بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Animal Production Science & Technology

Effect of Replacement Fishmeal with Water Spinach (*Ipomoea aquatica*) on Growth Parameters, Feed Conversion and Chemical Composition for Nile Tilapia fries (*Oreochromis niloticus*)

أثر تبديل مسحوق السمك بنبات الأركلة على عوامل النمو و التحول الغذائي و التركيب الكيميائي ليرقات سمكة البلطي النيلي

A dissertation Submitted to the College of Animal Production Science & Technology in Patrial Fulfillment of the Rrequirements for the Dagree of Bachelor of Science in Fisheries & Wildlife Science (B.Sc. Hon.)

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قال تعالي:

(وهو الذي سخر البحر لتأكلوا منه لحما طريا وتستخرجوا منه حلية تلبسونها وتري الفلك مواخرة فيه ولتبتغوا من فضله ولعلكم تشكرون**)**

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DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Bachelor Degree (B.Sc. Hon.) in Fisheries and Wildlife Science, which was approved by the Team of Examination on the date mentioned below
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DECLARATION OF THE STATUS OF THESIS

BY STUDENTS

The work described in this graduation project thesis was carried out in the Fish Hatchery in the Department of Fisheries & Wildlife Science at the College of Animal Production Science & Technology, Sudan University of Science & Technology from June 2018 to September 2018 under the supervision of Dr. Ramzy Ahmed Yousif.

The experimental work is original and the thesis has not been submitted partially or fully to any other University.

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DECLARATION OF THE STATUS OF THESIS

BY SUPERVISOR

The work described this graduation project dissertation was carried out in the fish hatchery in the Department of Fisheries & Wildlife Science at the College of Animal Production Science & Technology, Sudan University of Science & Technology from Julye 2018 to September 2018 under my supervision.

The experimental work is original and the thesis has not been submitted partially or fully to any other University.

Dr. RAMZY AHMED YOUSIF

Supervisor

October, 2018

DEDICATION

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To our fathers

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To our kind unfailing support mothers

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To our brothers

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For those who were in our heart and are still there

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Thank you for your support

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Abbreviations and acronyms

ABW	Absoute Body Wight
ANOVA	Analysais of Variance
AOAC	Association of Analytical Communisties
APHA	Animal and Plant Health Agency
CF	Crude Fiber
CP	Crude Protein
CSM	Cotton Seed Meal
DHA	Docosa Hexaenoic Acid
DO	Dissolved Oxygen
EAA	Essential Amino Acid
EE	Ether Exteract of fat
EFAs	Essential Fatty Acids
EPA	Eicosa Pentaeonic Acid
FAO	Food and Agriculture Organization of the United Nation
FM	Fish Meal
GNK	GrouNtnut Cake
HUFAs	Highly Unsaturated Fatty Acids
LWG	Live Wight Gaine
NFE	Nitrogen Free Exteract
NRC	National Research Council
OIE	Office International des Epizooties
PER	Protien Efficiency ratio
PUFAs	Polyunsaturated Fatty Acids
RDA	Recommande Dietary Allowances
SF	Sea Food
SGR	Specific Growth rate
TiLV	Tilapia Lake virus
WB	Wheat Bran
WM	Wheat Middling
WS	Water spinach
WRC	Water Research Commission

ABSTRACT

The experiment was carried out a period of 45 days; from15 July to 2 September 2018. The experiment design in plastic aquarium was developed at the freshwater hatchery department of fisheries science and wildlife, to determine the effect replacement of fishmeal with water spinach in the formulation of diets for Nile tilapia fry (*Oreochromis niloticus*). Design 150 Nile tilapia fries (average weight 3.25 g) Fish were distributed in flow through system of 15 plastic aquariums each containing about 20 liters, acclimatized to the hatchery conditions for 3 days. Before the beginning of the experiment, weak and abnormal fish were excluded and the remaining fish redistributed on aquariums at 10 fries / aquarium. The experiment included 5 treatments with 3 replicated aquariums for each. Feeds The diets replacing 0, 25, 50, 75 and 100% of fish meal protein content T0 T1, T2, T3 and T4 respectively.

fish indicated that Absolute body weight (ABW), live weight gain (LWG), specific growth rate (SGR), Feed conversion ratio (FCR), Protein efficiency ratio (PER) and survival rate of *Oreochromis niloticus* decreased with increasing level of water spinach in diets. AWG was found 36.90, 36.60, 33.10, 29.40 and 24.30 for T1, T0, T2, T3 and T4 respectively. LWG the higher in T3 (8.43) and the lower in T0 (1.02). The higher FCR in T4 (3.10) and the lower in T1 (1.96). The data were analyzed by one-way analysis of variance (ANOVA) and LSD for significantly different means at a significance level of 0.05 using SPSS 16.

Keywords: Growth parameters, Diets, treatments, Water spinach, *Oreochromis niloticus*.

ملخص البحث

اجريت هذه التجربة لمعرفة تبديل مسحوق السمك بنبات الأركلة في عليقة يرقات سمكة البلطي النيلي و اثره على عوامل النمو و التحويل الغذائي و التركيب الكيميائي ليرقات سمكة البلطي النيلي خلال 45 يوم في الفترة من 15يوليو - 2 سبتمبر 2018.

صممت هذه التجربة في أحواض بلاستيكية في مفرخ المياه العذبة التابع لقسم علوم الاسماك و الحياة البرية.

استعملت للتجربة مائة و خمسون من يرقات البلطي النيلي بمتوسط وزن 3.25 جرام.

قسمت الاسماك بشكل عشوائي في احواض بلاستيكية بلغ عددها 15 حوضاَ و وضعت في كل حوض 10 سمكات و كان سعة الحوض 20 لترا و تمت أقلمة الاسماك لمدة ثلاثة ايام قبل بدء التجربة.

قسمت التجربة الى خمس معاملات (المعاملات هي T0, T1, T2, T3, T4), حيث T0 تمثل التجربة بلا أضافة نبات الأركلة و T3, T2,T1 التجربة بإضافة نبات الأركلة و T4 تمثل التجربة بلا اضافة مسحوق السمك, بنسبة 25,0 ,50 ,75 ,100 % على التوالي.

أظهرت الدراسة ان الوزن المطلق المكتسب وجد كالاتي 24.30, 29.40, 33.10,36.60,36.90 علي التوالي.

أعلى وزن حي مكتسب في T3(4.98) و أقل وزن حي مكتسب في T0(1.02). أعلى معدل تحويل غذائي في T1(1.96) أقل معدل تحويل غذائي في T4(3.10).

أستخدم برنامج SPSS 16 لتحليل البيانات احصائيا و استخدم اختبار ANOVA. Test بمستوى معنوي0.05.

كلمات مفتاحية: عوامل النمو، علائق، معاملات، سبانخ مائي، بلطي نيلي.

CHAPTER I

INTRODUCTION

Aquaculture is the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated (FAO., 1997). Aquaculture also known as aqua farming, is the farming of fish, crustaceans, mollusks, aquatic plants, algae, and other organisms. Aquaculture involves cultivating freshwater and saltwater populations under controlled conditions, and can be contrasted with commercial fishing, which is the harvesting of wild fish (Garner., 2016).

