



**SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY**  
**COLLEGE OF ANIMAL PRODUCTION SCIENCE AND TECHNOLOGY**  
**DEPARTMENT OF FISHERIES AND WILDLIFE SCIENCE**



Effects of Soaked Pigeon Peas Seed Using graded Levels on the  
Growth performance and Food Utilization of Mon-sex Nile  
Tilapia, (*Oreochromis Niloticus*) Fingerlings Diets.

أثر إستخدام مستويات متدرجة من اللوبيا العدسية المنقوعة في علائق أسماك البلطي النيلي  
وحيد الجنس علي النمو.

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Rrequirement for the Degree of B.Sc. in Fisheries and Wildlife  
Science (Honor).

**Prepared By:**

- 1- Abdul naser Sidigg Ali Bhr.
- 2- Rufida Musa Mohammed.
- 3- Mubarak Musa Ali.

**Supervisor:**

**Dr. Hassan Mohammed Yagoub Adam**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الإستِـهال

قال تعالى:

(وجعلنا من الماء كل شيء حي)

(٣٠ الأنبياء)

يقول الله عز وجل:

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

[ سورة النحل الآية: 14 ]

## **DEDICATION**

**To my dear parents**

**Teachers**

**Brothers**

**Sisters**

**Family**

**Friends**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Our great thank to Allah almighty, the most merciful who gave us the health, strength and patience to complete this study

Thanks giving and the end of God Almighty. Then after him all tired of me (family particularly my father, my mother and my sister).

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## ABSTRACT:

This experiment was conducted out in the Hatchery of College of Animal Production Science and Technology the Sudan University of Science and Technology during the period from 10 July to 30 August 2018. The objective of the study was Effects of Soaked Pigeon Peas Seed Using graded Levels on the Growth performance and Food Utilization of Mono-sex Nile Tilapia, (*Oreochromis niloticus*) Fingerlings Diets. A total of fish 140 Nile tilapia, were randomly assigned to four dietary treatments (0, 25%, 50% and 75%), each treatment was composed of three replicates (10fish/replicate).

The study demonstrated that there were significant differences ( $p \leq 0.05$ ) in the (WG g/Fish), (DWG g/Fish) and (SGR) of mono-sex Nile tilapia (*Oreochromis Niloticus*) fed on diets with partially replaced by Pigeon peas. In Control, T1, T2% and T3% \days increase in control ( $3.4 \pm 0.01$  to  $9.93 \pm 0.73$ ), T1 ( $3.3 \pm 0.00$  to  $9.00 \pm 0.53$ ), T2 ( $3.2 \pm 0.00$  to  $7.60 \pm 0.26$ ) and T3 ( $3.3 \pm 0.00$  to  $7.13 \pm 0.03$ ) at ( $p \leq 0.05$ ), and the Lowest level of survival rate 69.12% was noted in fish fed with the diet containing the level of Pigeon Peas T2 (50%). and the highest increase was noted in fish fed with the diet containing the level of Pigeon Peas T1 (25%), it recorded the best effect in growth index.

## ملخص الدراسة:

أجريت هذه الدراسة في مفرخ الأسماك بكلية علوم وتكنولوجيا الإنتاج الحيواني - جامعة السودان للعلوم والتكنولوجيا - خلال الفترة من 10 يوليو وحتى 30 أغسطس / 2018م. وكان الهدف من الدراسة معرفة أثر استخدام مستويات متدرجة من اللوبيا العدسية المنقوعة علي أداء نمو أصبعيات البلطي (النيلي وحيد الجنس). حددت مجموعة بها 140 أصبعية بلطي نيلي بشكل عشوائي للمعاملات الغذائية الأربعة (0 ، 25% ، 50% و 75% عدسية) ، تتألف كل معاملة من ثلاثة مكررات (10 سمكات /مكرر).

أظهرت نتائج التجربة أن هنالك فروق معنوية ذات دلالة إحصائية ( $50.0 \geq$  ف) و ( $f \geq 10.0$ ) في زيادة الوزن المطلق ، زيادة متوسط وزن الجسم اليومي ، معامل التحول الغذائي والزيادة في معد النمو النوعي. في المعاملات (T0, T1, T2 , و T3 ) بنسبة أحلال جزئي لبدرة السمك (0 ، 25% ، 50% و 75%)

أوضحت نتائج الدراسة أن أدني مستويات معدل البغاء علي قيد الحياة 69.12% في الأسماك التي غذية علي الحمية المحتوية علي مستوي 50% من اللوبيا العدسية في المعاملة T2. وأظهرت نتائج الدراسة أيضا أن الأسماك التي غذية علي الحمية المحتوية علي مستوي 25% من اللوبيا العدسية في المعاملة T1 سجلت أفضل النتائج في مؤشر النمو.

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# CHAPTER ONE

## INTRODUACTION

A feed ingredient may appear from its chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be digested and absorbed in the target species. Knowledge of nutrient digestibility of the various feed ingredients used in formulating fish feeds is desirable so that effective substitution of one ingredient for another may be achieved. Together with chemical analysis, digestibility determination may allow a more thorough estimation of the nutritive value of a particular protein source in a complete feed for fish.

Fish are the most numerous vertebrates, with at least 20,000 known species, and more than 58% are found in marine environment (Thurman and Webber, 1984).

Nile tilapia (*Oreochromis niloticus*), which occurs throughout the country, is one of the most dominant and popular indigenous fish species in sub-Saharan water bodies and best candidate species for aquaculture, due to good adaptive abilities, fast growth rate, growth in adverse conditions, diverse feeding habit and preference by the consumers (Liti et al., 2006; Kassahun Asaminew et al., 2012; Zenebe Tadesse et al., 2012). It is also cultured in fresh and saline waters and in any climate zones especially tropical and temperate regions (Lim and Wabste, 2006). This fish has been farmed for subsistence or commercial purposes and most of the culture practices are semi-intensive culture systems due to flexible feeding habit and the ability to use more diversified feed resources (El-Sayed, 2006). Feeding of fish is the main role in aquaculture production systems and therefore, it accounts for 40-50% of the total production costs in semi-intensive culture systems (Craig and Helfrich, 2002; FAO, 2006). To develop aquaculture, search for cheaper and locally available feed stuffs is necessary. Industrial and agricultural by-products have been used as

sources of suitable and cheap feed sources (FAO, 2006; Munguti et al., 2006, Kassahun Asaminew et al., 2012). The production of fish increases by improving feeding quality and health of the fish through supplementing formulated protein rich feed stuffs with vitamins and minerals premix (Lim and Wabste, 2006). Vitamins and minerals are needed in small amounts for normal growth, health and metabolic activities (Carmen and Geoff, 2007). In Sudan, very few studies have been conducted on the effect of fish feeds of agro-industrial by products on the growth performance of fish (Kassahun Asaminew et al., 2012; Zenebe Tadesse et al., 2012).

Few studies were done on evaluating Pigeon pea (*Cajanus Cajan*) as replacement of fish meal in Nile tilapia diet globally and nothing done in Sudan. For those reasons this study was conducted in the Fisheries Laboratory and hatchery of Sudan University for Science and Technology to assess the growth Performance, carcass composition, feed utilization and condition factor (K) of Mono-sex Nile Tilapia fingerlings fed diets containing different levels of Pigeon pea (*Cajanus Cajan*) meal, as partial replacement of fish meal Protein Gain at 25%, 50% and 75% levels. Also to maximize the beneficial returns from the local returns from the local materials in order to reduce of feed cost.

Objectives:

1. To study the effect of additions of Pigeon peas (*Cajanus Cajan*) levels (25%, 50% and 75%) respectively in Mono-sex Nile tilapia (*Oreochromis niloticus*) diets on the growth performance, end carcass composition, feed utilization and condition factor K.
2. Effect of additions of Pigeon peas on length, to determine what feed suitability.

## CHAPTER TWO

### LETURTURE REVOW

#### 2.1. Pigeon pea (*Cajanus Cajan*):

Pigeon pea (*Cajanus Cajan* (L.) Huth) is one of the most common tropical and subtropical legumes cultivated for its edible seeds. Pigeon pea is fast growing, hardy, widely adaptable, and drought resistant (Bekele-Tessema, 2007). Because it is drought resistance it can be considered of utmost importance for food security in regions where rain failures are prone to occur (CropTrust, 2014).

#### 2. 1.1 Classification: (National Plant Data centre)

Domain:	Eukaryota
Kingdom:	plantae
Phylum:	spermataphyta
Subphaylum:	Angiopemee
Class:	dicotyledonae
Order:	fabales
Family:	fabaceae
Subfamily:	faboideae
Genus:	cajanus
Species:	<i>Cajanus Cajan</i> (L) Mill sp.

#### 2.1. 2 Morphology:

The fruit of *Cajanus Cajan* is a flat, straight, pubescent, 5-9 cm long x 12-13 mm wide pods. It contains 2-9 seeds that are brown, red or black in colour, small and sometimes hard coated (FAO, 2016a; Bekele-Tessema, 2007). An erect, woody, short – lived, perennial shrub, often grown as an annual, which shows considerable variation in form under different environmental and cultural conditions shows can vary in height from 2 to 12 ft(0.6-3.6m) and the spread of the branches normally ranges from less than one- quarter to more than one half of the height of the plant. The root system varies according to the type: tall upright cultivars produce long, vertical, deeply penetrating tap-roots, while spreading, bushy cultivars produce shallower more spreading roots the stems are angular hairy and branched the point on the main stem where branching begins (Daisy, 1979). Pigeon pea show great diversity in their habit growth period, and the colour shape and size of the pods and seeds. Some authorities recognize two separate varieties (i) *Cajanus Cajan* var. *flavus*; (ii) *C.cajan* var. *bicolor*. The former is earlier maturing, semi-dwarf in habit and as yellow flowers and green pods, usually with 2-3 seeds (Daisy, 1979).

### 2.1.3 Distribution:

The origin of *Cajanus Cajan* is either north eastern Africa or India (Ecocrop, 2016; van der Maesen, 1989). The centre of origin of the pigeon pea has been the subject of much discussion. Some authorities consider in Indian origin. There is evidence, however, that was cultivated in Egypt before 2000bBC and many authorities consider that it may have originated in the origin between Egypt and East Africa and the SE (Daisy, 1979). Its cultivation dates back to at least 3000 years (Mallikarjuna et al., 2011; van der Maesen, 1989). It is now a pan tropical and subtropical species particularly suited for rain fed agriculture in semi-arid areas thanks to its deep taproot, heat tolerance and fast growing habit (Mallikarjuna et al., 2011). *Cajanus cajan* can be found in both hemispheres from 30°N to 30°S and from sea level to an altitude of 2000 m (3000 m in Venezuela) (Ecocrop, 2016). It is very heat-tolerant and grows better in places where temperatures range from 20° to 40°C and which are deprived of frost (FAO, 2016a). In Sudan replaced bandied in states

in western Sudan and Algazira, middle Sudan and mean of yield about 750kg/h (Ali, 2007).

Although Pigeon pea is keeps growing at temperatures close to 0°C and tall plants can survive light frost. It performs better where annual rainfall is above 625 mm but it is highly tolerant of dry periods and, where the soil is deep and well-structured; it still grows with as low as 250 to 375 mm rainfall. Pigeon pea adapted to a wide range of soils ranging from sands to heavy black clays, with variable pH however, optimum or best pH range is within 5 - 7. It has low tolerance of soil salinity, but some cultivars were reported to tolerate high (6-12 dS/m) salinity (Duke, 1983). *Cajanus Cajan* sensitive to salt spray and water logging. Under shade, it shows reduced growth and bear thin, pale green foliage and few pods (FAO, 2016a).

#### 2.1.4 The nutritive values

Pigeon peas seeds are rich in protein feed, with about 18.30 – 20.13% DM crude protein, crude fibre 3,98 – 10.50 % NFE 35.66 -55.28 % and oil 1.50 – 2.42% (table 3.1), all amino acids essential for fishes except methionine (0,3 – 1.5%), Lysine (0.8 -7.8%), Phenylalanine (0.9 – 10.4%), ... etc (table 2.2), and 7 minerals table (2.1), also those component above looks like or similar to mother milk, and its energy similar to that found in corn, barley and wheat bran, etc.

