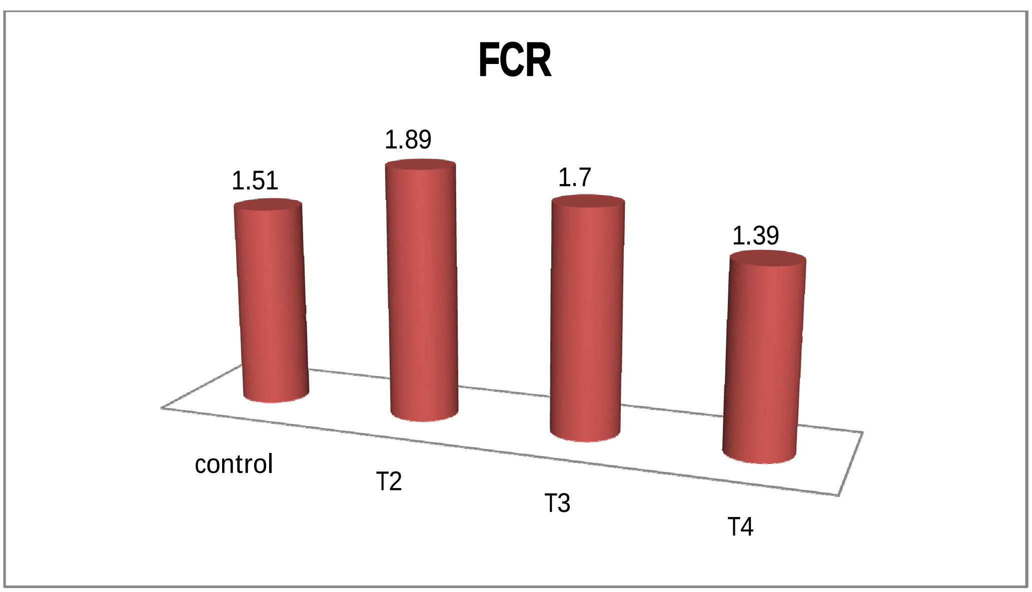

Figure (2): Food conversion ratio (FCR) of studied fish fed with difference diets containing different alfalfa meal level.

Table 4.2 Proximate chemical of *Oreochromis niloticus* feed at different levels of alfalfa meal.

Parameters **Treatment**	**Moisture**	**D.M**	**C.P**	**E.E**	**Ash**	**N.F.E**
T1 Control	73.50±0.70^{c}	26.50±0.70^{a}	31.00±0.14^{c}	6.85±0.07^{b}	2.05±0.07^{a}	33.60±0.42^{b}
T2	75.50±0.70^{b}	24.50±0.70^{b}	31.90±0.14^{c}	6.95±0.07^{b}	1.95±0.07^{b}	34.60±0.28^{a}
T3	73.50±0.70^{c}	26.50±0.70^{a}	32.45±0.35^{b}	7.35±0.35^{a}	2.15±0.07^{a}	31.85±0.35^{c}
T4	76.50±0.70^{a}	23.50±0.70^{c}	33.75±0.35^{a}	7.15±0.07^{a}	2.10±0.14^{a}	33.50±0.14^{b}

^{a,b,c} Means in the same column with superscripts are significant different at level ($p<0.05$).

whereas:

D.M= Dry matter, C.P= Crude protein, E.E= ether extract, N.F.E= nitrogen free extract.

CHAPTER SIX
DISCUSSION

Finding of table 4. 1 indicated that, increasing alfalfa level (5%, 10% and 15%) in *O. niloticus* diets were followed by a significant increase in weight gain (WG) and treatment containing 15% alfalfa level give higher weight gain compared with other treatments including control these result agreement with Olvera-Novoa (1990) who reported that the use of alfalfa leaf protein concentrate as a protein source in -45- diets of Nile Tilapia (*Oreochromis niloticus*).

In contrast to the above finding, Omar, *et. al*., (1994) showed that fish meal protein can substituted at 5, 10, 20 and 30% by alfalfa leaf meal protein.

Increasing alfalfa levels (from 0, 5, 10 and 15%) significantly ($P<0.05$) increased PI, and significantly improved FCR, PER and PPV (Table 4.1) which was subsequently followed by an increase in the growth performance this result with same line with Pantha (1982) who stated that diet utilization did not affected when diet containing 40.1% plant protein. Wee, K.L. and S.S. Wang (1987) showed that feed utilization of Nile Tilapia decreased with increasing Leucaena leaf meal levels in the diets.

Al-Asgha, *et.al.* (2003) reported that no significant differences were observed in feed conversion ratio of diets containing 15% of alfalfa meal. Furthermore, Omar, et. al., (1994) found that feed conversion ratio were higher in Nile Tilapia fish fed control diets as compared to fish fed on diets containing 5,10,20,and 30% protein from Alfalfa meal.

Ogino, et. al., (1978) indicated that alfalfa meal could replace up to 43-40% -51- of total protein in diets for fishes without any loss in feed conversion ratio when compared with casein control ration.

Increasing alfalfa levels (from 0, 5, 10 and 15%) significantly (P<0.05) difference in SGR in this study this result were agree with Abdel-Hamid et al.,(2000) whose indicated that SGR of Carps was greater (0.98 %/day) when fed on M.Sativa diets. Moustafa (1993) indicated that for Nile tilapia the SGR was 0.75; 0.944; 0.991 and 1.061 %/day for 20, 24, 28 and 32 % protein level in diets when fish were stoked at a rate of 120 fish/m3 in Cages.

Proximate analysis of *O. niloticus* which was feed by fed containing different level of alfalfa meal in Table 4.2 shown that all alfalfa levels significantly difference (P<0.05) in dry matter, lipid, fat, ash and N.F.E when compared to the control group this result were differ to El-Sherif, M. S., and A. M. EL-Feky (2008) indicated that, *O. niloticus* fed diet supplemented with alfalfa meal recorded a high level of dry matter and lipid content than control group with no effect on the ash content.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The results of the present study clearly indicated that the supplementation of alfalfa meal not only enhanced the growth performance and feed utilization of Nile tilapia (*O.niloticus*), Moreover, the supplementation of alfalfa meal had significant beneficial effects and there were significant interactions between increasing dietary of alfalfa meal level and proximate composition of the studied fish.

6.2 Recommendation

According to the results obtained from this study, this study recommends that:

More studies were needed to determine:

1/the effect of alfalfa meal supplementation on hematological and biochemical blood p777earameters.

2/ the effect of alfalfa meal supplementation on fatty acids profiles

3/ the effect of alfalfa meal supplementation on amino acids

4/the effect of alfalfa meal supplementation on fish fecundity.

5/the effect of alfalfa meal supplementation on digestibility.

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