Aquatic animal it's all life stage of fish, mollusks, crustaceans and amphibians originating from aquaculture establishment or removed from the wild for farming purposes, release into the environment and human consumption of ornamental purposes (OIE., 2010).

As of 2016, global production has been recorded for a total of 598 “species items” ever farmed in the world. A species item refers to a single species, a group of species or an interspecific hybrid. Species items recorded so far include 369 finfishes (including 5 hybrids), 109 mollusks, 64 crustaceans, 7 amphibians and reptiles (excluding alligators, caimans or crocodiles), 9 aquatic invertebrates and 40 aquatic algae (FAO., 2018a). This aquatic species and species groups ever farmed in inland freshwater, inland saline water, costal brackish water and marine water (FAO., 2017a). Farmed aquatic animals can be omnivores, herbivores or carnivore. Species themselves can be freshwater, marine or diadromous. Depending on the species being farmed, they can be fed (i.e. most finfish) or no fed (seaweeds and filter-feeding shellfish).The density of stock defines a production system as either extensive, semi-intensive or intensive, this species farming in "ponds, tanks, net or cages (SF., 2016).

Total fish production in 2016 reached an all-time high of 171 million tonnes, of which 88 percent was utilized for direct human consumption, thanks to relatively stable capture fisheries production, reduced wastage and continued aquaculture growth. This production resulted in a record-high per capita consumption of 20.3 kg in 2016. Global aquaculture production (including aquatic plants) in 2016 was 110.2 million tonnes, with the first-sale value estimated at USD 243.5 billion (FAO., 2018b).

In 2016, a total of 80 million tonnes of farmed food fish and 30.1 million tonnes of aquatic alge were produced in the world. Farmed food fish include 54.1 million tonnes of finfish, 17.1 million tonnes of molluscs , 7.9 million tonnes of crustaceans, and 938.5 thousand tonnes of miscellaneous aquatic animals such as tutles, sea cucmbers, frogs edible and jellyfish (FAO., 2018a).

Tilapia is one of the most popular aquaculture species and is farmed in more than 120 countries and territories. However, global tilapia aquaculture production is highly imbalanced, with the top ten countries in 2015 accounting for over 90 percent of the 5.7 million tonnes of global production (Cai *et al*., 2018). Tilapia is second-most important cultured finfish worldwide and farmed globally by many small holders. Nile tilapia (*Oreochromis niloticus*) ranks 6th among the most important cultured species, providing food, jobs, domestic and exporting earnings. Tilapia is an important protein source especially for poor consumer because they are an omnivorous diet, are tolerant to high density in aquaculture and relatively diseases resistant (FAO., 2017 b).

Fishmeal is recognized by nutritionist as high quality, very digestible feed ingredient that is favored for addition to the diet of most farm animals, especially fish and shrimp. Fishmeal carries quantities of energy per unit weight and excellent source of protein, lipids, minerals and vitamins, carbohydrate very little in fishmeal (Mile & Chapman., 2015).

Water spinach (*Ipomoea aquatic*) is wild plant that grows in water or moist soils and belongs to family *Convolvulaceae* (Salih., 1991). The leaves are also valuable as feed for domestic animals and common feed eaten by all social groups (Westphal., 1993). water spinach offer nutritive values with significant quantities of essential amino acids, nonessential amino acids, macro and micro salts crude fibers, fatty acids, organic acids and polyphenols (Doka *et al.*, 2014), the leaves of this plant is enriched with important vitamins (Igwenyi *et al.*, 2011 & Misra *et al*., 2014).

OBJECTIVES

The present study was designed to achieve the following goals:

1. Effect of Replacement Fishmeal with Water Spinach (*Ipomoea aquatica*) on Growth Parameters, Feed Conversion and Chemical Composition for Nile Tilapia fries (*Oreochromis niloticus*).

CHAPTER II

LITERATURE REVIEW

2.1 Fish Nutrition

Culture fish required protein, lipid, energy, vitamins and minerals in their diet for growth, reproduction, and other normal physiological functions. Nutrients for culture may come from various feed source, such as plankton, bacteria, insects and other fish from within the aquaculture ecosystem, and organic matter and processed feeds added to the ecosystem (Hancz., 2011). Natural foods are the best foods for fish and include algae (phytoplankton), zooplankton, detritus, snails, worms, insects and insect larvae, small plants like duckweeds and various other weeds and grasses that are found in a fish pond (WRC., 2010).

Artificial diets may be either complete or supplemental, complete diet supply all the ingredient (protein, carbohydrate, fats, vitamin, and minerals) necessary for optimal growth and health of the fish. Most fish farmers used component diets; the nutritional content of the feed depends on species of fish in being cultured and life stage. Supplemental diets are intended only to help support the natural feed (insects, algae, and small fish) normally available to fish in ponds or outdoor raceways. Supplemental diets do not contain a full complement of vitamins or minerals but are typically used to help fortify the naturally available diet with extra protein, carbohydrate, and/or lipids (Kuhn & Schwarz., 2017).

Supplemental feeds, which are usually rich in protein. Supplemental feed may be a single ingredient product such as rice bran or multi-ingredient processed feed, fish with highly specialized feeding habits, such as micro filterers, herbivores, carnivores, and omnivores (Hancz., 2011). Microbes such as rotifer, *Artemia*, and microalgae can be important substituent for fishmeal and fish oil (FAO., 2017c).

Green water systems provide natural feeds for the fish. The algae remove nitrogenous compounds and produces oxygen for the fish. The greener

the pond, the more natural food there is available and, depending upon stocking densities, little or no supplemental feeding may be required. In order to create a plankton bloom, it is necessary to add nitrogen and phosphorous (N and P, respectively) the two major limiting nutrients to plankton growth in water. The recommended application levels of these elements are 1–2 kg P/ha/day and 4 kg N/ha/day. Increase in fertilizer requirements throughout the growth period. Farmers producing more fish use higher densities and supplementary feeds, and fertilize the ponds (Bhujel., 2013). Microalgae areas source for zooplankton such as rotifer and *Artemia*, both the grow the zooplankton and enrich their nutritional value, they are continent long chain polyunsaturated fatty acids, resulting in high level of DHA, some species of yeast have been used probiotics, dietary supplements and source of pigment in aquaculture, bacteria are increasingly use in aquaculture as probiotics (FAO., 2017c). Rotifers live feed to the fries and fingerlings having small mouth size (Velasco & Corredo., 2011).

2.1.1 Fishmeal

Fishmeal is a generic terms for a nutrient rich feed ingredient primarily in diets for domestic animals, sometimes used as high quality organic fertilizer. Fishmeal can be made for almost any types of seafood but is generally manufactured from wild caught, small marine fish contain a high percentage of bone and oil. Cooking, pressing, drying, and grinding the fish make fishmeal. Fishmeal high quality of protein contents between 60% and 72% crude protein by weight, fishmeal is preferred animal protein supplement in the diets of farm animals and often the major source of protein in diets for fish and shrimp. Fishmeal including for essential amino acids that are A*rginine, Histidine, Isoleucine, leucine, Methionine, Phenyalalnine, Theronine, Tryptophan,* and *Valine* (Miles & Chapman., 2015).