Pigeon pea seeds are a source of protein and energy as they are rich in protein (18-28% DM) and carbohydrates (starch 37-45% DM), and relatively poor in fibre (crude fibre < 10% DM). The amino acid profile is close to that of soybean (Oshodi et al., 1993).

TABLE (2.1): Minerals in Pigeon peas seed:

Minerals	Unit	Avg	SD	Me	Max	Nb
Calcium	g/kg DM	3.6	3.7	1.2	9.6	8
Phosphorus	g/k DM	3.3	1.5	1.0	6.0	9

Potassium	g/kg DM	10.1	5.3	3.8	14.8	5
Magnesium	g/kg DM	1.4	0.2	1.2	1.7	5
Manganese	Mg/k DM	17	-	-	-	1
Zinc	Mg/kg DM	69	-	-	-	1
Copper	Mg/kg DM	22	-	-	-	1

Source (Obasa et al., 2003).

Table (2.2): Amino acids in Pigeon peas seed:

Amino acids	Unit	Avg	SD	me	Max	Nb
Alanine	% protein	5.2	1.4	3.6	6.3	3
Arginine	% protein	5.7	1.5	3.2	6.8	5
Aspartic acid	% protein	9.7	1.8	7.6	11.0	3
Cysteine	% protein	1.5	0.4	1.1	2.2	6
Glutamic acid	% protein	19.1	4.8	15.9	24.7	3
Wisteria	% protein	3.9	0.2	3.6	4.0	3
Histidine	% protein	3.2	0.5	2.2	3.8	7
Isoleucine	% protein	3.7	0.4	3.1	4.1	7
Lucien	% protein	7.2	1.1	5.5	8.6	7
Lysine	% protein	6.5	0.8	5.6	7.8	7
Methionine	% protein	1.0	0.4	0.3	1.5	7
Phenylalanine	% protein	8.9	0.9	7.7	10.4	7
Praline	% protein	4.4	-	3.5	5.4	2
Serine	% protein	4.5	0.3	4.2	4.8	3
Threonine	% protein	3.4	0.6	2.6	3.9	7
Tryptophan	% protein	0.6	0.2	0.3	0.9	5
Trosine	% protein	2.4	1.0	0.4	3.8	7
Valine	%protein	4.5	1.0	2.9	5.7	7

Source (Obasa et al., 2003).

### 2.1. 5 Uses of Pigeon peas:



Pigeon peas are used for human and animals. Dry pigeon peas (seeds) are common in Indonesian and Indian cuisines. In India, pigeon peas are soaked, dried, hulled and split to prepare dhal (is a term in the India subcontinent for dried, split pulses). In Indonesia, pigeon peas are fermented with *Rhizopus* mould then soaked, dehulled and cooked to produce tempeh. Fermentation with *Aspergillums oryza* yields a sauce similar to soy sauce (Orwa et al., 2009). The pigeon pea is an important protein food in many tropical areas in India it is consumed mainly in from of dhal. In Africa and Indonesia the mature seeds are usually soaked for several hours before being pounded and fried ,or steamed ,and eaten often in the form of a puree .The fresh green seeds are a very popular vegetable, particularly in the Caribbean area, where considerable quantities are processed(Daisy,1979).

Dry peas can be ground and mixed with wheat flour in order to increase the flour protein content. Immature pods may be cooked in curries and other relishes (Orwa et al., 2009). *Cajanus cajan* has numerous uses in animal feeding. The leaves and pods are valuable and palatable protein-rich fodder. Leaves are sometimes used to replace alfalfa in ruminant's diets where alfalfa cannot be grown. Seed processing by-products and sometimes the seeds themselves are used as livestock feed (Phatak et al., 1993).

The seeds can be fed to poultry; and mixtures of pigeon pea with maize grain were successful in animal feed in Hawaii (Orwa et al., 2009). Plant breeders have created varieties adapted to drier conditions, more resistant to diseases and suited to different production systems and cropping cycles (Valenzuela, 2011). Since the 1990s there has been a great scope for selecting cultivars with not only higher grain yields but also higher forage yields and crude protein (Phatak et al., 1993).

#### 2. 1.6 Harvest:

In Sudan seeds are harvested manually where the plant in cut left to dry then seeds harvested. Seeds yield in amounted to in Sudan about ton/hectare (Ali, 2007).

Pod harvest can be done by hand-picking over a long period in gardens and hedge crops. The harvest begins when about 75% of the pods have turned brown. In small farms, the plant is traditionally cut at the base with a sickle. It is possible to use a Combine-harvester if the plants have matured uniformly and the pods are at a uniform level above the ground (CIMMYT, 2011; van der Maesen, 1989). The cut branches are dried on the field or tied in bundles which are stacked upright in order to dry, and then threshed with wooden flails, by cattle trampling or with a threshing machine (FAO, 2016a; Singh et al., 1992).

Another harvesting method consists in hand-picking pods once, then letting the plant regrow, and hand-picking a second time and sometimes a third time if the quantities of pods make the practice profitable (Singh et al., 1992). If hand-picking is not possible, it is advised to cut the upper parts of the stems bearing the mature pods and let enough foliage so that the plant can re grow (Singh et al., 1992). In Colombia, pigeon peas are cultivated for feed but once for beans and once for forage: during the first year, the peas are used for poultry rations, they are cut at 0.5 m high and, in the second year, they are cut at 1 m high and cattle are then allowed to browse the forage re growth (FAO, 2016a).

## 2.2. Biology Of *Oreochromis niloticus*

### 2.2.1 Historical background

The Nile tilapia (*Oreochromis niloticus*) is a species of tilapia, a cichlid fish native to Africa from ancient Egyptian times as depicted on bas-relief from an Egyptian tomb dating back over 4000 years, which showed the fish held in ornamental ponds, south to East and Central Africa, and as far west as Gambia. It is also native to Israel, and numerous introduced populations exist outside its natural range. The distribution occurred during the 1960s up to the 1980s. In 1974 Nile tilapia was introduced to Bangladesh from Thailand ( Wikipedia ) and in 1978 to China from Sudan 22 of them, which leads the world in tilapia production and consistently

produced more than half of the global production in every year, FAO (2015), it is also commercially known as mango fish, nilotica, or boulti.

### 2.2.2 Scientific Classification

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciform

Family: Cichlidae

Genus: *Oreochromis*

Species: *Oreochromis niloticus*

Binominal name: *Oreochromis niloticus* (Linnaeus. 1758).

#### 2.2. 2.2. Description:

The Nile tilapia has distinctive, regular, vertical stripes extending as far down the body as the bottom edge of the caudal fin, with variable coloration, the body compressed; caudal peduncle depth equal to length. Scales cycloid. A knob-like protuberance absent on dorsal surface of snout. Upper jaw length showing no sexual dimorphism. First gill arch with 27 to 33 gill rakers (figure 2.4.3). Lateral line interrupted. Spinous and soft ray parts of dorsal fin continuous. Dorsal fin with 16-17 spines and 11 to 15 soft rays. Anal fin with 3 spines and 10-11 rays. Caudal fin truncated. Colour in spawning season, pectoral, dorsal and caudal fins becoming reddish; caudal fin with numerous black bars FAO (2015). Adults reach up to 60 cm (24 in) in length and up to 4.3 kg (9.5 lb). It lives for up to 9 years. It tolerates brackish water and survives temperatures between 8 and 42 °C (46 and 108 °F). pH ranging from 5-10 but do best performance at 6-9. In recent

research done in Kenya, this fish has been shown to feed on mosquito larvae, making it a possible tool in the fight against malaria in Africa.

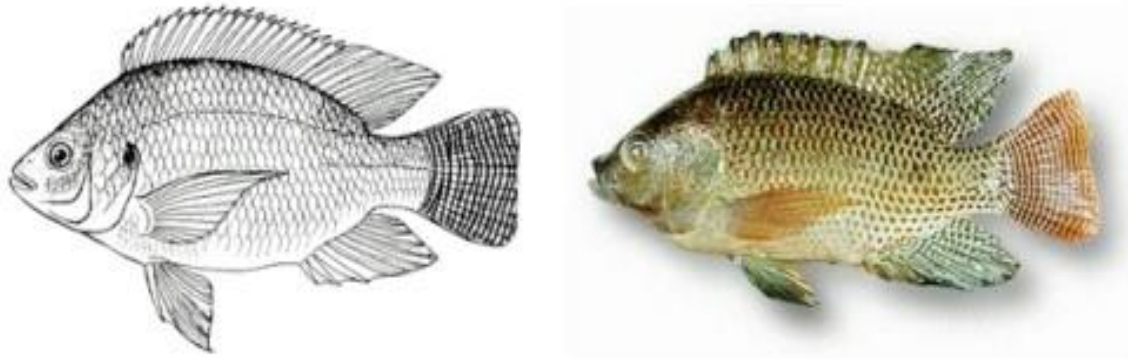


Figure (2.1) Images of the *Oreochromis Niloticus* (Source: FAO 2012).

### 2.2.3 Natural distribution and habitat:

*O. Niloticus* is native to central and North Africa and the Middle East (Boyd 2004) (Figure 2). It is a tropical freshwater and estuarine species. It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation (Picker & Griffiths 2011).

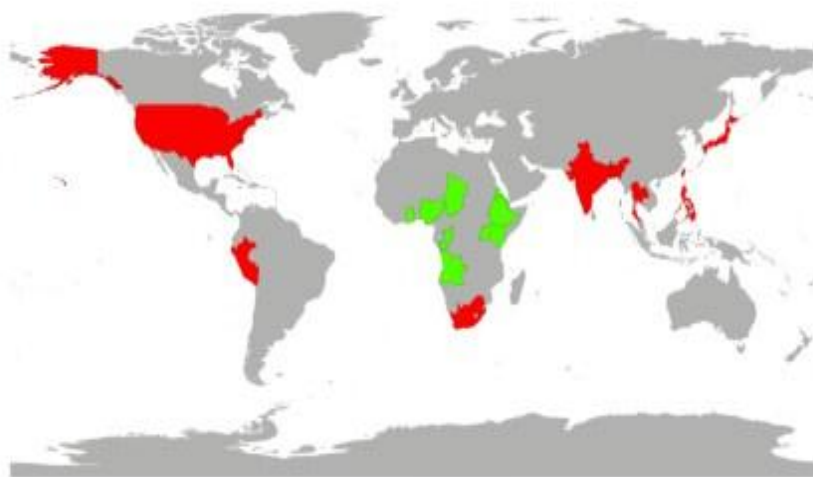


Figure (2.2). Native (green) and introduced (red) ranges of *O. niloticus* globally (Data source: GISD 2012). Please note this map does not indicate country wide presence, but merely that the species is categorised as an alien within that country.

### 2.2.4. History of domestication

Nile tilapias have been farmed for centuries. Depictions on an Egyptian tomb (dated at 4000 years) display the fish in ornamental ponds. The culture of the tilapia genus on a global scale, primarily *Oreochromis mossambicus*, began in the 1940s. However, it was not until the 1960s that *O. niloticus* was exported worldwide (FAO 2012).

Aquaculture was heralded as the perfect protein production technique for developing countries during the 1960s and 1970s. Aid organisations promoted aquaculture as a means of improving food security with low grain to feed conversion rates, and minimal environmental impacts (Canónico et al. 2005).

This global popularity has led to a number of important developments in culture techniques. Initially, tilapias were allowed to breed freely. However, farmers and scientists observed that this led to the production of smaller fish. In the 1960s, attempts were made to produce male mono sex populations through hybridisation between different tilapia species (Hickling 1963). This proved problematic and gradually females reappeared in the progeny (Wohlfarth 1994). Major technological developments in the 1970s allowed for the successful production of all-male populations through the use of sex reversing hormones which resulted in higher returns from tilapia farming. Following this, and further research into culture processes, the industry boomed (FAO 2012).