The amino acid profile of fish meal is makes this feed ingredient as attractive as protein supplement. Fish lipids are highly digestible by all species of animals and excellent source of the essential polyunsaturated fatty acids (PUFA) in both the omage-3 and omage-6 families of fatty acids. The predominant omage-3 fatty acids in fishmeal and fish oil are linolenic acid,

docosahexaenoic acid (DHA), and eicosapentaeonic acid (EPA). Lipids in fishmeal not only excellent profile of essential fatty acids but also provide a high content of energy to the diet. Since there is very little carbohydrate in fishmeal, the energy content of fishmeal relates the percentage of protein and oil contents. The ash content of good quality fishmeal averages between 17%_25%. More ash indicates a higher minerals content, calcium, phosphor and magnesium (Miles & Chapman., 2015).

The vitamin of fishmeal is highly variable and influenced by several factor, such as origin and composition of the fish, fishmeal processing method, and product freshness. Fishmeal is considered to be a moderately rich source of vitamins of the B-complex especially cobalamine (B12), niacin, pantothenic acid, and riboflavin (Miles & Chapman., 2015).

2.1.2 Water spinach

2.1.2.1 Scientific classification

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Sub class : Asteridae
Order : Solanales
Family : Convolvulaceae
Guns : *Ipomoea*
Species : *I. aquatic*

2.1.2.2 Natural distribution and nutritional value

Water spinach (*Ipomoea aquatic)* is an aquatic vegetable distributed in Southern Asia, India, and China. The aerial parts of *I. aquatic* is a common vegetable eaten by different social groups of South-eastern Asia, India, China, Southwestern Pacific Islands, and African countries, namely Sudan, Nigeria, Tanzania, and Somalia (Doka *et al*., 2014 & Igwenyi *et al*., 2011 & Umar *et al*., 2007). Water spinach *(Ipomoea aquatic*), locally known in western Sudan as alarkala is wild plant grow in water or moist soil and belong to family *Convolvulaceae*. In Sudan the herb is used to treat stomach and intestinal

troubles. Until recently, little attention has been given to the use of wild plants as food in Sudan. By learning more about the protein, fat and mineral content of each plant, one can better assess their importance in the nutritional well-being of the communities (Doka *et al*., 2014).

Water spinach leaves contained high amount of essential amino acids (4765 mg/100 g) and non-essential amino acids (11669 mg/100 g) representing a total amino acids content of 16434 mg/100 g. The most abundant components of essential amino acids were leucine (1365 mg/100 g), Tyrosine + phenylalanine (1124 mg/100 g), lysine (682 mg/100 g) and threonine (606 mg/100 g). The leaves contained high amounts of the amino acids lysine and tryptophan, which are lacking in cereals, hence can complement the cereal-based staple diets in the area. Moreover, this species has the potential to be a resource for supplementation of some essential amino acids as it represented 45.5% in leucine, 45% in Tyrosine + Phenylalanine and 40.4% in threonine of the Recommended Dietary Allowances (RDA) for the essential amino acid uptake of adults, considering a 100 g serving size for a 18–30 year-old male of 70 kg (FAO/WHO/UNU., 2007). Non-essential amino acids (Asp, Ser, Gly, Ala, Arg, Pro), macro salts (K, Ca, Mg, Na, P), micro salts (Zn, Fe, Mn, Cu, Co), crud fibers, carbohydrates, fatty acids, organic acids and *polyphenols* (Doka *et al*., 2014). Total saturated fatty acids were 52.45 and total unsaturated fatty acids were 47.55% Monounsaturated fatty acids represented 23.03% whereas polyunsaturated fatty acids accounted for 24.52% Palmitic acid (40.22%) and oleic acid (22.37%) were the most abundant saturated and unsaturated fatty acids respectively (Kavishree *et al*., 2008).

The leaves of this plant is enrich with important vitamins, namely thiamine, riboflavin, niacin, pyridoxine, cyanocobalamin, ascorbic acid, a- tocopherol, and phylloquinone (Igwenyi *et al*., 2011 & Misra *et al*., 2014).

2.2 Tilapia Nutrition Requirement

The Nile tilapia belongs to the taxonomic family of the *Cilchlidae*. Cichlids are one of the most species rich fish families comprising more than 1600 valid taxa (Dunz & Chliewen., 2013). These include the important aquaculture species *Oreochromis niloticus* (Dunz & Chliewen, 2013 & Schwarzer *et al*., 2009). While larger Nile tilapias feed as omnivorous grazers

on plankton, aquatic plants and benthic fauna as well as on detritus and bacterial films, juvenile Nile tilapias mainly feed on plankton. However, adult Nile tilapias seem to be highly opportunistic feeders, occupying a large variation of different feeding niches (Rakocy., 2016).

The advantages of using Tilapia are that this species can digest natural food organisms, such as plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, larva fish, detritus, and decomposing organic matter (Sorphea., 2010). Tilapia fry accept feed immediately after yolk-sac absorption. There is no need to feed live feeds such as algae, Artemia and rotifers. Normally, tilapia fry are fed with fishmeal, rice bran or oil cakes separately or in combination, either in a powdered form or as dough (Bhujel., 2013). With regard to the total sulphur amino acids (namely tyrosine, cystine, methionine and phenylalanine), tyrosine and cystine are best considered semi-essential in that the fish can utilize cystine as a precursor for the biosynthesis of methionine and phenylalanine, thus reducing the dietary requirement for these two essential amino acids. The optimum gross dietary lipid requirements for Nile tilapia ranges between 10 and 15 percent (White *et al.*, 2018).

2.2.1 Protein Requirement

Protein the building blocks of any living organism and they consist of 20 protein genic amino acids. Crud protein content represent more or less an approximation for the true protein content of a sample and true protein may differ from crud protein content by 10%_20% (NRC., 2011). Protein are present in the tissues of fish as actin and myosin in muscle tissues, as enzymes, as structural important collagens, as immunoglobulin's as membrane protein structural in scales. Tilapia in the general, require lower inclusion levels for dietary protein than many other fish species. The recommend dietary protein levels for fish weighing less than 20g is 40% for Nile tilapias (NRC., 2011). The gross protein requirements of Nile tilapia were established. First feeds for fry should contain a protein ration of 45–50 percent and fry in the weight range of 0.02 g to 1.0 g have a requirement of 40 percent gross protein. This is reduced to 35–40 percent in fingerlings (weight range 1 to 10 g), to 30–35 percent in juveniles (10–25 g), and finally to 28–30 percent in adults (White *et al.*, 2018).

Nile tilapia have been shown to require the same ten essential amino acids as other fish species. With regard to the total sulphur amino acids (namely tyrosine, cysteine, methionine and phenylalanine), tyrosine and cysteine are best considered semi-essential in that the fish can utilize cysteine as a precursor for the biosynthesis of methionine and phenylalanine, thus reducing the dietary requirement for these two essential amino acids (White *et al.*, 2018). Amino acids are small molecules consisting of a carbon skeleton, nitrogen containing amino-group and carboxyl-group. The dietary essential amino acid (EAA) requirements for Nile tilapia are 1.2, 1.0,1.0, 1.9, 1.6, 1.0, 1.6, 1.1, 0.3 and 1.5% of dry matter for Arginine, Histidine, Isoleucine, Leucine, lysine, Methionine+Cystine, Phenylanaline+tyrosine, Threonine, Tryphthophan and valine, respectively (NRC., 2011).

2.2.2 Lipids Requirement

Dietary lipids are important sources of highly digestible energy and the only source of essential fatty acids needed by fish for normal growth and development. Lipids carry and assist in the absorption of fat-soluble nutrient such as sterols and vitamins. Lipids, especially phospholipids, are the main constituents of cellular structure that are important for maintenance of membrane flexibility and permeability. Other important functions of dietary lipids are as precursors of steroid hormones and prostaglandins, improving the flavor of feeds and affecting feed texture. Requirements of fish vary among species. In general, freshwater fish like tilapia, which possess the ability to desaturate and chain elongate C18 EFAs to longer-chain highly unsaturated fatty acids (HUFAs) (18:2n-6 to arachidonic acid, 20:4n-6; and 18:3n3 to eicosapentaenoic acid, 20:5n-3 and docosahexaenoic acid, 22:6n-3), only have a requirement for linoleic acid or linolenic acid, or both. The qualitative of essential fatty acids (EFA) requirement of fish vary among species. Fatty acids requirement has shown that linoleic series fatty acids are dietary essential for tilapia. The optimum dietary level of the n-6 acids have been estimated at about 0.5% for Nile tilapia. Dietary lipids have a spring effect on the utilization of dietary protein. Lipid levels of 5-12% are optimum in tilapia diets (Aksoy., 2016).

2.2.3 Carbohydrates Requirement

Carbohydrates constitute the largest proportion of farm animal diets they are the main source of dietary energy in most animal diets. Most fish species are unable to utilize dietary carbohydrates due to lack of adequate gut micro biota especially amylase that digests starch (Abro., 2014).

The superiority of omnivores as compared to carnivores in terms of carbohydrate utilization is due to presence of higher levels of amylase. Amylase in the digestive enzyme involved in the metabolism of starch (Mizutani, *et al*., 2012). The level of protein in diets for Nile tilapia can be reduced from 33.2 to 25.7% by increasing dietary lipids from 5.7 to 9.4% and carbohydrates from 31.9 to 36.9% (Aksoy ., 2016).

2.2.4 Vitamins Requirement

Vitamins are organic compound required in trace amounts and are essential for normal fish growth, reproduction and general health. Minimum requirement for most of the 15 essential vitamins have been established for Nile tilapia. Although determined generally for fingerling fish, the requirement is probably sufficient for larger fish. Vitamins are relatively unstable and matter of major concern in feed processing, handling and storage. Some vitamins (e.g. C, A, and D) are highly vulnerable to destruction during processing and storage while others (e.g. E and the B-complex) are not (Hancz., 2011). Water-soluble vitamins include B vitamin, *inositol*, *choline* and vitamin C. Fat-soluble vitamin include vitamin A, D, E and K (Craig & Helfrich., 2011).

2.2.5 Minerals Requirement

Mineral are inorganic elements necessary in the diets for normal body function. They can be divide two groups, macro minerals and micro minerals. Common dietary Macro minerals are calcium, sodium, chloride, potassium, chlorine, sulphur, phosphorous and magnesium. Common micro minerals are iron, copper, chromium, iodine, manganese, zinc and selenium (Craig & Helfrich., 2011).

Fish require up to 22 different minerals for tissue formation, metabolic process and maintain osmotic balance between their internal fluids and their water environment. Most freshwater fish can absorb sufficient calcium from the water unless calcium

carbonate content of water is below 5 mg/l. However, supplemental phosphorus is required in the feed. The available phosphorus requirements for Nile tilapia are 0.60%. Some required minerals, such as sodium and potassium (Hancz., 2011).

2.3 Water quality

Tank culturists need equipment that analyzes the minimum basic water quality parameters of dissolved oxygen, temperature, pH, ammonia, nitrite, alkalinity, chloride concentration, and calcium hardness (DeLong *et al*., 2009) Good water quality is essential to the health of fish at all stages of development. Water- quality requirements differ between species and between the different life stages as the fish develop. Many of the water-quality parameters are interlinked and a change in one feature can have an effect on another. Therefore, it is important to understand the various parameters that may affect the health of cultured fish (WRC., 2010). Feed contains protein utilized by fish to build muscle but protein contains nitrogen that, in digestion, generates ammonia that is released to the environment as dissolved nitrogenous waste excreted through fish gills; and nitrogenous waste that is released in particulate form as feces which can stay in the water column or settle onto the lake or pond bottom and seabed and when overfeeding occurs protein (nitrogen) rich feed settles on the lake or pond bottom and seabed. Particulate material on the lake/pond bottom and seabed is broken down by bacteria and this process can increase further concentrations of ammonia (as well as phosphorus and other nutrients) to the water column. Ammonia (NH_3) is of specific concern because it can be extremely toxic to fish at low concentrations. Ammonia is naturally oxidized, or broken down, by bacteria to ammonium (NH_4^+) which less toxic, into nitrite (NO_2^-) which can be toxic, and eventually to relatively non-toxic nitrate (NO_3^-). In the process of breaking down particulates or dissolved components like ammonia, bacteria consume large quantities of oxygen that can reduce the oxygen concentration available for fish. Unfortunately, lower oxygen also slows the process of ammonia oxidation, leading to increased concentrations of ammonia in the water in a potentially spiraling effect. It is therefore critical to have good control of feeding and feed use, and to limit feed waste (White et al., 2018).

2.3.1 pH

Dagree to which water is acid or alkaline is described by the pH scale, which ranges from 0-14. Acid substances have a pH from 0-7, 7 is neutral (neither acidic or alkaline) and alkaline is between 7-14. A change in one pH unit represents a large change in water quality and fish generally prefer water that is neither to acidic or alkaline and should be maintained within one unit from neutral (pH 6-8). pH levels can change depending on the amount of oxygen available in the water. At night, plants and algae in the pond use carbon dioxide and make oxygen. Carbon dioxide is acidic and causes the pH of the water to decrease. If not carefully monitored and possibly controlled, the pH may drop to levels that are dangerous to the fish. Excess carbon dioxide can be removed from the water by agitating the water using aerators or paddlewheels. Water with low pH affects the fish's gills, making it difficult for them to remove oxygen from the water. Tilapia can tolerate a pH from 3.7 to 10.5 but below pH 5, they are stressed and will not eat. The percentage of poisonous waste-products (such as ammonia) that is toxic to fish is also dependent on the pH. As pH increases, the percentage of toxic ammonia increases (WRC., 2010).

2.3.2 Dissolved Oxygen

The oxygen available to them is that which is dissolved in the water and measured in mg/l. oxygen enters the water through the surface of the water and amount that is capable of entering the water can also be expressed as the percentage of saturation. A normal dissolved oxygen level is approximately 7-9 mg/l in 25ºC freshwater at sea level. An extremely important thing to remember regarding water quality is the relationship between dissolved oxygen concentration and temperature. Low oxygen levels occurring at higher temperature. Plants and algae in the pond will produce oxygen during the day, and then this can be used by fish (WRC., 2010).

2.3.3 Ammonia

Ammonia is probably is the next most important water quality factor after dissolved oxygen. Ammonia comes from decomposition material, such as plants and dead fish. It also come from the fish as part of their normal metabolism and excreted through the gills. Ammonia is present in two forms: ionized (NH_4) and un-ionized or free ammonia (NH_3). Only NH_3 is directly toxic and its toxicity increase with an increase in temperature and/or pH, ammonia is measured in mg/L (WRC., 2010).