Today, tilapias are often farmed with multiple species in the same pond, such as shrimp and milkfish. This not only optimises the financial return if space is limited, but also helps prevent the growth of harmful bacteria and serves to remove excess organic matter in the water (Troell 2009). Genetic modification of the species has also been undertaken to maximise farming efficiency. For example, the Genetic Improvement in Farmed Tilapia (GIFT) project in the Philippines created strains of *O. niloticus* that grew up to 60% faster than their relatives (Eknath & Acosta 1998). However, in Africa, the use of improved stock lines is rare due to concerns regarding genetic modification. As a result, many tilapia

farms use brood stock which underperforms by 20-40% relative to wild individuals. There is great scope for improvement in this regard, either by rotational mating or the introduction of improved strains (Brummet & Ponzoni 2009).

#### 2.2.5. Reproduction:

Male fish initiate breeding with the creation of a spawning nest, which is fiercely guarded. When the water temperature increases above 24°C, a female will lay her eggs into the nest. These are then fertilized by the males before the female collects them in her mouth (known as mouth brooding).

The eggs and the fry which then hatch are incubated and brooded in this manner until the yolk sac is fully absorbed two weeks later (FAO 2012).

The number of eggs a female will produce is dependent on body size. This can range from 100 eggs (produced by a 100 g fish) to 1500 eggs (spawned by a 1 kg fish). The females will not spawn while brooding. Males on the other hand fertilise the eggs of multiple females continuously given optimal environmental conditions (FAO 2012).

#### 2.2.6. Mono-sex Tilapia: (*Oreochromis Niloticus*)

The plentiful fish, nicknamed the chicken of the sea as it can be cooked and prepared in so many different ways, is found naturally in sea and fresh water and is successfully farmed in all climates. It is fast replacing varieties of fish whose numbers are becoming scarce in the wild, particularly in America where it is now one of the top five fish dishes in the country. tilapia has numerous advantages as an aquaculture species (Teichert-Coddington et al.,1997) such as rapid growth, omnivorous fish can use high proportion of inexpensive plant sources in their feeds, stands well in wide range of environmental conditions (Such as temperature, salinity low dissolve oxygen, etc.), resistance against stress and disease, short generation interval and low supplementary feed require in natural environment and can take the Commercial feed immediately after yolk.-sac absorption, El-Sayed (2006).

Unfavourably, the uncontrolled breeding of tilapia in ponds (Early sexual maturity 3 – 4 month) which led to excessive recruitment about 70%, as a result competition in space and food within the target fattening species (Yosif et al ,2010), stunting and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish, Has resulted in various techniques being developed to control unwanted reproduction, Under favourable conditions they will continue to reproduce, the offspring competing with the initial stock for food (Phelps and Popma, 2000). There are a number of ways to control reproduction in mixed sex Population so the development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male mono-sex population to be raised to uniform, marketable sizes.

According to FAO (2015), all male culture of tilapia is preferred because of their faster growth. Several techniques have been adopted for production of mono-sex (all male ) tilapia: Manual sexing Guerrero (1982); hybridization Hickling (1960); genetic manipulation (pandian and Varadaraj,1988); and sex reversal through sex hormone administration (Shelton et al., 1978; Guerrero, 1982); sex reversal by oral administration of feed incorporated with 178 methyl testosterone is probably the most effective and practical method for the production of all male tilapia (figure 2.6),which consistently grow to a larger, more uniform size than mixed sex or all-female tilapias. This is the most common method of sex reversal in most countries Cagauan et al., 2004).

Hormonal sex reversal has been particularly effective in cichlids because the gonadal differentiation takes place early in the life history. Tilapia species that have been successfully sex reversed are mouth brooding species where hormone treatment begins within a few days after hatching (Phelps and Popma, 2000). In addition, research on nutrition and culture systems, along with market development and processing advances, let to rapid expansion of the industry since mid 1980s. Several species of tilapia are the cultured commercially, but Nile tilapia is the predominant cultured species worldwide FAO (2015).

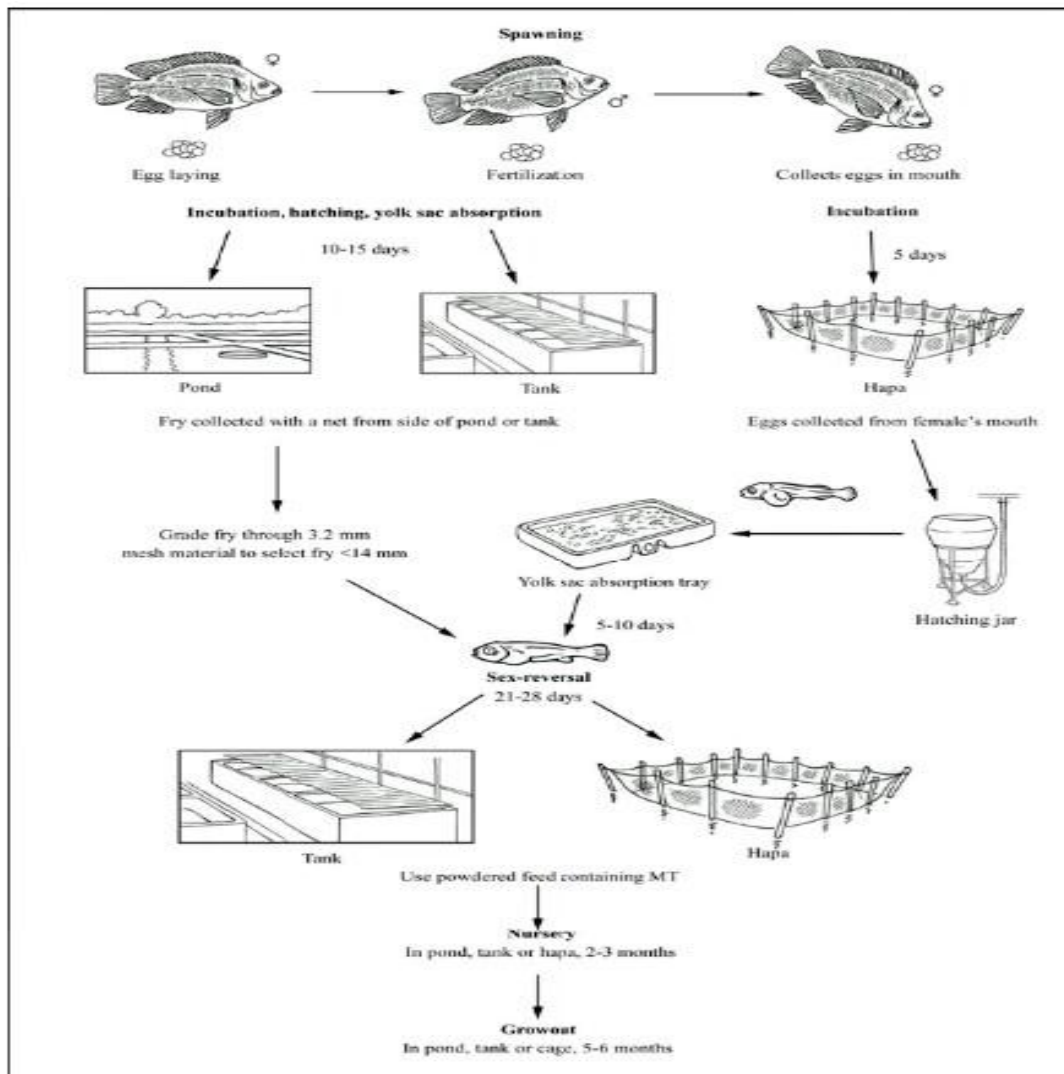


Figure (2.3): production cycle of mono-sex Nile tilapia (*Oreochromis Niloticus*) source FAO (2015).

### 2.3 Global Aquaculture and Fish Meal

As the global population continues to grow and forecasts exceeding 8 billion by the year 2030. The seafood consumption is predicted to reach between 150-160 million tons per year in 2030 FAO (2005). The decline in wild fish catches due to overexploitation of our fisheries resources; aquaculture will be called upon to fill this gap. In the last three decades (1980–2010), world food fish production of aquaculture has expanded, (fish farming business increases more than other animal production enterprise) by almost 12 times, at an average annual rate of 8.8 percent at 60 million tons (excluding aquatic plants and non-food



products), with an estimated total value of US\$119 billion. Global aquaculture production has continued to grow, when farmed aquatic plants and non-food products are included, world aquaculture production in 2010 was 79 million tones, worth US\$125 billion, Freshwater fishes dominate global aquaculture production 56.4 percent, 33.7 million tones. About 600 aquatic species are raised in captivity in about 190 countries for production in farming systems of varying input intensities and technological Sophistication. These include hatcheries producing seeds for stocking to the wild, particularly in inland waters FAO (2012). Global production of farmed Nile tilapia was 1.66 million metric ton (MMT) and 2.54 MMT in 2005 and 2009, respectively, including other cichlids the production was 3.1 MMT out of global aquaculture production of 55.1 MMT FAO (2010). Thus tilapia and other cichlids totally contribute about 5.6% of total aquaculture production. With the amount of sales for year 2000, 2005 and 2010 are 1.744.000, 457.312.000,>5.000.000.000 U\$ respectively Antony (2010). According to the Fishery Statistical Yearbook of Bangladesh, tilapia production was 136,000 metric tons in 2012. It appears that tilapias are likely to be higher rank in global aquaculture production next to carp production, According to El-Sayed (2006), requires minimum fish meal, suitable for different culture system either in marine or fresh water Fitzsimmons (2010).

The major problems in using fish meal in formulation of fish feed are its rising cost, the price has increased more than two fold in recent years FAO (2013). In Asia alone, the production of fish feed increased from 40% in 2000 to 60% in 2008 while the fishmeal consumption for tilapia increased from 0.8 million tons to 1.7 million tons during same period (Tacon and Metian, 2008), Within the animal husbandry subsectors, aquaculture is the largest user of fishmeal and fish oil, uncertain availability, currently up to 36% of the world's total fisheries catch each year is ground up into fishmeal and oil to feed farmed fish, livestock, chicken and pigs (Jacquet et al., 2010), variation in quality and its high inclusion 40 -70% in fish feed and it is expensive.

#### 2.4. Food and feeding habits:

Nile tilapia are known to feed on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films (FAO 2012) and even other fish and fish eggs. Depending on the food source, they will feed either via suspension filtering or surface grazing (GISD 2012), trapping plankton in a plankton rich bolus using mucus excreted from their gills (Fryer & Iles 1972). *O. niloticus* have been observed to exhibit trophic plasticity according to the environment and the other species they coexist with (Bwanika et al. 2007).

One of the key factors to successful fish culture is the understanding of some Biological fundamentals especially food and feeding behaviour. Nile Tilapia is an omnivore, feeding of plankton as well as on higher plants, the juvenile and adult Nile tilapia is reported to filter phytoplankton (Northcott et al. 1991). Since Nile tilapia use algal protein raising tilapia for food at lower trophical level can be a cost-efficient culture method. fish food consumption might be influenced by many environmental factors such as water temperature, food concentration, stocking density, fish size and fish behaviour (Houlihan et al. 2001).

The feeding behaviour of Nile tilapia begins at sunrise continues during the daytime size to size, time to time, and actually it positively correlated to the occurrence and the richness of nature food components. So the fullness degree insignificantly differed. On the other hand, plankton is quickly digested and passed to the mid-gut and subsequent to hind-gut. Also; evacuation time (rate) may affect the composition percentage of phytoplankton in fish stomach.

In this concern Yada (1982) reported that Nile tilapia could change its feeding periodicity according to the fluctuation of phytoplankton concentrations. Moreover, Focken et al (2000) reported that the natural food is ingested for 4 - 5 hours in the morning and 5-7 hours in the afternoon, and the flow of natural food from the stomach is low and fast subsequently. It could be supposed that each phytoplankton organisms has its own evacuation rate.

## 2.5. Feeding rates and Growth:

Nile tilapia can live longer than 10 years (GISD 2012). Food availability and water temperature appear to be the limiting factors to growth for *O. Niloticus* (Kapetsky & Nath 1997). Optimal growth is achieved at 28-36°C and declines with decreasing temperature (Teichert-Coddington et al. 1997, FAO 2012). The ability to vary their diet may also result in variation in growth (Bwanika et al. 2007).

In aquaculture ponds, *O. Niloticus* can reach sexual maturity at the age of 5-6 months (FAO 2012).

It has been evident from several studies that feeding rate and meal frequency can influence the production performance of tilapia. The feeding rate relative to the body weight decreases as fish size increases, however, the rate of food consumed increases per individual (Wang et al. 1989).

(Brummett, 1995; Saha and Dewan, 1979) observed that the amount of phytoplankton in Nile tilapia stomachs decreased as fish size increased. Study with polyculture farming of tilapia, common carp and silver carp showed that growth performance, body fat and gross energy gain increased as Feeding rate 0 to 5% and to apparent satiation increased (Abdelghany and Ahamed 2002a).

Tambaqui showed better outcome using 10% feeding rate and 3 meals per day at growth phase (Silva et al., 2007). Research from pikeperch (6.4 gm) gave enhanced growth at 2% feeding rate and 3 meals/day (Wang et al., 2009).

Yuan et al. (2010) found increased growth performance, protein and lipid contents with increasing feeding rate. And Riche et al. (2004) reported that growth efficiency of tilapia increased if they are allowed for four hours satiation feeding increased daily feeding rate from 30 to 60% of body weight for juvenile tilapia (12 mg body weight) gave significantly higher growth (Santiago et al., 1987). A study with red tilapia showed that best growth can be achieved near satiation, feeding rate (Clark et al., 1990).

According to El-Saidy et al (2005) tilapia with average weight 61.9g showed cost effective and affordable feed strategy at 2% feeding rate. Similarly, Storebakken and Austreng (1987) found that Atlantic salmon showed increased meal frequency provided better carbohydrate utilization for hybrid tilapia (Tung and Shiau, 1991). Imageperiod also influences the growth of tilapia and El-Saidy and Kawanna (2004) stimulated the growth of tilapia by the using longer Imageperiod.

## 2.6 Length- Weight relationship:

Tilapia growth can generally be broken into three distinct phases such as:-

1. Exponential or Accelerating Phase : In this phase the young fry is ravenous eaters and they can consume feed that is equivalent to up to 25% of their body weight daily. As a result, the fish grow very fast when measured in percent of body weight per day. However, since the fish are initially very small, the total weight gain is initially low. This rate of weight gain (in grams per day) increases until the fish weigh approximately 100 grams, at which point they enter the Linear Phase of growth.
2. Linear Phase: In this Phase, Tilapia eat approximately the same amount each day and the growth remains fairly linear. Their feeding rate does not change drastically during this period because, although the fish are growing, they eat less feed as a percent of their body weight as they grow. The duration of the linear growth phase differs dramatically with the species and strain of tilapia. Some inferior strains may show growth deceleration at sizes of less than one pound.
3. The Plateau Phase : At this point(When fish growth begins to decelerate) it begins to take more food and time to achieve a given amount of growth, this can be very frustrating for the aquaculturist who is trying to get fish to market. Cheaper fingerlings often become more expensive in the long run due to poor genetics. In fishes, generally the growth pattern follows the cube law

Beverton and Holt (1957) stated that major deviations from isometric growth are rare. Such cubic relationship for fishes will be valid when Fish grows isometrically. But in reality, the actual relationship between the variables, length and weight, may depart from this, either due to environmental conditions or condition of fish (Le Cren, 1951). According to Martin (1949) the value of the exponent 'b' in the parabolic equations usually lies between 2.5 and 4. Depending upon the deviation of 'b' values from '3' fishes can be classified into three groups (i)  $b=3$  where the body form of fish remains constant at different lengths (isometric) (Allen, 1938), (ii)  $b<3$  when fish becomes more slender as the length increases and (iii)  $b>3$  (allometric) when fish grows more stouter with increase of length (Growner et.al., 1976).

## 2.7. Fish nutritional requirements:

Nutrition is the most expensive component in the intensive aquaculture industry, where it represents over 50% of operating costs. Moreover protein itself represents about 50% of feed cost in intensive culture, therefore, the selection of proper quantity and quality of dietary protein is a necessary tool for successful tilapia culture practices.

### 2.7.1. Protein requirements:

Several factors including fish size or age, dietary protein source, energy content, water quality and culture conditions have been reported to affect protein requirements of tilapia (Elsayed, 2003 P.29). Many studies indicated that protein requirement for maximum performance of tilapia during larval stages is relatively high (35 – 50%) and decreases with increasing fish size (Winfrey and Stickney, 1981; Jouncey and Ros, 1982; Siddiqui et al., 1988; Elsayed and Teshima, 1992) for Tilapia Juveniles the protein requirement ranges from 30-40% while adult tilapia Require 20-30% dietary protein for optimum performance; on the other hand tilapia brood stock requires 35-45% dietary protein for optimum reproduction, spawning

efficiency and larval growth and survival (Gunsekera et al., 1996a, b; Siddiqui et al., 1998; Elsayed et al., 2003).

#### 2.7.1.1. Amino acid requirements:

Despite that tilapia require the 10 essential amino acids (arginine, Lysine, histidine, threonine, valine, leucine, isoleucine, methionine, Phenylalanine and tryptophan) which content in protein, requirement,

This follows the concept that protein requirement and ensure maximum Growth.

#### 2.7.1.2. Animal protein sources:

##### 2.7.1.2.1. Fish meal:

Fishmeal (FM) has been traditionally used as the main protein source in the aqua feed industry. However the increased demand for FM coupled with a significant shortage inglobal and the supply of fishmeal is not growing worldwide (Rumsey, 1994; Barlow, 1997).

FM production has created sharp competition for its use by the animal feed industry. As a result, FM has become the most expensive protein commodity in aquaculture feeds in recent years (Tacon, 1990 P.30). Moreover the price of fish meal is often high, these necessitate replacing FM with cheaper protein sources (Shepherd, 1998) therefore, many attempts have been made to partially or totally replace FM with less expensive, locally available protein source.

##### 2.7.1.2.2. Fishery By-products:

Despite the fact that large amounts of fishery by-products and by catch are produced annually in the world, little attention has been paid to the commercial use of this by-product for tilapia. The exception is fish silage and shrimp meals, where several studies have considered their use as a FM replacer in tilapia feeds.

The results indicated that between 30-75% fish silage can be successfully incorporated in tilapia feeds, depending on fish species and size, silage source, and diet composition (Fagbenor 1994; Fagbenor and Jauncey, 1994). It is evident that fish silage has potential as a protein source for tilapia.

Nile tilapia utilized shrimp head meal at up to 15 and 60% of the diet without adverse effects on their performance (Toledo et al., 1987; Nwanna and Daramola, 2000) and Elsayed (1998) reported that shrimp meal can replace FM in Nile tilapia diet at 50% and 100% respectively without significant retardation in weight gain and feed efficiency.

#### 2.7.1.2.3. Poultry by-product meal (PBM)

Poultry by-product meal (PB) is made of ground rendered powder of the carcass of slaughtered poultry. PBM has been tested at varying success so far in profiles (Sadiku and Jauncey, 1995; Elsayed, 1998). Fowler (1991) and Sevgili (2002) reported PBM could replace about 50% of fishmeal in the diets for Rainbow trout. Hassan and Amin (1997) found that processing techniques greatly affected the nutritional quality of PBM. They reported that autoclaved product showed better growth performance than sun dried and or oven dried PBM and there is a deficiency of EAA (lysine) in PBM (NRC, 1983; Tacon and Jackson, 1985).

Belal et al. (1995) fed *O. Niloticus* fingerlings (10.8 g) test diets containing 20% chicken offal silage (COS) made from chicken viscera, as a replacement of fishmeal. They found that the growth and body composition of fish fed COS up to 20% level were similar to that of fish fed a FM based diet.

#### 2.7.1.2. Plant protein sources:

##### 2.7.1.2.1 Oil seed plants:

##### 2.7.1.2.1.1 Soybean meal (SBM):

SBM contains the highest plant protein content and has the best EAA profile, but it is deficient in sulphur, containing amino acids (methionine, lysine, cysteine) and contains endogenous anti-nutrients including protease (trypsin) inhibitor SBM can be used as total or partial protein source for farmed tilapia, depending on fish size, dietary protein level and processing methods.

#### 2.7.1.2.1.2 Cotton seed cake:

Cottonseed cake (CSC) is one of the best plant protein sources for tilapia in developing countries due to its high availability, relatively low price, good protein content 26.54% depending on processing methods and amino acid profile (FAO, 1998 P.29). However, it is deficient in some EAA such as cysteine, lysine and methionine in addition to its high content of gossypol that may limit the use of CSC in tilapia feeds. Results on the use of CSC indicated that replacement of more traditional protein sources at between 50-100% can be effective in tilapia feed depending on CSC source, processing method and fish species and sizes.

#### 2.7.1.3 Wheat Meal

Although primarily a crop of temperate countries, wheat is also grown in parts of the tropics where there is a long period of relatively cool weather. Where it is not grown, it is imported for flour production. Wheat products are, therefore, available in practically every country. Wheat, often used as an ingredient of compound fish feed in temperate countries is seldom used in the tropics because of its high cost. Damaged grain is, however, frequently available and when fed to fish is at least equivalent to maize in feed value. Milling of wheat produces three major by-products, two of which are almost exclusively used as feed for livestock. These are: wheat bran and wheat middlings (or fine bran). The third, wheat germ, has higher commercial value as a food item for humans. Occasionally, damaged flour is also available as feed.



#### 2.7.1.4 Wheat bran Meal

Wheat bran is the primary coat of the wheat grain. It has fairly high protein content and polyunsaturated fatty acids (PUFA). Because it is also high in fibre, it has a laxative effect when fed at excessive levels to animals. Such an effect, however, will be more difficult to determine in fish. Nevertheless, wheat bran has been successfully fed at fairly high levels to various species of fish without adverse effects on growth.

Too much wheat bran in a formulation results in pellets with poor water stability due to the water absorption characteristics of fiber, and it is grown primarily for human consumption. The use of grains is somewhat restricted in fish diets because of generally lower tolerance for dietary carbohydrates by fish. This effect appears to be more pronounced among cold-water species such as the trout than among warm-water species (FAO 2014).

#### 2.7.1.5. Aquatic plants:

Several studies have been conducted on the use of aquatic plants in tilapia feeds, among these plants the Duck weed is the most promising and its excellent food source for tilapia due to its good protein content (35-45%) and amino acid and mineral profiles. In addition to that plant protein is cheaper per unit of nutrient than animal protein (Alcest, 2000). Other aquatic plants including *Azolla pinnata*, *Hydrodictyon reticulatury* and others.

#### 2.7.1.6. Grain legumes:

Many leguminous or cereal plants and by-products can be used as partial protein sources for tilapia. Among these leucaena leaf meal, Brewery wastes, Corn products, Cassava leaf meal, green legume and other.

#### 2.7.2. Carbohydrate:

Fish need small amounts of carbohydrate the digestible energy requirement of economically optimum growth have been estimated in the fry (2.5g) with 35% protein with a digestible energy, protein ratio (DE/P) of 8.2 Kcal/g of protein. Large fish (7.5g) need 9.4 Kcal (Sinfree and Stiekeny, 1981).

#### 2.7.3. Fat:

Fish require long chain fatty acids. The optimum dietary levels of fatty acids have been estimated about 5% for *O. Niloticus* and 1% of *T. Zillii* (Takeuchs, 1983).

#### 2.7.4. Vitamins and minerals:

Tilapia require vitamins and minerals as most fishes. Most of the tilapia culture satisfies their vitamin needs from the natural foods, and it needs supplementary vitamin under extensive conditions in ponds.

Minerals needs in many functions like structure of skeletons and soft tissues, nerve impulse and transmission and muscle contraction (Jauncey, 1992).

#### 2.8 Formulated Feeds:

High quality formulated feeds are used to achieve high yields and large sized fish (600-900 g) within a short period of time. The maximum size at harvest of Nile tilapia reared in ponds that are only fertilized is generally less than 250 g after 5-6 months of on growing. Under semi-intensive farming systems, most tilapia farmers in Asia fertilize their ponds and use formulated feeds. However, in intensive pond and tank culture systems or in cages, tilapia farmers mainly depend on commercial pelleted feeds. The nutrient inputs used and the yield and weight of tilapia at harvest in several Asian countries are summarized by Dey (2001). In terms of pond yields, Dey (2001) reported that overall, the average yield of pond farming in Taiwan, Province of China is very high (12 to 17 tones/ha) while ponds in Bangladesh, China, the Philippines, Thailand and Viet Nam produce around 1.7, 6.6, 3.0, 6.3 and 3.0.