2.3.4 Nitrite (NO_2)

The forming of nitrite (NO_2) is the step between the conversions of ammonia to nitrate. In systems where ammonia levels are high, high levels of nitrite may be found. Nitrite is measured using water test-kits and measured in mg/l. High levels of nitrite can reduced the oxygen-carrying ability of the fish's blood. This cases the gills to change from red to brown (WRC., 2010). Avoid concentrations greater than 5 mg/L nitrite nitrogen if chloride is low (DeLong *et al* , 2009).

2.3.5. Nitrate (NO_3)

The final stage of the breakdown of ammonia is the formation of nitrate (NO_3). Nitrate also comes from farming fertilizers that run off the land into the water (WRC, 2010). Nitrate toxicity can occur if level in water reuse system exceeds the 300 to 400 mg/L nitrate- nitrogen range (DeLong *et al* , 2009).

2.3.5 Temperature

Temperature is hotness or coldness of something and probably the most important water quality variable. Temperature affects growth rate and feed convention rate, with each species having an optimal temperature for growth. Temperature also affects the metabolism and reproductive ability of fish. Tilapia, carp and catfish thrive in warm ponds of up to 33 ºC. Tilapia are more adversely affects by too low temperatures, and usually die if the water goes below 12-13 ºC for length period. The optimal temperature range 20-35 ºC for Nile tilapia (WRC., 2010).

CHAPTER III

MATERIALS AND METHODS

3.1. Preparation of experimental diets

In this study, firstly proximate composition and amino acid profile of water spinach meals used in fish feeds were analyzed (Table 1) and then feasibility of replacing fishmeal with water spinach for Nile tilapia *Oreochromis niloticus* fry by replacing fishmeal protein by water spinach protein were find out. In this experiment five isonitrogenous (30% CP) and isocaloric (3.95 kj/g) diets replacing 0% (Diet 0), 25% (Diet 1), 50% (Diet 2), 75% (Diet 3) and 100% (Diet 4) fishmeal protein by water spinach protein were formulated (Table 2). All diets were isonitrogenous (30% crude protein); out of which 10% protein was contributed by fish meal.

Crude protein content in the diet was fixed at 30% on the basis of earlier available information (Abdelghany, 2000). All the ingredients were weighed and blended in a Hobart electric mixer thoroughly. These were then steam cooked at 80°C in a volume of hot water. Oil, mineral and vitamin premixes were added to the lukewarm bowl one by one with constant mixing at 60°C. The final diet with bread dough consistency was poured into a Teflon-coated pan, cut in the form of small cubes and stored until used. The amino acid profiles of the experimental diets used in experiment were also analyzed and are given in Table 7.

Table (1): Proximate composition profile of ingredients

Ingredients	FM	WS	GNC	CSM	WM	WB
Proximate composition						
Protein %	45	21	43,7	38	17	13,7
Fat %	7.5	13.21	16.81	14.87	4.0	7.72
Moisture %	7.0	9.36	6.25	13.69	11	4.12
Ash %	21.3	2.53	10	10.40	4.5	4.37
Fibre %	0.8	6.83	18.38	12.21	7.5	10.47
energy kj/g	14.25	10.35	18,79	18,09	13.85	12,01

Table (2): Diet composition of the experiment

Ingredients (g/ 100 g dry diet)	T0	T1	T2	T3	T4
Fish meal[1]	40.00	30.00	20.00	10.00	0.00
Water Spinach[2]	0.00	10.00	20.00	30.00	40.00
Groundnut Cake[3]	20.00	20.00	20.00	20.00	20.00
Cottonseed Meal[4]	3.00	3.00	3.00	3.00	3.00
Wheat middling[5]	20.00	20.00	20.00	20.00	20.00
Wheat bran[6]	11.00	11.00	11.00	11.00	11.00
Oil	3.00	3.00	3.00	3.00	3.00
Mineral premix[7]	1.50	1.50	1.50	1.50	1.50
Vitamin premix[8]	1.50	1.50	1.50	1.50	1.50
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Protein (%)	33.0±0.3	30.6±0.0	28.2±0.2	25.8±0.05	23.4±0.01
Calculated gross energy (kJ g^{-1}, dry diet)	15.22±0.1	14.83±0.3	14.44±0.5	14.05.10	12.20±0.2

[1]Fishmeal 45% CP; [2]Water Spinach 21%; [3]Groundnut Cake 43.7% CP; [4]Cottonseed Meal 38%; [5]Wheat Middling 17% CP and [6]Wheat bran 13.7%.[7]Mineral mixture (g/100g dry diet) calcium biphosphate 13.57; calcium lactate 32.69; ferric citrate 02.97; magnesium sulphate 13.20; potassium phosphate (dibasic) 23.98; sodium biphosphate 08.72; sodium chloride 04.35; almunium chloride.$6H_2O$ 0.0154; potassium iodide 0.015; cuprous chloride 0.010; mangnous sulphate H_20 0.080; cobalt chloride. $6H_2O$ 0.100; zinc sulphate. $7H_2O$ 0.40 (Halver, 2002). [8]Vitamin mixture (g/100 dry diet) choline chloride 0.500;inositol 0.200; ascorbic acid 0.100; niacin 0.075; calcium pantothenate 0.05; riboflavin 0.02; menadione 0.004; pyridoxine hydrochloride 0.005; thiamin hydrochloride 0.005; folic acid 0.0015; biotin 0.0005; alpha-tocopherol 0.04; vitamin B_{12} 0.00001; LobaChemie, India (Halver, 2002).

3.2. Experimental System and Animals

Fry of *Oreochromis niloticus* were procured from Hussien Fadoul Fish Farm, Soba-Khartoum, Sudan. These were transported to hatchery of the Department of fisheries and Wildlife Science, Sudan University of Science & Technology, Khartoum, Sudan, transport of fry in polyethylene sac and stocked in fiber glass for two days in this period fry no feed. After that use of small deep net to caught fries and then weight fries and standardized then transfered to medium aquarium (water volume 20 L).

During this period, the fish were fed to apparent satiation by feeding diet consisting of cotton seed meal, wheat bran and wheat middling in the form of dried powder diet twice a day at 8:00 a.m. and 04:30 p.m.. For conducting the experiments, *Oreochromis niloticus* fry (0.36±0.2g; 3.6±0.2 cm) for experiment. were sorted out from the above acclimated lot and stocked in triplicate groups in 70-L circular polyvinyl tanks (water volume 20 L) fitted with a continuous water flow-through (1-1.5 L min-1) system at the rate of 10 fish per tank for each dietary treatment. Fish were fed test diets in the form of powder diet to apparent satiation twice daily. No feed was offered to the fish on the day they were weighed. Initial and weekly weights were recorded on a top-loading balance. The feeding trial lasted for 7 weeks. Faecal matter and unconsumed feed, if any, were siphoned off. The unconsumed feed was filtered on a screen soon after active feeding, dried and weighed to measure the amount of feed consumed.