## 2.9 Inclusion of Pigeon pea (*Cajanus Cajan*) in Fish Diet.

Pigeon pea is a potential source of protein with a well-balanced amino acid profile that could replace fish meal in fish diets; but the presence of ant nutritional factors may limit their use (Ndau et al., 2015).

Pigeon pea seeds, raw or soaked, have been included to replace 15, 30 or 45% fish meal in is nitrogenous juvenile diets. Inclusion of pigeon pea, either raw or soaked decreased animal growth rate compared to the control diet. However, growth performance of Nile tilapia (*Oreochromis Niloticus*) fed on soaked pigeon pea diets were closer to that of fish fed on control diet, and it was concluded that soaked pigeon pea could replace fishmeal in fish diets in order to reduce feed costs (Ndau et al., 2015). When pigeon pea meal (pressure-cooked and sun-dried) was used to replace soybean meal, it was reported that 60% replacement resulted in better nutrient utilization and better growth in fish (Obasa et al., 2003).

In a study conducted to assess the Effect of soaked Pigeon peas on the growth of Nile tilapia; *Oreochromis Niloticus* was then evaluated for a period of 54 days. Seven diets (three diets of raw pigeon peas and soaked pigeon peas and one control diet) were formulated to contain about 30% crude protein. Fishmeal was replaced by 0% (control), 15%, 30% and 45% for both raw and soaked pigeon peas. Results obtained in this study showed that growth and feed utilization of fish fed diets containing raw Pigeon peas seed meal decreased significantly ( $P>0.05$ ) with increased dietary levels of raw Pigeon peas While significant results ( $P<0.05$ ) were are shown for fish fed soaked Pigeon peas seed meal with 45% level inclusion performing best next to control. It was therefore concluded that whenever the cost of Pigeon peas is lesser than fish meal, Pigeon peas can be soaked for 24 hours with a ratio of 1:3 w/v to replace up to 45% fish meal in fish diets as a way of reducing the current demand pressure on fish meal.

In Nigeria, channel catfish juveniles could be fed on diets containing 40% protein and where pigeon pea meal replaced up to 100% soybean meal (Hammed et al.,

2013). In a comparison of raw and heat-processed seeds showed that raw pigeon peas had deleterious effects on growth and on health parameters (hemoglobin, hematocrit, red blood cells and white blood cells were all reduced). Fried pigeon pea and boiled pigeon pea did not yield satisfactory weight gains but had no significant effect on health parameters. Only 16h-soaked pigeon peas resulted in higher weight gains and had no effect on blood parameters. It was concluded that soaked pigeon peas could be included in channel catfish larvae at up to 65% dietary level (Ogunji et al., 2005).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1. Locality:

This study was conducted in the fresh water Fisheries laboratory and Hatchery, Department of Fisheries and Wildlife Science, College of Animal Production Science and Technology, Sudan University of Science and Technology. The duration of experiments was one month and half, starting from 10 July to 30 August 2018.

#### 3.2. Experimental Diets:

Diet ingredients were obtained from the local market (Kuku market). Twelve experimental diets incorporated with different levels of Pigeon peas seed 0, 25, 50, and 75 in diets To, T1, T2, T3, respectively. All feeds were formulated with ingredients commonly used, including fish meal, groundnut cake, wheat bran, mineral mix and vegetable oil, as presented in Table 3.3. The Pigeon peas seed were cleaned and soaked in tap water for one day dried and some were milled in the home blender in Kuku market into powder, and Chemical Analysis of Pigeon peas are presented in Table 3.1 below.

Table 3.1: Chemical composition of Pigeon pea (DM %).

Type	DM	CP%	EE%	CF%	Ash	NFE
Fresh	91.63	20.13	2.42	10.50	3.47	55.11
treated(soaked)	79.22	18.40	1.99	3.98	2.43	35.66

DM= Dry Matter. CP= Crude Protein. EE= Ether Extract. CF= Crude Fibre. NFE= Nitrogen Free Extract.

M.E: calculate value according to equation of (Iodhi *et al*; 1974)

#### 3.2.1. Method of preparation:

Dry ingredients presented in Table 3.3, were first sieved to be very fine particles then were weighted by using electrical balance (Model; 2003, Max; 200g cl; 0.001g, AC; 220W/50HZ, S/N; 11g, SF; 400) and thoroughly all dry matters were mixed well for homogeneity and the vegetable oil was added to the mineral and then mixed with the previous components. Tap water was boiled added (depending on the ingredient) to obtain an excellent past with about 10% moisture content. pellets were obtained by using manual meat grinder also 0.5 mm - diameter and later were dried in the lab for 48 hours and subsequently broken into crumbled form and each diet packaged in plastic bags and kept under the ambient room temperature until starting the experiment.

Table (3.2) the Ingredients of the Experimental Diets /100g.

Treatment Ingredients	Control	25%	50%	75%
Fish Meal (49cp)	24	18	12	18
Pigeon pea (18,4cp)	0,0	6	12	6
Groundnut Cake (44,8cp)	30,5	35	39	43,5
Wheat Bran (15,7cp)	28	27	25	22
Dora fitrita (11,5cp)	17	13,5	11,5	10
Mineral Mix	0,3	0,3	0,3	0,3
Vegetable Oil	0,2	0,2	0,2	0,2
Total crude protein in take	30,0	30,2	30,1	30,0

### 3.2.2. Experimental fish:

Fingerlings of Mono-sex Nile Tilapia (*Oreochromis Niloticus*) used in the study were purchased from Hussein Fadol private Farm., Soba West Agricultural Scheme (south Khartoum States) at the age of approximately 2 months old and average 2.6 - 3.9g (these Fish were stocked in a high densities and feeding them just to keep them alive without growing till they are sold) they were held in oxygenated plastic bag. About 250 fingerlings; after receiving were acclimated for about 30 minutes in the surface of the plastic tanks filled by tap water and then those

experimental fish were kept in plastic tanks (Aquarium) in fisheries laboratory and fed on a basal diet for 9 days (from 1-9 July). the reasons of acclimatization period, and once the adaptation period was completed some initial sample was taken to the laboratory of Animal Production College (University of Khartoum) for the chemical analysis as presented in table 3.3 below .

Table (3.3); carcass composition (DM %) of Mono-sex Nile tilapia (*Oreochromis Niloticus*).

Type	Moisture	DM	CP%	EE%	CF%	Ash	PH
Caracas	74.73	25.26	19	3.16	-	3.06	6.30

DM= Dry Matter. CP= Crude Protein. EE= Ether Extract. CF= Crude Fibre.

### 3.2.3. Experimental Design:-

The experimental fish (10/Aquaria) were distributed randomly to 12 plastic Tanks (Aquarium) 36L (36 x 34 x31 CM) according to the complete randomization (using paper method), filled with precipitated tap water (in Hatchery) fish were bulk, weighted, and their initial weight, and total length (from the tip of the mouth till the end of the caudal fin) for random sample were recorded, with average as  $3.3 \pm 0.01$ ,  $3,4 \pm 0.01$ cm respectively using digital scale (electronic kitchen ,auto zero, power 15v\*2.AA battery 5000g\*1g /177oz\*0.10z) and ruler respectively.

The fish in each aquarium were fed on the experimental diets according to the feeding regime four times/daily (at 8.30 -11.30 -3.30o, clock), for 10 days at rations 15% of body weight for the first 10 days, then reduced to 12% in the second 10 days, reduced to 9% in third 10 days, reduced to 6% in fourd 10 days and 3% in the last 10 days for 51 days. The daily feed allowance was adjusted every 10 days (sampling) after bulk weighing the fish, and also at the end of the feeding trial each fish were weighted individually by using electrical balance and the total length were measured for some sample randomly selected, also some fish sample were taken from each treatment and kept in the freezer for the chemical analysis.

All plastic tanks were siphoned twice (accessories and personal hygienic Adopt, no disease appears during experiment), before feeding daily in order to prevent accumulation of fecal matter and waste, also they were brushed during the week according to the situation of the aquarium. Then cleaned, fresh water was added approximately 8% (1.5 - 2.5 litter/one times) to reset water volume and also for cleaning at each sampling and filled again with completely new water. During the day of sampling no feed will be provided for fish.

### 3.2. Growth performance Parameters:-

$$\text{Weight gain (WG \%)} = w_2/w_1 \times 100$$

$$\text{Average daily gain (ADG) (g/fish day-1)} = \text{total gain /duration period (days).}$$

$$\text{Average daily feed intake (ADF/IN)} = \text{Total feed intake /duration period.}$$

$$\text{Specific growth rate (SGR\% day-1)} = 100 \times (\text{Ln } W_2 - \text{Ln } W_1)/n.$$

Where: Ln: Natural logarithms, n: is the duration period.

$$\text{Relative weight gain (RWG \%)} = \text{Weight Gain /initial weight} \times 100.$$

$$\text{Survival Rate (\%)} = 100 \times (\text{Final Number of Fish /Initial Number of Fish})$$

$$\text{Condition Factor (K)} = 100 \times W/L^3$$

Where:

W: Weight of the Fish in Gram.

L: Total Length of Fish in Centimetre.

#### 3.2.1. Feeds Efficiency:

1. Feed conversion ratio (FCR) = dry matter intake (g)/ total gain (%).

2. Protein efficiency ratio (PER) = total gain (g)/protein intake (g).

3. Protein productive value (PPV %) =  $(P_2 - P_1) \times 100 / \text{protein intake (G)}$ .



Where:

P1: Protein content at the start.

P2: Proteins content in fish Caracas at the end.

4. Energy Retention Efficiency ERE (EU %) =  $(E_2 - E_1) \times 100 / \text{Energy intake (Kcal)}$ .

Where:

ET: Energy in fish carcass at the end.

EL: Energy in fish carcass (Kcal) at the start.

5. Energy retention efficiency (%) =  $\{\text{Final body energy (Kcal)} - \text{Initial body energy (Kcal)}\} / \text{Dietary energy consumption (Kcal)} \times 100$

### 3.3. Statistical Analysis:

Feed ingredients experimental feeds and carcasses were analyzed for proximate composition following AOAC standard procedures (2003).

Statistical analyses of data were analyzed by SPSS (version16) 2010 (general model multivariate). Duncan's multiple range tests were used to evaluate specific differences between treatment means at (P0.01) and (P0.05). Excel sheet were used to describe the data in figures.

## Chapter Four

### Results:

#### 4.1. Growth performance Pigeon pea:

The study demonstrated that there were significant differences ( $p \leq 0.05$ ) in the (WG g/Fish), (DWG g/Fish) and (SGR) of mono-sex Nile tilapia (*Oreochromis Niloticus*) fed on diets with partially replaced by Pigeon peas. The growth parameters were analyzed and presented in Table (1).

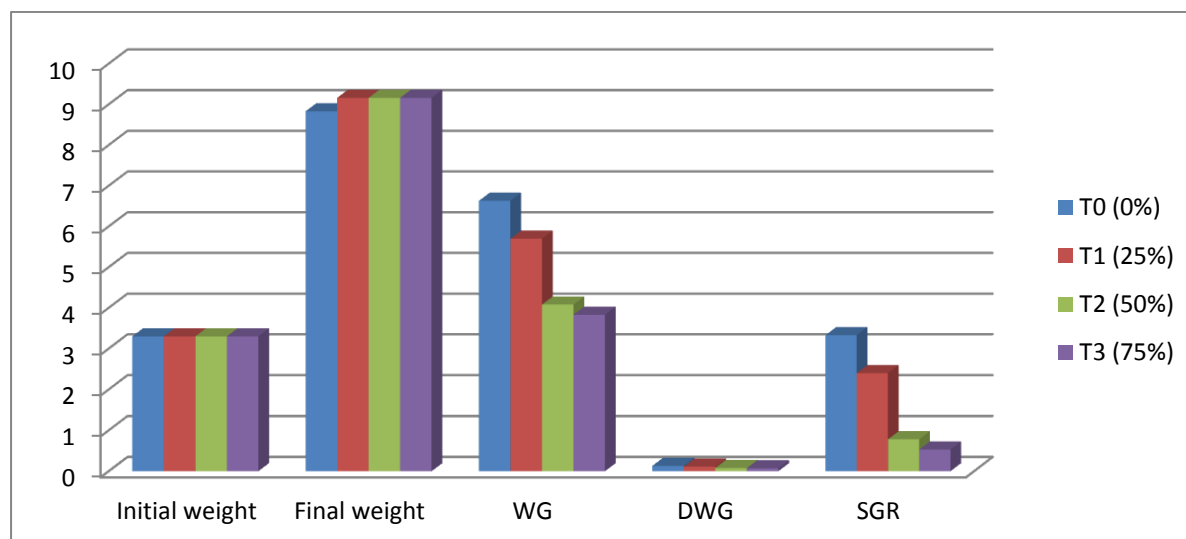
**Table 4.1. Growth performance of Nile tilapia (*O. Niloticus*) fed diets containing different levels of meal.**

Treat	Initial weight	final weight	WG	DWG g/d	SGR%
0%	3.4±0.01	8.83±0.44 <sup>a</sup>	6.63±0.73 <sup>a</sup>	0.13±0.01 <sup>a</sup>	3.33±0.73 <sup>a</sup>
25%	3.3±0.00	9.16±0.33 <sup>a</sup>	5.70±0.53 <sup>a</sup>	0.11±0.01 <sup>a</sup>	2.40±0.53 <sup>a</sup>
50%	3.3±0.00	8.50±0.28 <sup>ab</sup>	4.08±0.08 <sup>ab</sup>	0.08±0.00 <sup>b</sup>	0.78±0.08 <sup>b</sup>
75%	3.3±0.00	7.33±0.44 <sup>b</sup>	3.83±0.03 <sup>b</sup>	0.07±0.00 <sup>b</sup>	0.53±0.03 <sup>b</sup>

WG = weight gain, DWG = daily weight growth and SGR% = specific growth rate

Data are represented as mean of three samples replicates ± standard error.

\*: Means in the same Colum with the same letter are Significant different at ( $p \leq 0.05$ )



**Figure 1. Effect of Pigeon pea on weight of Nile tilapia (*O. Niloticus*) at different levels.**

#### 4.2. The increment weight:

Result in Table 4.2 show that the result of mean ± SD of increment weight of Mono-sex Nile tilapia (*Oreochromis Niloticus*) fed the experimental diet

Control, T1, T2% and T3% \days increase in control (3.4±0.01 to 9.93±0.73), T1(3.3±00 to 9.00±0.53), T2 (3.2±00 to 7.60±0.26) and T3(3.3±00 to 7.13±0.03).

**Table 4.2: The Increment Weight of Mono-sex Nile tilapia (*Oreochromis Niloticus*) (g/Fish) as affected with Pigeon pea Incorporation in Diets/Days.**

Diets	Weight of fish (g) at different times						
	0 times	10 days	20 days	30 days	40 days	50 days	Increment (g)
T(0.0%)	3.4±0.01	4.37±0.29 <sup>ab</sup>	5.60±0.26 <sup>a</sup>	8.35±0.27 <sup>b</sup>	9.52±0.24 <sup>a</sup>	9.93±0.73 <sup>a</sup>	6.63±0.73
T (25%)	3.3±00	4.74±0.33 <sup>a</sup>	6.36±0.27 <sup>a</sup>	9.46±0.23 <sup>a</sup>	9.74±0.12 <sup>a</sup>	9.00±0.53 <sup>a</sup>	5.70±0.53
T(50%)	3.2±00	4.14±0.04 <sup>b</sup>	4.60±0.27 <sup>b</sup>	7.38±0.26 <sup>c</sup>	7.60±0.26 <sup>b</sup>	7.38±0.08 <sup>b</sup>	4.08±0.08
T(75%)	3.3±00	4.06±0.02 <sup>b</sup>	4.78±0.08 <sup>b</sup>	5.68±0.36 <sup>d</sup>	5.51±0.41 <sup>c</sup>	7.13±0.03 <sup>b</sup>	3.83±0.03

Data are represented as mean of three samples replicates ± standard error.

\* Means in the same row with the same letter are not significant difference (P>0.05)

### 4.3: Feed Efficiency:

Results in Table 4.3 indicate that the mean ± SD of feed intake, food conversion ratio, protein productive value, condition factor (K%) and survival rate. The lower significant value of feed intake (The death fish is not lower values of the mortality rate excluded) was (55.88±0.00 and 60.79±0.00) was obtained by fish diet T0 (give low FCR), and T2 ( which give high FCR). The highest (p<0.01) value of the K was (1.89 and 1.46) in T3 and T0. The highest survival rate was in T2 and T1.

**Table 4.3: Growth and Food Utilization Efficiency of Mono-sex Nile tilapia (*Oreochromis Niloticus*) Fingerlings Fed the Experimental Diets.**

Treat / Item	T0 (0.0%)	T1 (25%)	T2 (50%)	T3 (75%)	Sig
Feed intake	55.88±0.00 <sup>c</sup>	67.60±0.00 <sup>a</sup>	60.79±0.00 <sup>b</sup>	45.15±0.00 <sup>b</sup>	**
FCR	8.60±0.85 <sup>c</sup>	12.08±1.19 <sup>b</sup>	14.91±0.30 <sup>a</sup>	11.77±0.10 <sup>b</sup>	**
PPV	39.33±0.19 <sup>a</sup>	34.33±0.00 <sup>a</sup>	33.66±0.00 <sup>b</sup>	29.00±0.19 <sup>c</sup>	**
K (%)	1.46±0.16	1.19±0.16	1.21±0.11	1.89±0.36	NS
SR (%)	94.27± 7.9 <sup>a</sup>	71.10± 10.8 <sup>b</sup>	69.12±8.6 <sup>b</sup>	89.59±11.6 <sup>c</sup>	**

Data are represented as mean of three samples replicates ± standard error.

\*\* : Means in the same row with the same letter are Significant different at (p≤0.01)

NS: NO Significant different at ( $p \geq 0.05$ )

#### 4.4: Feeding Rate (Regime %):

Results in Table 4.4 indicate that the mean  $\pm$  SD of feeding regime % for Mono-sex Nile tilapia (*Oreochromis Niloticus*) fed the experimental diets /Sample decreasing of feeding intake as feed rate decrease, however the initial highest feed intake was in T2( $13.80 \pm 0.58$ ) and T0 ( $13.78 \pm 1.16$ ), Although they showed the lower percentage at the final feeding regime T3 ( $2.50 \pm 0.40$ ) and T0 ( $4.88 \pm 0.95$ ) respectively (the differences was due to electricity problems, losing their appetite and at the end the mortality rate increased).

**Table 4.4: Feeding Rate (Regime %) Intake by Mono-sex Nile tilapia (*Oreochromis Niloticus*) / Sample.**

Diets	Feed intake (%) at different sample				
	1(15%)	2(12%)	3(9%)	4(6%)	5(3%)
Control 0(0.0)	13.78 <sup>c</sup>	14.15 <sup>a</sup>	12.25 <sup>b</sup>	9.78 <sup>c</sup>	4.88 <sup>c</sup>
T1	12.80 <sup>d</sup>	13.86 <sup>b</sup>	15.39 <sup>a</sup>	15.40 <sup>a</sup>	7.84 <sup>a</sup>
T2	13.80 <sup>a</sup>	14.17 <sup>a</sup>	12.63 <sup>c</sup>	12.63 <sup>b</sup>	6.56 <sup>b</sup>
T3	12.99 <sup>b</sup>	13.98 <sup>b</sup>	7.48 <sup>d</sup>	6.20 <sup>d</sup>	2.50 <sup>d</sup>

Data are represented as mean of three samples replicates  $\pm$  standard error

\*\*: Means in the same Colum with the same letter are Significant different at ( $p \leq 0.01$ )

#### 4.5 Body Composition:

The mean  $\pm$  SD of the chemical composition of the whole fish body as affected by partial replacement at the end of the experimental period are illustrated in table 4.6. In all groups including Pigeon peas in the diets, fish body dry matter and protein content was improved as compare a fish fed diet containing 75% diet which has high protein and control diet which has lower body dry matter, Ether Extract and crude fibre. Fish fed diet containing 25%, 50% and 75 Pigeon peas showed the highest significace ( $P < 0.01$ ) in protein body content, body dry matter, moisture and crude fibre respectively.

**Table 4.5: The End Carcass Composition (DM %) of Mono-sex Nile tilapia (*Oreochromis Niloticus*) Fed Experimental Diet.**

NO	Mot	DM	CP	EE	CF	Ash	PH	Sig
T0 (0%)	75.80±0.26 <sup>a</sup>	24.20±0.26 <sup>d</sup>	18.20±0.10 <sup>c</sup>	2.96±0.11 <sup>c</sup>	00±00 <sup>d</sup>	3.03±0.05 <sup>d</sup>	6.36±0.05 <sup>a</sup>	**
T1 (25%)	71.36±0.05 <sup>b</sup>	28.63±0.05 <sup>c</sup>	19.90±00 <sup>bc</sup>	3.43±0.05 <sup>b</sup>	1.90±00 <sup>c</sup>	3.40±00 <sup>c</sup>	5.90±00 <sup>b</sup>	**
T2 (50%)	70.36±0.23 <sup>c</sup>	29.63±0.23 <sup>b</sup>	20.00±00 <sup>b</sup>	3.63±0.05 <sup>a</sup>	2.30±00 <sup>b</sup>	3.63±0.05 <sup>b</sup>	5.70±00 <sup>c</sup>	**
T3 (75%)	28.36±0.05 <sup>d</sup>	31.63±0.05 <sup>a</sup>	21.30±0.10 <sup>a</sup>	3.76±0.05 <sup>a</sup>	2.66±0.05 <sup>a</sup>	3.90±00 <sup>a</sup>	5.53±0.05 <sup>d</sup>	**

Data are represented as mean of three samples replicates ± standard error

\*\* : Means in the same Colum with the same letter are Significant different at ( $p \leq 0.01$ ).

#### 4.6 Length during Experiment:

Results in Table 4.6 indicated that mean ± SD of the initial length (cm) and final length (cm) of Mono-sex Nile tilapia (*Oreochromis niloticus*) fed control, 25%, 50% and 75% diets showed no significant differences ( $P > 0.05$ ) initial length and showed the highest significance ( $P < 0.05$ ) in final length.

**Table 4.6: Length of Mono-sex Nile tilapia (*Oreochromis niloticus*) During the Experiment.**

Item	T0 (0.0%)	T1 (25%)	T2 (50%)	T3 (75%)	Sig
IL (cm)	3.4±0.00	3.5±0.00	3.3±0.00	3.4±0.00	NS
FL (Cm)	8.83±0.44 <sup>a</sup>	9.16±0.33 <sup>a</sup>	8.50±0.28 <sup>ab</sup>	7.33±0.44 <sup>b</sup>	*

Data are represented as mean of three samples replicates ± standard error

\*: Means in the same row with the same letter are Significant different at ( $p \leq 0.05$ )

NS: NO Significant different at ( $p \geq 0.05$ )

IL = initial length, FL = final length.

## CHAPTER FIVE

### DISCUSSION:

The present study demonstrated the potentials of Pigeon peas for inclusion in commercial mono-sex Nile tilapia (*Oreochromis Niloticus*) feeds as fish meal, as well as being of importance for feed production in Sudan. There is little information in the scientific literature concerning the use of it in Nile tilapia feeds, particularly at the level of research and the feeds produced under commercial conditions. most tilapia utilize starch efficiently from 22 to 46% dietary starch while 22% is considered as optimum level for juvenile tilapia (**Wang et al., 2005**). It could be noticed that, the initial body weight of tilapia in all treatments were approximately similar, growth performance in terms of body weight gain (WG), specific growth rate (SGR) and daily weight gain (DWG) are presented in Table: 4.1 ,The study demonstrated that there were significant differences ( $p \leq 0.05$ ) in the (WG g/Fish), (DWG g/Fish) and (SGR) of mono-sex Nile tilapia (*Oreochromis Niloticus*) fed on diets partially replaced by Pigeon peas. The experimental fingerlings fed actively on each diet showed increase in weight of fish was observed in the final weight from each diet.