3.3. Water quality parameters

Water temperature, dissolved oxygen, NO_2, NO_3, pH, and total ammonia during the feeding trial were recorded following standard methods (APHA 1992). The range of water temperature, dissolved oxygen, NO_2, NO_3, pH, and total ammonia over the 7 weeks feeding trial, based on weekly measurements, were 25.60-29.60 ºC, 4.06-5.55 mg L^{-1}, 0.00 mg L^{-1}, 40- 80 mg L^{-1} and 8.00-8.28, 0.22 mg L^{-1}, respectively.

3.4. Proximate analysis

Assessment of proximate composition and fatty acid profile of ingredients, diets and carcass was made using standard techniques (AOAC 1995). All the analyses were done on quadruplet basis (n=4x1). Details for each analysis are as under:

3.4.1. Moisture

A known quantity of sample (2 g) was taken in a pre-weighed crucible and placed in a hot air oven at 105±1°C for 24 hours. After complete drying, the sample cooled at room temperature in a desiccator and was reweighed. The loss in weight gave an index of water from which its percentage was calculated.

3.4.2. Ash

A known quantity of dried powdered sample (2 g) was taken in pre-weighed silica crucible and incinerated in a muffle furnace (650 °C) for 2-4 hours or till the sample became carbon-free and completely white. The crucible was cooled in a desiccator and reweighed to estimate the quantity of ash. The result was expressed as percentage on dry weight basis.

3.4.3. Fat

Crude fat estimated by continuous soxhlet extraction technique (Socs Plus, SCS 4, Pelican equipments, Chennai, India) using petroleum ether (40-60 °C B.P.) as solvent. Finely powdered and dried sample (5 g) was placed in fat extraction thimble and placed in the soxhlet apparatus. A clean, dry soxhlet receiver flask was weighed and fitted to the soxhlet assembly on a boiling water bath for extraction, which was continued for 2-3 hours. After extraction the flask removed and kept in hot air oven (100 °C) to evaporate the traces of solvent, and then transferred to a desiccator, cooled and reweighed. The difference between the weight of the flask before and after gave the quantity of crude fat extracted from the unknown amount of the sample. The result expressed as percentage on dry weight basis.

3.4.4. Crude protein

The estimation of crude protein (N x 6.25) done using an auto Kjeldahl system (Kjeltec Foss Tecator 2300 TM, Hoganas, Sweden) after acid digestion with an auto-digester (Foss, Tecator, Hoganas, Sweden). A known quantity of samples were taken in Kjeltec digestion tubes. To this, 0.8 g of copper sulphate, 7.0 g potassium sulphate and 12 ml. of concentrated sulphuric acid added. The content digested in pre-heated (420°C) digestion block of the instrument. The process of digestion continued for 60 minutes until clear blue/green solution obtained. Now the digested sample cooled at room temperature and titrated automatically in distillation unit of the instrument. The level of protein displayed on the screen noted down.

3.4.5. Crude fibre content

To determine the crude fibre content of the collected samples (feed, ingredient, fish muscle, wholebody), triplicate samples of each feedstuff weighing approximately 5g were taken in a heat resistant volumetric flask (Borosil). Now 25 ml of H_2SO_4 (10%) solution was added and the volume was raised up to 125 ml by adding 100 ml of double distilled water to each flasks. Then flasks were placed for boiling at 110°C for 30 minutes. After continued boiling for 30 minutes, the samples were washed with boiling water to remove traces of acids. Then again 25 ml of 10% NaOH solution was added, the content was top up with 100 ml of double distilled water and boiled for 30 minutes. After this, again the samples were washed with boiling water to remove the traces of alkali. Finally the contents were acetone dried and placed in a thermostat for drying at 60°C. After complete drying, the samples were kept in a muffled furnace for ashing at 600^{o}C for 2 h. The CF was quantified by expressing the loss in weight after ashing as a percentage of the original weight of the sample.

3.4.6. Nitrogen-Free-Extract (NFE)

Nitrogen Free Extracts estimated by difference as 100-(Crude protein (CP) + Ether extract or fat (EE) + Crude fibre (CF) + Ash.

3.4.7. Gross energy

Gross energy was determined on a ballistic bomb calorimeter (Gallenkamp, Loughborough, England). Prior to estimate, a known quantity of dried powdered sample (0.5-1.0g) was taken in metallic crucible and compacted carefully to increase the rate of combustion at 25 lb oxygen pressure. The heat generated upon combustion was read on the modulated galvanometer scale, and converted to energy equivalent, worked out earlier using the thermo chemical grade benzoic acid (24.41 kJ g^{-1}) as a standard. The gross energy was expressed as kJ g^{-1}.

3.4.8. Amino acid analysis

Amino acid analysis of experimental diets was carried out with the help of Hitachi L-8800 Amino Acid Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) by hydrolyzing 0.3 mg sample in 1 mL of 6 N HCl for about 22 h. The sample thus obtained was diluted in 0.02 N HCl. The hydrolyzed samples were filtered using microfilter (Cellulose acetate membrane, 0.45 μm, Corning, Japan) and then injected in an automatic Amino Acid Analyzer (Hitachi L-8800). Recovery hydrolysis of tryptophan was performed in 4 N-methanesulfonic acid instead of 6 N HCl followed by

the decomposition at 110°C for 22 h. After this, 4 N NaOH was added to adjust the pH to approximately 2. This was then diluted again in 0.02 N HCl. However, the recovery hydrolysis of sulphur amino acids methionine and cystine was performed in 2 mL of performic acid for 4-24 h. After this, 0.3 mL of 48% HBr was added, and the decomposition was performed at 110°C for 22 h. The samples were then dried solid under reduced pressure. After this, 1 mL of 0.2 N NaOH was added, and sample was then left stand still for about an hour. Lastly, the pH and volume of the sample was adjusted using 0.05 N HCl and 0.1 N HCl.

3.5.Growth Parameters

The effects of replacement fishmeal with water spinach in diets on growth and conversion efficiency of fries *Oreochromis niloticus* during the present experiment was evaluated using following indices:

Live weight gain (LWG; %) = Final individual body weight-Initial individual body weight/Initial individual body weight × 100

Absolute weight gain (g/fish) = Final individual body weight-Initial individual body weight

Feed conversion ratio (FCR) = Dry feed fed/Wet weight gain

Protein efficiency ratio (PER) = Weight gain/Protein fed

Specific growth rate (SGR; %/day) = ln Final body weight-ln Initial body weight/No. of days × 100

Per cent survival = (Final number of fish/Initial number of fish) × 100

The proximate content analysis of carcass and feed was done. The results from the replicates for each feed samples were used to provide the data for the statistical analysis**.** All growth data were subjected to analysis of variance (Snedecor and Cochran 1968; Sokal and Rohlf 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at a $P<0.05$ level of significance (Duncan 1955).