The Results in Table 4.2 show the increment weight of Mono-sex Nile tilapia (*Oreochromis Niloticus*) fed the experimental diet Control, T1, T2% and T3% \days increase in control ( $3.4 \pm 0.01$  to  $9.93 \pm 0.73$ ), T1( $3.3 \pm 0.0$  to  $9.00 \pm 0.53$ ), T2 ( $3.2 \pm 0.0$  to  $7.60 \pm 0.26$ ) and T3( $3.3 \pm 0.0$  to  $7.13 \pm 0.03$ ) has significant differences ( $p \leq 0.05$ ). the Recent study using Pigeon pea seeds, raw or soaked, have been included to replace 15, 30 or 45% fish meal in nitrogenous juvenile diets. Inclusion of pigeon pea, either raw or soaked decreased animal growth rate compared to the control diet. however, growth performance of Nile tilapia (*Oreochromis niloticus*) fed on soaked pigeon pea diets were closer to that of fish fed on control diet, and it was concluded that soaked pigeon pea could replace fishmeal in fish diets in order to reduce feed costs (**Ndau et al., 2015**). When pigeon pea meal (pressure-cooked

and sun-dried) was used to replace soybean meal, it was reported that 60% replacement resulted in better nutrient utilization and better growth in fish (**Obasa et al., 2003**).

The Results in Table 4.3 shows the mean  $\pm$  SD of feed intake, food conversion ratio, protein productive value, condition factor (K%) and survival rate. The lower significant value of feed intake The dead fish of (lower values of mortality rate was excluded) was ( $55.88\pm 0.00$  and  $60.79\pm 0.00$ ) was obtained by fish diet T0 (gave low FCR), and T2 gave high FCR). The highest ( $p < 0.01$ ) value of the K was (1.89 and 1.46) in T3 and T0. The highest survival rate was in T2 and T1. Similar Results were also reported by (**Ndau and Madalla, 2015**) for seven diets (three diets of raw pigeon peas and soaked pigeon peas and one control diet) were formulated to contain about 30% crude protein. Fishmeal was replaced by 0% (control), 15%, 30% and 45% for both raw and soaked pigeon peas. Results obtained in this study showed that growth and feed utilization of fish fed diets containing raw Pigeon peas seed meal decreased significantly ( $P > 0.05$ ) with increased dietary levels of raw Pigeon peas while significant results ( $P < 0.05$ ) were shown for fish fed soaked Pigeon peas seed meal with 45% level inclusion performing best next to control. It was therefore concluded that whenever the cost of Pigeon peas is lesser than fish meal, Pigeon peas can be soaked for 24 hours with a ratio of 1:3 w/v to replace up to 45% fish meal in fish diets as a way of reducing the current demand pressure on fish meal.

The Results in Table 4.4 indicate that the mean  $\pm$  SD of feeding regime % for Mono-sex Nile tilapia (*Oreochromis Niloticus*) fed the experimental diets decreasing of feeding intake as feed rate decrease, however the initial highest feed intake was in T2 ( $13.80 \pm 0.58$ ) and T0 ( $13.78 \pm 1.16$ ), though showed the lower percentage at the final feeding regime T3 ( $2.50 \pm 0.40$ ) and T0 ( $4.88 \pm 0.95$ ) respectively.

Results in Table 4.5 shows the chemical composition of Fish fed diet containing 25%, 50% and 75 Pigeon peas replacement, showed the highest significant ( $P < 0.01$ ) in protein body content, body dry matter, moisture and crude fibre respectively. The study showed significant differences ( $p \leq 0.05$ ) in final length.



## CHAPTER SEX

### **Conclusions and recommendations:**

#### **6.1. Conclusions:**

concluded that the inclusion of 25%, 50% and 75% Pigeon peas seed replacement is a good plant protein source, which could be partially replacing fish meal in Mono-sex Nile tilapia *Oreochromis niloticus* for its positive effects in growth performance , feed utilization.

Based on the results obtained from dietary composition of Pigeon peas, processing of Pigeon peas in cold water (1:3w/v) for 24 hours under room temperature has no significant influence on the nutritive value of Pigeon peas. However slightly reduction in dry matter, crude lipid and ash content has occurred.

The results of this study show that processed Pigeon peas seed can be included in Nile tilapia diets at 75% level without harmful effect in terms of growth performance and feed utilization of Nile tilapia fingerlings. It is therefore concluded that whenever the cost of Pigeon peas is lesser than fish meal, fish farmers can use soaked Pigeon peas to replace up to 75% fish meal in Nile tilapia diets as a way of reducing the current demand pressure on fish meal.

#### **6.2. Recommendations:**

The study recommended:

1. More research in feeding Pigeon peas seed for Nile tilapia and other culture species.
2. Rephcation of the experiment in different aquaculture systems (e.g. Earth pond, concrete ....etc) and in the present of the different fertilizations.

3. The availability of water analyses Kits to monitor and analyze the parameters during the experiment e.g. ammonia ( $\text{NH}_3$ ) which had the most lethal effect in fish aquaculture system.
4. More research is needed to examine good plant resources as fish meal replacement from local material.

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## Appendixes:

### A- Figures:

Figure (1) Growth performance and survival rate of Tilapia

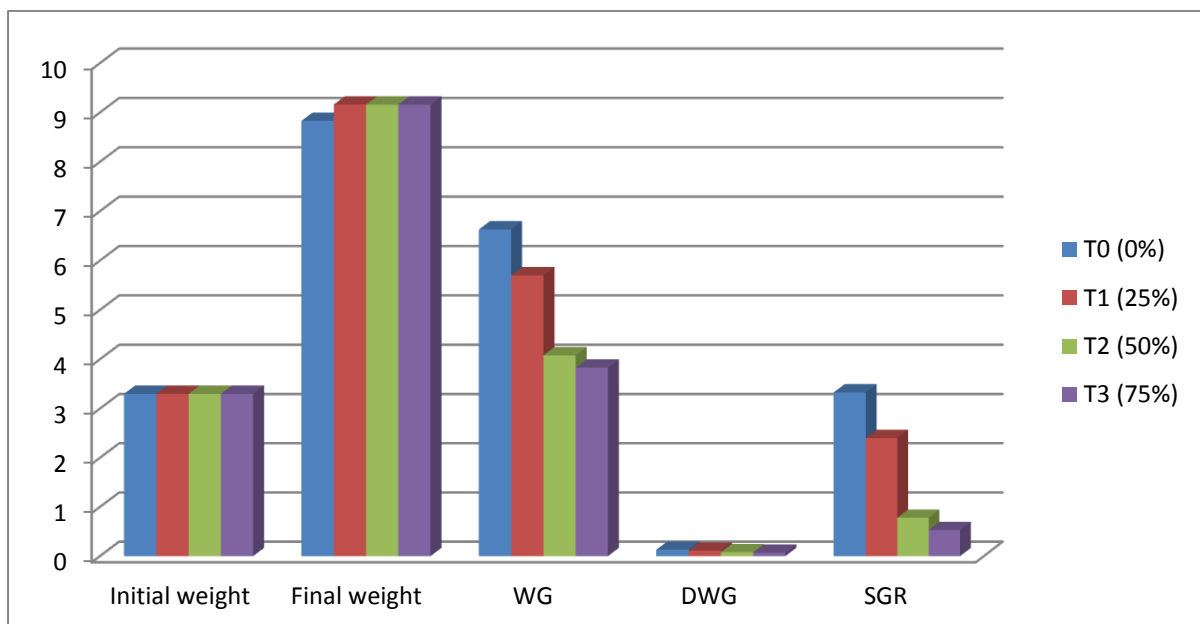
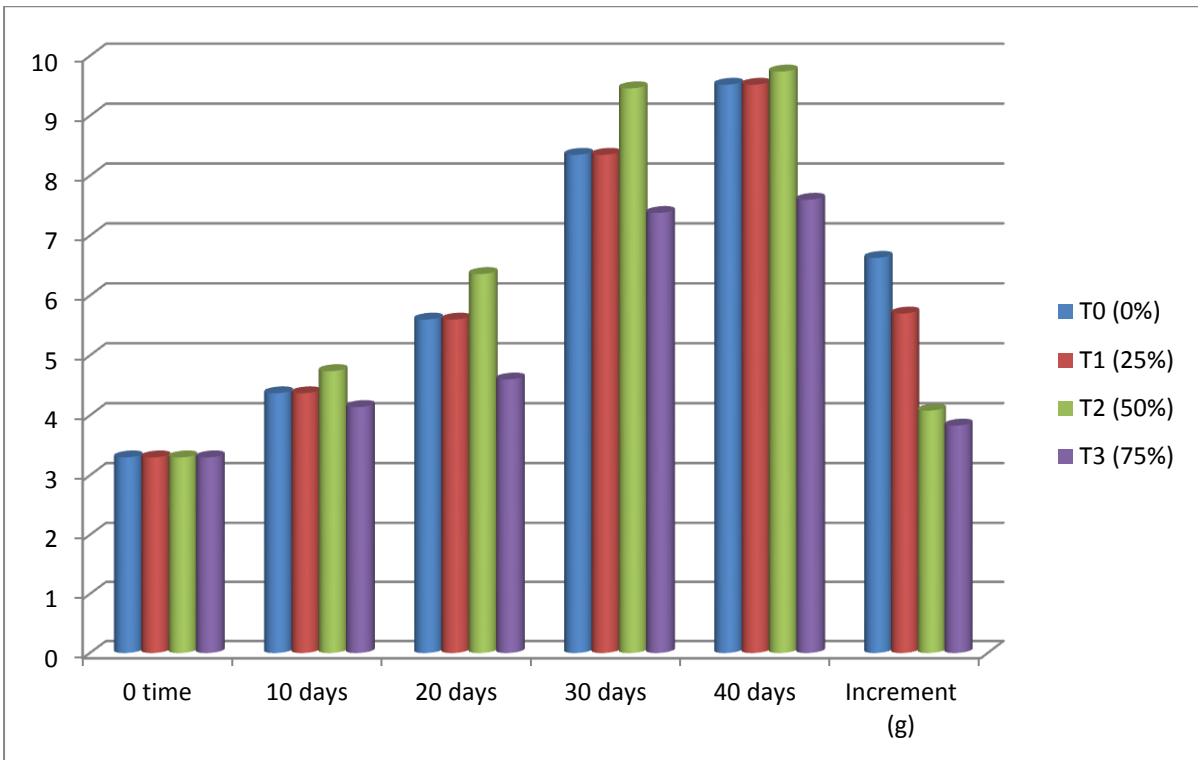
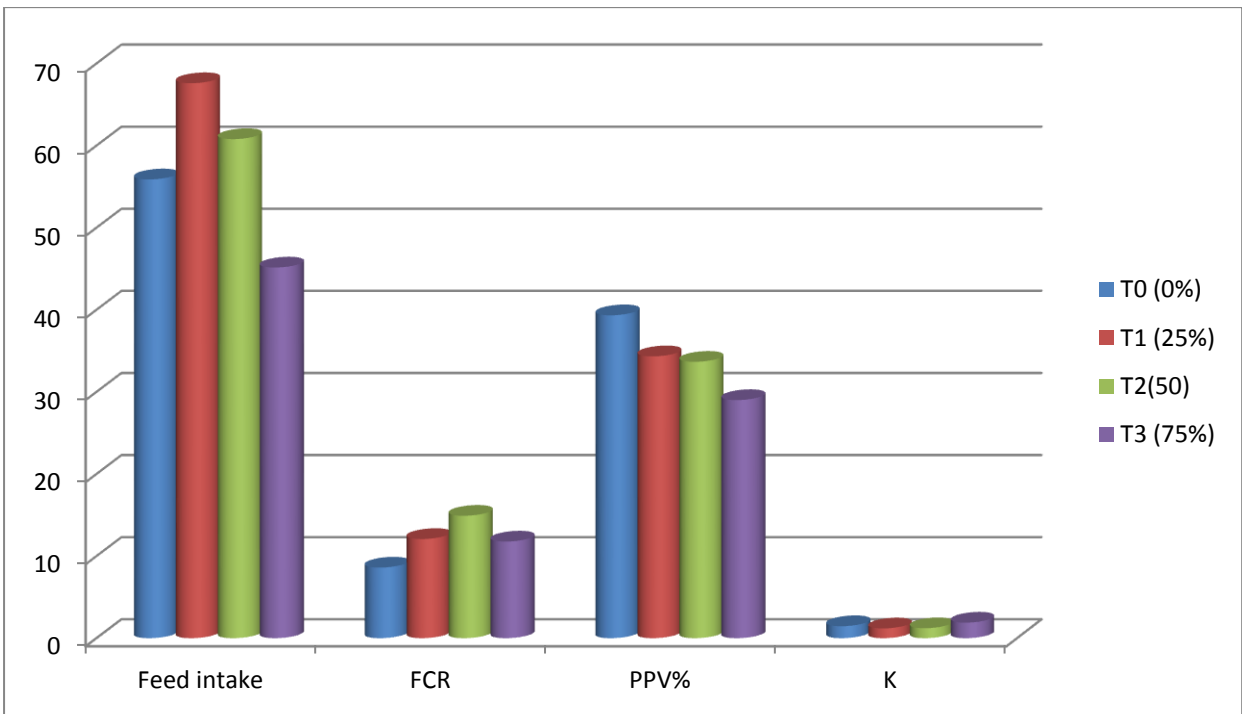