CHAPTER IV

RESULTS

Table 3: Comparment of weekly weight of all treatment for seven weeks

	T 0	T 1	T 2	T 3	T 4
Initial weight	3,60±0,10^{a}	3,5±0,36^{a}	2,53±0,51^{b}	3,5±0,30^{a}	3,13±0,84^{a}
W 1	7,00±0,56^{a}	7,73±0,40^{a}	5,60±0,61^{b}	4,93±0,57^{c}	6,77±1,59^{b}
W 2	9,53±0,40^{b}	11,67±0,55^{a}	8,27±2,25^{b}	7,27±0,25^{c}	9,63±1,48^{b}
W 3	12,77±1,56^{b}	16,50±0,56^{a}	12,07±3,51^{b}	9,53±1,01^{c}	13,37±1,72^{a}
W 4	17,43±3,40^{a}	19,43±1,07^{a}	15,83±3,35^{b}	13,93±1,45^{c}	17,17±0,93^{a}
W 5	21,57±4,05^{b}	25,13±2,38^{a}	19,43±6,41^{b}	18,27±1,10^{b}	21,50±0,87^{b}
W 6	29,40±4,73^{b}	35,43±3,66^{a}	26,80±7,99^{c}	24,87±2,74^{c}	25,37±3,91^{c}
W 7	40,20±9,43^{a}	40,40±3,22^{a}	35,63±8,95^{b}	32,90±3,38^{b}	27,43±6,36^{c}

^{a,b,c} Mean values followed by the same superscript in each column are not significant different ($p>0.05$)

In thesis experimental, the higher initial weight in T_0 (3.60g) and lowest in T_2 (2.53g) . the higher weight in T1 (7.73 g) and the lowest T_3 (4.93g) in week one. The higher weight in T1 (7.73 g) and the lowest T_3 (4.93g) in week one. The higher weight in T1 (11.67 g) and the lowest T_3 (9.53g) in week three. the higher weight in T1 (19.50g) and the lowest T_3 (13.93g) in week four. The higher weight in T1 (25.13g) and the lowest T_3 (18.27g) in week five. The higher weight in T_1 (35.43g) and the lowest T_3 (24.87g) in week six. The higher weight in T_1 (40.40g) and the lowest T_4 (27.43g) in week seven.

Table 4: Growth, survival and feed utilization of Nile tilapia fed experimental diets

	T_0	T_1	T_2	T_3	T_4
IW (g)	3,60±0,10[a]	3,5±0,36[a]	2,53±0,51[b]	3,5±0,30[a]	3,13±0,84[a]
FW (g)	40,20±9,43[a]	40,4±3,22[a]	35,63±8,95[b]	32,90±3,38[b]	27,43±6,36[b]
AWG (g)	36,60±9.46[a]	36,90±3,50a	33,10± 8,70[b]	29,40±3,39[b]	24,30±6,42[b]
LWG %	1,02±296,73[b]	1,07±212,08[b]	1.32±341,42[b]	8.34±107,34[a]	8,12±311.00[a]
SGR	4,66±1.30[b]	5,44±0,38[a]	5,19±1,49[a]	4,98±0,29[b]	4,83±0.73[b]
FCR	2,05±0,5[a]	1,96± 0,18[a]	2,28±0,68[b]	2,47± 0.27[b]	3,10± 0.78[b]
PER	1,54± 0,41[b]	1,65±0,16[a]	1,64±0,43[b]	1,56±0,19[b]	1,47±0,39[b]
Survival %	83,33±11,55	83,33±5,77	93,33±5,77	93,33±5,77	80,00±26.50

[a,b,c] Mean values followed by the same superscript in each column are not significant different ($p>0.05$)

The highest (36.90 g) Absolute weight gain was recorded in T_1, which was followed by T_0 (36.60g), T_2 (33.60g) and T_3 (29.40g). Whereas, the lowest Absolute weight gain (24.30g) was in T_4. The highest live weight gain was in T_3 (8.34%). This was followed by T_4 (8.12%), T_3 (1.32%), T_1 (1.07%) and in T_0 (1.02%). The live weight gain in treatment was ($p<0.05$) significantly different. The SGR was significantly higher (5.44) in T_1 as compared to other treatments. The lowest SGR (4.66) was found in T_0. Higher FCR (3.10) in T_4 as compared to other treatments and the lowest FCR (1.96) was found in T_1.

Table 5: Mean physical-chemical parameters of the test concentrations water spinach on Water Quality

	T_0	T_1	T_2	T_3	T_4
pH	8.20±0.00[b]	8.20±0.00[b]	8.17±0.07[c]	8.16±0.08[c]	8.28±0.10[a]
Ammonia	0.22±0.19[a]	0.22±0.19[a]	0.22±0.19[a]	0.22±0.19[a]	0.22±0.19[a]
NO_2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
NO_3	40.00±0.00[b]	40.00±0.00[b]	80.00±0.00[a]	80.00±0.00[a]	80.00±0.00[a]
DO	5.55±2.29[a]	4.06±0.33[b]	5.09±1.63[a]	5.42±1.90[a]	5.38 ± .25[a]
Temperature	29.60±0.51[a]	29.60±0.51[a]	25.60±0.51[b]	26.60±0.51[b]	29.60±0.51[a]

[a,b,c] Mean values followed by the same superscript in each column are not significant different ($p>0.05$)

During the experiment, the average water temperature ranged between 25.60 - 29.60 °C). the mean values of pH were in the range of 8.54 to 8.59 in different treatments The highest (8.28) and lowest values of pH were in T_3 (8.16). the average water Nitrite ranged between 40.00 – 80.00 mg/L. the average Ammonia 0.22 mg/L. Dissolved oxygen is very important for fish growth and other physiological functions. The high DO in T_0 (5.55 mg/L) and lower DO in T_1 (4.06).

Table 6. Carcass Composition of *Oreochromis niloticus* fed graded concentrations of Water Spinach

	Experimental diets				
	T_0	**T_1**	**T_2**	**T_3**	**T_4**
DM%	25.91 ± 0.20^{b}	25.10 ± 0.81^{b}	25.78 ± 0.76^{b}	26.06 ± 1.95^{a}	23.65 ± 0.32^{c}
Fat%	5.26 ± 0.50^{c}	5.36 ± 0.30^{c}	7.14 ± 0.83^{b}	11.11 ± 0.10^{a}	10.00 ± 0.01^{a}
CP%	70.5 ± 0.50^{a}	68.43 ± 0.40^{a}	67.05 ± 0.50^{a}	61.95 ± 0.2^{b}	60.11 ± 0.7^{b}
Ash	1.92 ± 0.30^{c}	6.00 ± 0.26^{b}	8.11 ±0 .71^{b}	8.33 ± 0.29^{b}	15.00 ± 0.25^{a}

Means in the same row with different superscripts are significantly (P<0.05) different.

Carcass Composition before experimental very important for nutritional value of Nile tilapia. Dry matter in The experimental ranged from 23.65 to 26.06. The fat in body ranged between 5.26% in T_0 to 11.11% in T_3. Crude protein in body of fish depended to essential amino acid in the diets. Generally, crude protein decrease with decrease of fish meal, high Crude protein in T_0 (70.5%) and lower crude protein in T_4 (60.11%). The Ash content between 1.92 in T_0 to 15.00 in T_4.