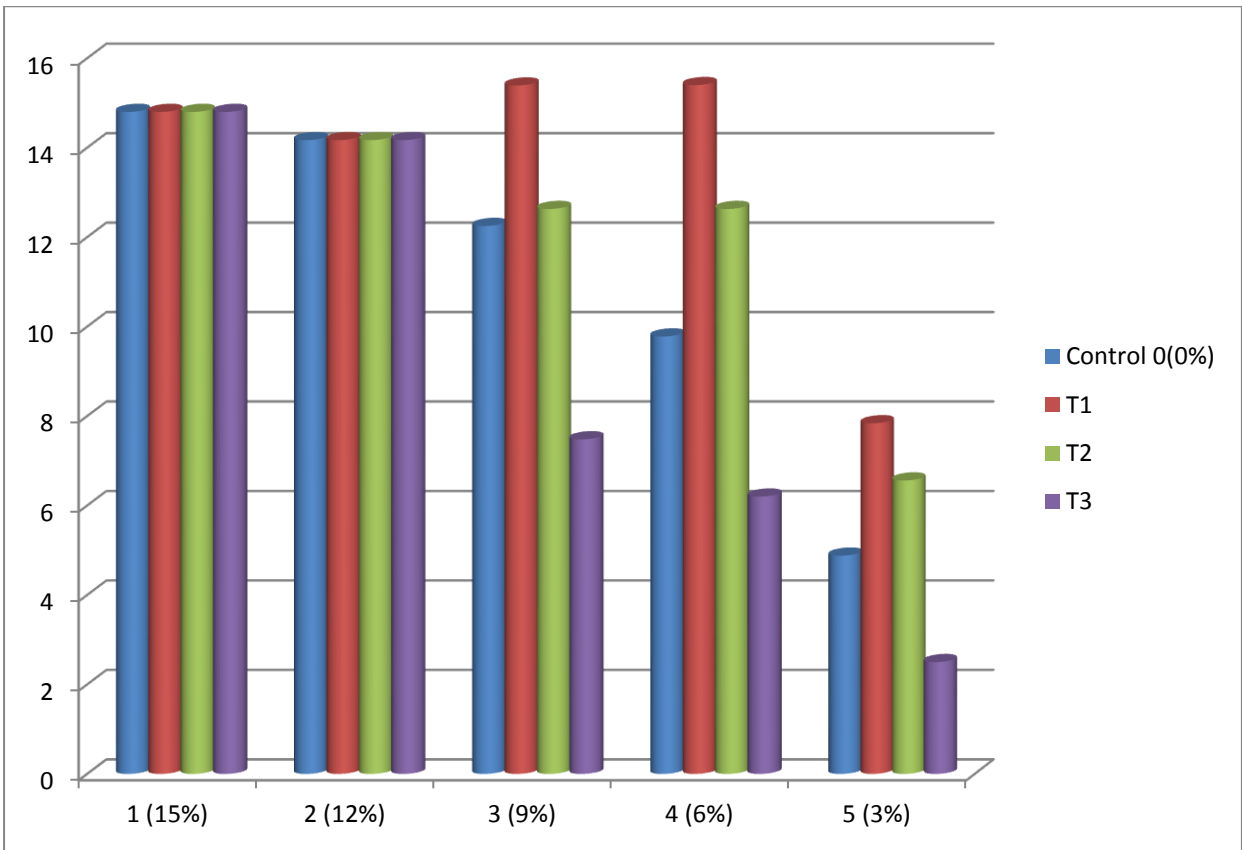
Figure (2) the increment Wight of Nile Tilapia (g/fish) as affected with pigeon pea addition in Diets/days:



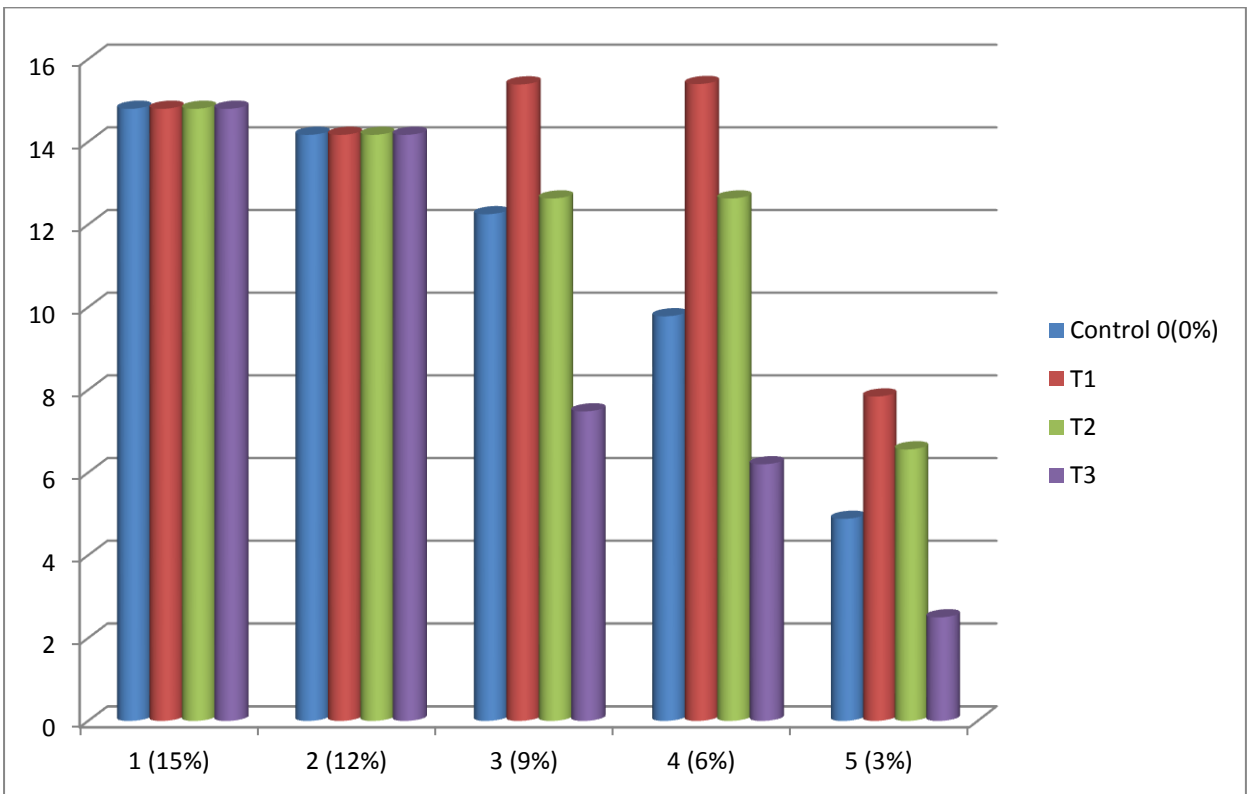
**Figure (3) growth and food utilization efficiency of Nile Tilapia feed experimental diets:**



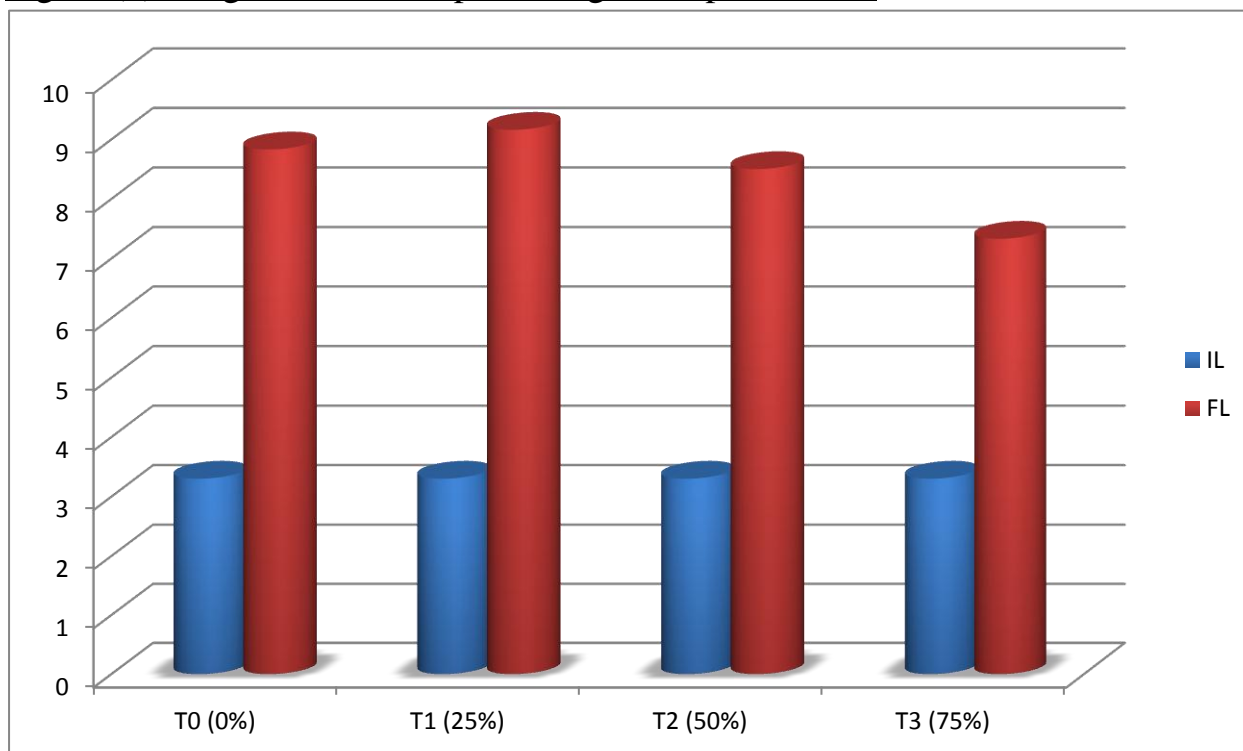
**Table (4) feeding rate (Regime) % intake by Nile Tilapia/sample**



**Figure (5) the end of carcass composition (DM %) of Nile Tilapia fed experimental Diets:**



**Figure (6) Length of Nile Tilapia during the experimental:**



**B- Images:**



**Image: fish Before the Experiments.**



**Images:** Fish after experimented.





Image: showed fish weighted.



Image: Transporting of Mono-sex Nile tilapia (Hussein Fadol Farm)



Image: The Experimental Plastic Tanks (Aquarium).



Image: The Experimental Diets.