Table (7): Amino acid Profile of the diets

	T0	T1	T2	T3	T4
Arginine, %	2.43	2.93	2.89	2.57	3.34
Histidine, %	1.22	1.28	1.33	1.39	1.44
Isoleucine %	0.78	1.80	2.08	2.37	2.65
Leucine %	2.52	1.83	3.24	3.61	3.99
Lysine %	2.45	1.14	2.64	2.75	2.53
Methionine %	0.78	0.51	0.84	0.89	0.92
Cystine %	0.38	0.37	0.42	0.90	2.52
Phenylalnine %	1.48	1.30	2.10	2.44	2.76
Tyrosine %	1.05	0.96	1.58	1.87	2.14
Threonine %	1.43	1.63	1.65	2.03	2.23
Tryptophan %	0.37	0.43	0.49	0.55	0.62
Valine %	0.88	2.10	2.31	2.54	2.76

The high Arginine in T4 (3.34 g/100) and the Lowe in T0(2.43 g/100) . The high Histidine in T4(1.44 g/100) and the Lowe in T0 (1.22g/100). The high Isoleucine in T4 (2.65 g/100) and the Lowe in T0 (0.78 g/100). The high Leucine in T4 (3.99 g/100) and the Lowe in T1(1.83 g/100). The high Lysine in T3 (2.75 g/100) and the Lowe in T1 (1.14 g/100). The high Methionine in T4 (0.92 g/100) and the Lowe in T1 (0.51 g/100). The high Cystine in T4 (2.52 g/100) and the Lowe in T1 (0.37 g/100). The high Phenylalnine in T4 (2.76 g/100) and the Lowe in T1(1.30 g/100). The high Tyrosine in T4 (2.14 g/100) and the Lowe in T1 (0.96 g/100). The high Threonine in T4 (2.23 g/100) and the Lowe in T1 (1.43 g/100). The high Tryptophan in T4 (0.62 g/100) and the Lowe in T0 (0.37 g/100). The high Valine in T4 (2.76 g/100) and the lowe in T0 (0.88 g/100) .

CHAPTER V

DISCUSSION

Selection of feed ingredients is one of the most important factors for the formulation and commercial production of supplemental quality feed for any aquatic species (Zamal *et al*., 2008; Koumi *et al*., 2009). Although, fish meal is the widely used feed ingredients as animal protein source and accepted for its higher protein composition and essential amino acids; but it is rather expensive than the available plant protein sources (Vechklang *et al*., 2011). Beside this, the availability of fish meal is decreasing day by day due to its high demand in other than aquaculture industry like livestock, poultry etc. The decreased supply of fish meal in future will dramatically affect the fish production. Considering this, it is essential to partially reduce or eliminate fish meal in fish diet. One approach to reduce fish meal from fish diets is to replace it with alternative less expensive and easily available plant protein, which will allow for continued expansion of aquaculture. In view of this, a number of plant protein source has been evaluated for the replacement of fish meal (Alceste & Jory., 2000 & Yue & Zhou., 2008 & Francis *et al*., 2001). The proximate composition of water spinach leaf meal used in the experiment revealed that the crude protein content were high compared to the result (Doka *et al.*, 2014). These differences might be due to different environmental conditions such as soil type, local varieties, and processing methods. All the experimental feeds were actively fed upon and accepted by the fish throughout the experimental period which could be as a result of palatability of the feed indicating that the levels of incorporation of water spinach did not affect the palatability of the diets. Growth rates in weight and were calculated from measurements all sample of 10 fish, but also by the survival rate of the fish population. This varied among treatments, and thus in some cases the feed available per fish was influenced by the numbers of fish surviving in the aquarium. For this reason productivity was measured as the growth performance, expressed as weight of fish at the end of the experiment high the weight at the beginning. Using this criterion it was clear that the survival rate had a determining effect on fish

productivity compere to the results of (Sorphea., 2010). Growth rate measured on weight of all fish in the aquarium weekly at 7 week. High weight in final week, in the present study, there were no significant differences in AWG, LWG, SGR, and PER between the 5 treatments, but there was a difference in survival, suggesting that *I. aquatica* cultivation significantly affected survival rather than the growth of *Pelodiscus sinensis* in ponds. Although this is the first study to report the effects of *I. aquatica* cultivation on *P. sinensis* growth and survival in pond culture, similar observations on cultured fish species, such as crucian carp *Carassius auratus* (Chen *et al*., 2010).

Water temperature is one of the most influencing environmental factors affecting growth of fish. The optimum temperature for feeding, growth and reproduction of tilapia is between 22 and 30 °C (Caulton., 1982). In the present study, the water temperature varied between 25.60 °C to 29.60 °C. Like water temperature, other water quality parameters (i.e. pH, DO, Ammonia, nitrite and Nitrate) were also within the favorable range for good aquaculture. Therefore, the growth variations in different treatments could not be assigned to water quality. This might be due to the quality of the feed given to experimental fish because the protein levels in different experimental diets were significantly different. Similar results were also reported by (Al-kenaway *et al*., 2008).

In the present study, the inputs of fish meal replacement with water spinach meal (0-100%) have been evaluated on Nile tilapia fry. The highest absolute weight gain (36.90g), live weight gain (1.07g) and SGR (5.44) was noticed in T1 (Table 4) as compared to treatments. While comparing the treatments, it was the growth performance of experimental fish had negative impact of increasing water spinach meal in fish diet without T1. Similar results were also reported by (Yee Lin *et al*., 2004 & Xu *et al.,* 2012) four isonitrogenic and isocaloric diets which contained 100-75% fish meal. After 7 weeks feeding period, no significant ($P>0.05$) difference was found in live weight gain, feed conversion ratio and protein efficiency ratio among fish fed different experimental diets. (Yee Lin *et al*., 2004). Weight gain, feed conversion ratio and survival rate compared to fish fed of all the treatment ($p<0.05$) in the present study, the highest absolute weight gain was recorded in T1 (36.90) which was followed by T0 (36.60), T2 (33.10 g), T3 (29.40 g)

whereas, the lowest absolute weight gain (24.30g) was in T4. The recorded absolute weight gain was statistically different between treatments. SGR was non significantly, higher (5.44) in T1 as compared to other treatments. Whereas lowest SGR (4.66) was found in T0. FCR was also no significant higher (1.91) in T1 as compared to other treatment. Whereas, lowest FCR (3,10) was found in T4. The Nile tilapias fed with T0, T1, T2, T3 and T4 diet with no replacement or replacement of fish meal with water spinach meal attended an average net weight of 36.90 respectively. Thus, best growth of Nile tilapia fry was reported when fed with the T1 diet. Nile tilapia provided the diet with 25 per cent replacement of fish meal with water spinach attended an average weight of 36.90±3.50 which was reported to be the highest in fish meal replaced diet. Thus, fish meal replaced by water spinach meal had effect on growth of Nile tilapia compared to the result (Fabusoro *et al*., 2014).

CONCLUSION AND RECOMMENDATION

Conclusion:

The experiment showed that feeds were actively consumed by the experimental fish; Nile tilapia which brought an increase in weight. Since there was no significant difference ($P>0.05$) among the means of the treatments, it shows that any of the inclusion level can be used up to 50% inclusion level of water spinach. However, 25% inclusion level of water spinach produced best result in terms of growth. It is therefore recommended that water spinach plant can be incorporated at 50% inclusion without compromising fish growth. It is therefore recommended that water spinach plant can be incorporated at 25% & 50% inclusion without compromising fish growth. There are various alternative protein sources that can be used in aquaculture diets, with water spinach meal (WSM) being the most wide used plant protein ingredient.

Recommendations:

1- The best result of replacement fish meal with water spinach on growth parameter, feed conversation and chemical composition of Nile tilapia fries in T_1 (25%).

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